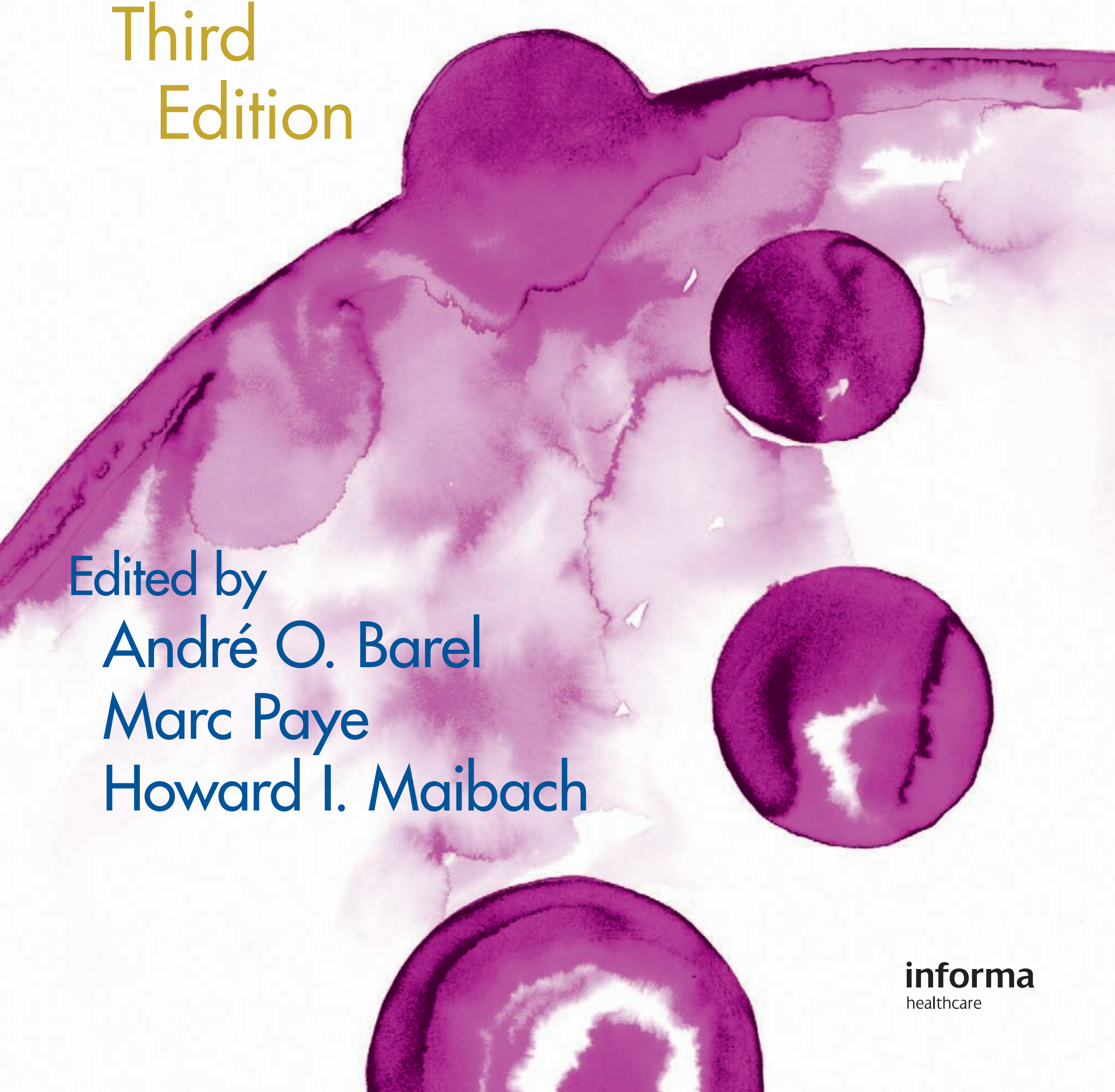
Handbook of Cosmetic Science and Technology

Third Edition



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Preface

Thanks to the contribution of leading experts in cosmetology, the first editions of the *Handbook* were successful and received excellent reviews. The editors appreciate the excellent author contributions.

The first edition, published in 2001, reviewed the multiple facets of the cosmetic field including the physiology of cosmetics targets and the safety, legal and regulatory context worldwide. It gave a broad overview of cosmetic ingredients, vehicles and finished products, and described the main methodologies used for microbiology, safety and efficacy testing. In the second edition (2006), we examined the future of cosmetology by the addition of chapters related to new ingredients, new delivery systems and new testing methodologies, but also by asking the previous authors to update their chapter with their speculation about the future in their field of expertise. To make the information more accessible, chapters were significantly reorganized.

Cosmetic science is a fast moving area. Furthermore, rapid and extensive changes in the worldwide regulatory context of cosmetics, increasing constraints and limitations in the choice of cosmetic ingredients and regular pressure from the media force the cosmetic formulator to think differently about his products. For all those reasons and due to more and more demanding and educated consumers asking for additional benefits from their cosmetic products, we have been asked to initiate the third edition of the *Handbook*.

Several chapters, from previous authors, are key in *Handbook of Cosmetic Science and Technology* and have been updated with the latest developments in the given field. However, it is the intention of the editors to give this version a new and important dimension that will complement the previous editions; a focus on the mechanism of interaction of the products or ingredients with their target.

Today, cosmetic products are of a high quality. If we want to further improve their quality, this will inevitably pass through an even better understanding of how those products or ingredients work to improve the appearance, protect their target or help maintain its natural functions. So, with the outstanding evolution of instruments to investigate in depth the skin or the hair, great progress is made daily in the understanding of the mechanisms of action of cosmetics. This understanding has been extensively covered in the third edition, which concentrates on skin, nail and hair cosmetics.

In the third edition, emphasis has been given to:

- Skin types, their relationship with age, sex, ethnic differences and the concept of sensitive skin.
- New bioengineering techniques for studying hydration of the skin – such as skin capacitance imaging and confocal raman spectroscopy – and for investigating skin friction and wettability.
- New developments in the description of skin aging and anti-aging treatments.
- In vitro skin tests using 3D reconstructed skin models.
- Specifically targeted cosmetics (decorative products, cooling and revulsive ingredients) and new forms such as oral cosmetics.
- An overview of the regulatory context for cosmetic preparations in the USA and in Europe, and of important ethical considerations in human testing.
- Finally, and controversially, the values and limitations of bioengineering measurements for the substantiation of efficacy claims.

The editors are grateful not only to the authors who contributed to previous editions and updated their chapters for the third edition, but also to the new authors who openly shared their "know how" in key areas.

Finally, we would like to invite readers' comments, criticisms and suggestions for improvements in order to ensure the continuous improvement of the *Handbook of Cosmetic Science and Technology.*

André O. Barel
Marc Paye
Howard I. Maibach

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1 Introduction

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Although cosmetics for the purpose of beautifying, perfuming, cleansing, or rituals have existed since the origin of civilization, only in the 20th century has great progress been made in the diversification of products and functions and in the safety and protection of the consumer.

Before 1938, cosmetics were not regulated as drugs, and cosmetology could often be considered as a way to sell dreams rather than objective efficacy; safety for consumers was also sometimes precarious. Subsequently, the Food and Drug Administration (FDA), through the Federal Food, Drug, and Cosmetic Act, regulated cosmetics that were required to be safe for the consumer.

With industrialization, many new ingredients from several industries (oleo- and petrochemical, food, etc.) were used in preparation of cosmetics, offering a list of new functions and forms. For a better control of these ingredients, U.S. laws required ingredient classification and product labeling since 1966.

In Europe, the Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the member states relating to cosmetic products ("Cosmetics Directive") was adopted in 1976 to ensure the free circulation of cosmetic products and improve the safety of cosmetic products by placing the responsibility of the product on the cosmetic manufacturer.

In 1991, the Cosmetics Directive was amended for the sixth time and prohibited the marketing of cosmetic products containing ingredients or combinations of ingredients tested on animals, as of 1998.

With the seventh amendment of the European Cosmetic Directive in 2003, a testing ban on finished cosmetic products was applied after 11 September 2004, whereas the testing ban on ingredients or combination of ingredients will be applied as soon as alternative methods are validated and adopted, with a maximum deadline of 11 March 2009, irrespective of the availability of alternative non–animal tests. For some endpoints (repeated-dose toxicity, reproductive toxicity, and toxicokinetics), a maximum deadline of 11 March 2013 was set up.

With regard to products, the latest innovation in the field of cosmetics is the development of active cosmetics (cosmeceuticals in the United States). Currently, cosmetics intend not only to improve the appearance or odor of the consumer but also to benefit their target, whether it is the skin, hair, nail, mucous membrane, or tooth. With this functional approach, products became diversified and started to claim a multitude of biologic actions. The cosmetic market then greatly extended with millions of consumers worldwide. The competitive environment pushed manufacturers to promise more to the consumers and to develop cosmetic products of better quality and higher efficacy. Today, many cosmetic products aim at hydrating the skin, reducing or slowing the signs of aged skin, and protecting the skin barrier and the skin in its entity against the multitude of daily environmental aggressions. For cosmetic products to support these activities, raw materials became more efficacious, safe, bioavailable, and innovative, while remaining affordable. With the continuous improvement of basic sciences and the development of new sciences, new sources for pure raw material have been found. Raw materials are not only produced from natural sources and are highly purified, but they can also be specifically synthesized or even produced from genetically manipulated microorganisms. However, the availability and use of these sophisticated and active ingredients are not always sufficient for them to be optimally delivered to their targets and to sustain their activity. The cosmetic vehicle is also crucial to obtain this effect, and the role of the formulator is to combine the right ingredient into the appropriate vehicle. Cosmetology has thus become a science in its own, and the cosmetologist is not only a formulator chemist anymore but also a real-life science scientist who needs to fully understand the interaction of his or her products and ingredients with their

targets to deliver the promised benefits. This is the reason why, in this third edition of the "*Handbook of Cosmetic Science and Technology*," the priority has been given to explaining the mechanism of action of cosmetic ingredients and products with their target.

Additional sciences also developed at parallel to active cosmetology and contributed significantly to its rise; this is the case for biometric techniques, which have been developing for more than two decades and allow a progressive and noninvasive investigation of many skin properties. Instruments and methods are available to objectively evaluate and measure cutaneous elasticity, topography, hydration, and turnover rate or even to see directly in vivo inside the skin through microscope evolution. Major innovations in the field are reported by the International Society for Biophysics and Imaging of the Skin. Guidelines for the appropriate usage of instrumental techniques and the accurate measurement of skin function and properties were published by expert groups such as the Standardization Group of the European Society of Contact Dermatitis or the European Group for Efficacy Measurement of Cosmetics and Other Topical Products (EEMCO). Any claimed effect of a cosmetic on the skin should today find appropriate techniques for a clear demonstration. Several other books describe in details all these methods, and so purposefully we have been very selective in this edition to cover only some very new, and maybe not so well known today, bioengineering methodologies that are emerging or are complementing other chapters of this handbook.

For better protection of the consumer against misleading claims, national or federal laws prohibit false advertisement of cosmetic products. In Europe, the sixth amendment of the European Directive on Cosmetic Products requires manufacturers to have readily available a dossier with the proof of the claims made on their products. The seventh amendment of the European Directive, published in March 2004, among several other requirements explained later in this book, also made information about the product more easily accessible to the public by any appropriate means, including electronic means.

Currently, big changes in the regulatory context are taking place and will greatly impact the cosmetic market. A recast of the European Cosmetic Directive has been adopted and is waiting for implementation very soon; this will strengthen consumer protection by limiting further the use of some ingredients and implementing stricter rules of postmarketing surveillance. The implementation of REACH (Registration, Evaluation, and Authorization of CHemicals) will also have implications by limiting the number of ingredients available to the cosmetic industry and creating high pressure on small and middle-size enterprises (SMEs). At a later stage, we may also expect changes in ingredient availabilities at a global level, with the set up of the global harmonization system (GHS). All the changes in the regulatory context are often an "affair of specialists," and we are proud to have real experts who have accepted to discuss the latest developments in that field for the purpose of this handbook.

Another topic that is clearly of interest today is the replacement of animal testing by alternative methods for testing the safety of cosmetic ingredients. The cosmetic industry, by separate activities or via its association, the COLIPA (The "European Cosmetic, Toiletry, and Perfumery Association"), has been extremely active in developing in vitro methods and strategies for confirming the safety of their ingredients. Even if much work has still to be done, great progress has been realized. Some updates on method developments are described in this book, although it has not been possible to cover all of them.

Finally, cosmetology has become a science based on the combination of various expertise domains: chemistry, physics, biology, bioengineering, dermatology, microbiology, toxicology, statistics, and many others.

Because of such a complexity in cosmetic science, it was not possible to cover in a useful manner all the aspects in one book. Details in most of the above fields are covered in the different volumes of the "*Cosmetic Science and Technology*" series. In the first edition of the "*Handbook of Cosmetic Sciences and Technologies*," we especially aimed at producing a useful formulation guide and a source of ideas for developing modern cosmetics. Four years later, with the second edition of the handbook, about 20 chapters were added, while the others were updated by trying to cover the most recent innovations in terms of ingredients and cosmetic vehicle forms that should orient the type of products of the future. The third edition is very different from the first two. A few chapters were updated from the first editions, but most are new, and the outstanding contributors were asked to deeply explain the science behind the products, ingredients, or methodology. Thus, the third edition may be seen, in some instances, as complementary to the two first editions.

The third edition of the handbook has been reorganized and subdivided into nine sections, including several chapters written by different authors. It may seem to some as too many chapters, but the editors chose this format intentionally to guarantee that each subject be described by a recognized expert in his/her field who is well aware of the latest development in the topic. Also, authors were selected worldwide. Indeed, cosmetology is universal, but there exists some regional specificity, which had to be addressed.

The first part of the handbook provides the reader with an overview of the different kind of skin types and their specificities. This is especially important at a time when cosmetic products become more and more diversified and targeted to ethnic skin, sensitive skin, elderlies, or others.

"Skin Hydration" (part II), "Skin Barrier" and "Skin pH" (part III) are then addressed from product or ingredient, mechanism, and assessment perspectives. Part IV ("Skin Aging and Sun Care products") covers the latest development in terms of skin aging and sun care products, which represent a large contribution to the current cosmetic business.

Today, consumers are not satisfied anymore with the claims made on cosmetic products; they also want to see or perceive any claimed property of their product. This is why part V, devoted to skin perception, has been introduced with recent developments in measuring what has long been considered as subjective and not measurable. Covering various aspects of skin tolerance is an important section of the handbook (part VI) and provides the reader with up-to-date information on the mechanism of skin irritation, last developments about in vitro predictive methods, specificities related to body sites or skin types, and expert view on allergenicity and allergens.

The sections "Targeted Cosmetics" (part VII) and "Cosmetic Vehicles" (part VIII) have been considerably reduced in the third edition and intentionally focused on emerging products that will represent, for most of them, new trends in cosmetology. For more conventional cosmetic products, the reader is referred to some excellent chapters from the two first editions. Finally, the last section, "Ethics and Regulations" (part IX), provides a clear overview of the quickly evolving worldwide regulatory context and ethical requirements that should always lead any development and testing of new products.

Given the number of contributions, it has been a challenge to edit this third edition, only four years after the second; if it has been possible, it is because of the dedication of the authors and great support of Mrs. S. Beberman and D Bigelow from Informa Healthcare Inc. We thank all of them for making this enormous task easy, enjoyable, and fascinating.

2 Biophysical Characteristics of the Skin in Relation to Race, Sex, Age, and Site

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INTRODUCTION

The skin mainly intends to protect human beings against environmental aggressions.

It fills this "barrier" part through a complex structure whose external part is made up by the stratum corneum—a horny layer covered with a hydrolipidic protective film. This function is only ensured when this horny layer made up of the accumulation of dead cells is properly moisturized as the water is the keratin plasticizer.

The underlying epidermis also enables to reinforce the skin's defense capacity by ensuring the continuous and functional regeneration of the surface state (keratogenesis) and skin pigmentation (melanogenesis).

The dermis also plays this part and appears as a nutritional structure whose function is also particularly important for the maintenance, coherence, elasticity, and thermoregulation of the whole skin.

Finally, the hypodermis has a protective and reserve function.

According to its state, activity, and defense capacity, the skin can have different appearances directly related to the water and fatty content of the hydrolipidic film.

Fatty deficiency, indispensable for retaining water in the teguments, favors its evaporation and therefore skin drying, whereas an excess of lipidic components favors a state defined as oily.

Among the numerous skin classifications that are proposed, the one most closely connected with cosmetological requirements distinguishes four different types: normal, oily, dry, and mixed.

However, in practice, such a classification must be used cautiously. In fact, the words used are ambiguous and lead to various interpretations; the criteria of selection to define each category are difficult to standardize since they vary from one case to another, some observations can even correspond to opposite clinical profiles.

So, for example, severe changes in epidermal water content associated with superficial pH changes can modify the skin's appearance and lead one to establish a visual diagnosis of dry skin, whereas it may be actually an oily skin.

For a long time, the research undertaken to try to understand the mechanisms leading to structural modifications of the skin have been limited, the researchers focused more on the practical consequences than on the causes.

From now on, more recent works would lead to progress significantly, but presently the different classifications taken as the authority are still based on the clinical observation instead of being based on the measurement of the biological parameters involved.

Dry skin would mainly correspond to structural and functional modifications of the components of the epidermis.

Oily skin would result from an excessive seborrheic production, invading skin surface and possibly hair.

Resulting from totally independent processes, oily skin and dry skin therefore correspond to two states that must not be opposed to each other, as some skins can be "dry" or "oily" and dehydrated at the same time.

The biophysical characteristics of skin also vary according to sex and age and can differ for the same subject according to the anatomical site considered.

Finally, the distribution of these different types of skin widely varies according to the ethnical group we are referring to.

A standardization of the skin typologies based only on visual criteria therefore appears difficult and would require in the more or less long term to resort to other quantitative means of identification, notably referring to biochemical and biophysical data.

After a quick reminder of the parameters on which the traditional classifications are based, an overview of the incidence of race, age, sex, and anatomical site on the measurement of the various skin biophysical characteristics will be proposed to show the limits of any kind of classification.

CLASSIFICATION BY THE SKIN TYPES

With a weight of about 4 kg and a surface of about 1.8 m^2, the skin is the widest organ of the organism: Its constitution is approximately the same at qualitative level and on the whole body. However, it undergoes notable variations especially concerning its thickness, its components, and above all, the way and variety of implantation of the skin appendices. These variations enable the skin to have a perfect functional adaptation.

In addition to its main protective part, the skin also ensures numerous other essential functions such as permeation, metabolism, and thermoregulation and actively contributes to the sensorial function. This structural and functional diversity is influenced by intrinsic factors related to subjects, their ethnic group, their age, and their physiological, psychological, and pathological state and by extrinsic factors related to the immediate environment such as the dryness level, sun exposure, temperature, and wind.

Numerous skin classifications have been proposed; they are all privilege-specific criteria. So, from a cosmetic point of view, the reference criteria are the users' feelings and therefore the surface state of their skin and their capacity for seduction and even attraction. There is a connotation of well-being and pleasure. This selective criterion generally leads to classification of the skin into four main types, which still remain to be clearly defined, i.e., normal skin, dry skin, oily skin, and mixed skin.

These denominations, based more on the feeling than on the causes, are imprecise and even erroneous and entertain in practice significant misunderstandings between biologists and consumers, which will have to be progressively raised.

The improvement of the knowledge of the mechanisms involved actually leads one to progressively better differentiate what corresponds to an evolutionary process from a particular and immutable skin typology. If it is true, for example, that the dry skin often has a genetic component (1), most of the people experienced it at a given moment of their life (because of the climatic conditions, etc.). In the same way, most of the people at a given stage of their hormonal and sexual development had to face the troubles related to an oily or mixed skin.

Normal Skin

Contrary to all expectations, it is worth noting that there is no definition of normal skin, the latter being qualified in comparison with the other skin types: a normal skin is not a dry skin, not an oily skin, not a mixed skin, and no more a pathological skin.

A brief analysis of its structure and of its functions enables to draw a more positive definition of the normal skin.

At the more external level, there is a very thin protective epithelium that constitutes *the epidermis*. It plays the main part in protecting the organism against external aggressions, notably ensured through the cohesion of epithelial cells and the keratinocytes that undergo a specific process of differentiation as they migrate from the dermoepidermal junction to the skin surface. This cohesion results from intercellular ties caused by the desmosomes, which are mainly responsible for the very great mechanical resistance of the epidermis. However, the migration of the keratinocytes remains possible since these desmosomal ties are submitted to a continuous process of dissolution and reconstitution associated with a progressive decrease in their adherence strength.

Keratinization corresponds to the most important structural and biochemical change that the epithelial cells undergo. Through this process they synthesize keratin, a fibrous complex protein whose structure evolves during cell differentiation. This process starts at basal level and ends with the transition between keratinocytes and corneocytes, which are cells mainly

full of a fibrous material. Corneocytes in degradation and intercellular lipids form a horny cover that reinforces the solidity and mechanical resistance of the stratum corneum, which also depends on the corneocytar supply in water.

In addition to this mechanical protection, the epidermis also has, through its structure and the presence of specialized cells such as the melanocytes, Merkel cells, and Langerhans' cells, other more complex functions, among which are the regeneration of tissue, the exchanges with the medium, and the active defense against external aggressions.

At intermediate level, *the dermis*, a dense conjunctive tissue, is much thicker than the epidermis to which it is connected by the dermoepidermal junction, which is the area not only of cohesion but also of intense exchanges.

This conjunctive tissue is globally made up of an amorphous extracellular substance in which more or less mobile cells float, the whole being kept together by a frame of elastic and collagen fibers. Numerous vessels, nerve fibers, and appendices with main functions, notably the sweat and sebaceous glands and the hair follicles, go through the fundamental substance.

Among the cells, it is worth noting the presence of fibrocytes with proliferative capacity, responsible for the synthesis and maintenance of the extracellular material, of histiocytes, mast cells, and leukocytes, involved in nonspecific defense and in immune supervision.

Because of its structure and the distribution of its components, the dermis is generally divided into two areas. The *reticular dermis*, thicker than the dermis and mainly made up of an interlacing of collagen fibers, is the place where the lower parts of the appendices are located, ensuring the hypodermal junction. It mainly has a mechanical function through its capacity for deformation (extensibility and compressibility). The *papillary dermis*, at the dermoepidermal junction, fairly loose, much vascularized, and rich in nerve fibers and endings. It therefore has multiple functions: enabling the nutritional exchanges with the epidermis and regulating the capacity for percutaneous absorption through its vascular and lymphatic networks, providing protection against aggressions and mechanical deformations through its fibrillar texture, ensuring sensory perception by the presence of most of the nerve endings, providing defense against foreign bodies by participating in the immune inflammatory and phagocytic processes through the existence of specialized cells, and maintaining tissue reconstruction.

Finally, at the most internal level, the *hypodermis*, which consists of loose conjunctive tissue, is linked to the lower part of the dermis by expansions of collagen fibers and elastic fibers of different thickness according to the anatomical areas. This tissue mainly contains adipocytes full of triglycerides, histiocytes, and mast cells. Its vascularization and innervation also vary according to the anatomical locations.

The hypodermis mainly has the function of protecting and reserving fat. Its mechanical properties are very badly known, but by enabling the skin to move as a whole on the underlying levels, this skin layer plays a main part in the breaking of the external strengths of deformation. In fact, it has been observed that the cicatricial elimination of the hypodermis results in a significant increase in the constraints of skin extension or friction due to a loss of mobility (2).

Therefore, considering its structure and its functions, a normal skin should be a smooth skin, pleasant to touch, because of the cohesion of the cells of its more superficial layers; a firm and supple skin because of the existence of a dense supportive tissue and of the presence of numerous elastic fibers of good quality; a mat skin through its balanced seborrheic production; a clear and pinkish skin because of the perfect functionality of its microcirculatory network.

In reality, a skin complying with all these characteristics would only exist in the healthy child before his/her puberty (3).

At cosmetological level, we must be content with a less strong definition and consider normal skin as a young skin, structurally and functionally balanced and requiring no care apart from those necessary for its cleaning.

Dry Skin

The concept of dry skin has also never been clearly defined. The term "dry skin" conceals several complementary or opposite points of view (4). It remains completely different from the way it is approached. People connect this notion to the effects observed and to their sensorial dimension. Therefore, for them it is first of all a feeling of drying along with loss of skin suppleness and elasticity, characterized by a rough appearance often associated with an

important desquamation, and leading to a certain discomfort they intend to correct by using moisturizing products.

For the biologist, the xerosis would be first the consequence of a change of the coherence and functionality of corneocytes, the water deficiency of the superficial layers of the stratum corneum, when it exists, only resulting from it.

As a matter of fact, the physiopathogeny of most xerosis is still badly known, and it remains difficult to distinguish the causes from the consequences of these skin abnormalities (5).

As it has been said before, in normal condition, the corneal layer is made up of a regular assembly of corneocytes, forming a structure of modulated thickness with unique physical qualities (5).

Each corneocyte contains dampening substances called NMFs (natural moisturizing factors), resulting from the enzymatic degradation of the fillagrines, which fix a certain quantity of inter-corneocytar water and therefore exert a decreasing osmotic pressure as they migrate to the surface (5).

Any decrease in the enzymatic function therefore plays an important part on the NMF content and consequently on the osmotic pressure and on the opening of corneosomes, consequently easing a disorganized desquamation as it is observed with xerosis (5).

This dysfunction actually depends on a qualitative and quantitative change of enzymes and/or on an inadequate change of the pH of the corneum (6). The inter-corneocytar cohesion also depends on a complex mixture of lipids that constitute the lamellar structure interposed between the corneocytes (made up of fatty acids, sterols, and ceramides coming from the keratinosomes) (5).

Whereas most of the research focused on the study of the change of the function of the horny layer and of its constitution and led to the theory of moisture balance (7–12), few works have been undertaken to better understand the components of the epidermal cells that are involved in skin drying. Such works will enable better understanding of the mechanisms that lead to xerosis.

Previous studies have shown the importance of four factors predisposing to dry skin:

1. the lack of water of corneocytes, directly depending on the presence of NMF;
2. the epidermal hyper-proliferation, resulting from a deficiency in the renewal process of the keratinocytes;
3. the change of lipidic synthesis at cell level; and
4. the deterioration of the functionality of skin barrier, following a degradation of intercellular cohesion.

All these factors are interdependent.

Consequently, dry skin should be characterized by its rough appearance, without referring to its hydration level (13).

Recent research have actually questioned some established ideas notably the influence of the inflammatory process or of the content in calcium ions of the epithelial cells in skin drying. In fact, experimental results have shown that the supply of nonsteroidal anti-inflammatory agents (14) or of calcic regulators (15) did not significantly modify the skin's state. On the other hand, the use of specific inhibitors of tryptic proteases, and particularly of "plasminogen activation system," showed a capacity for restoring the normal state of the skin and for simultaneously suppressing all the changes related to skin drying, notably against the mechanisms of cell regulation and differentiation, of increase in transepidermal water loss (TEWL) of the horny layer, of acceleration of its renewal, and the epidermal thickness resulting from it (16).

From now on, these works enable to confirm that skin drying does not correspond to an irreversible state but results from a dysfunction involving the traditional "balance moisture theory" (17) and the "protease regulation theory," resulting from these new research (16).

As already seen, dry skin depends on numerous biological factors (13); its reparation implies the restoration of the epidermal barrier, actually damaged by the loss of fat and dehydration of the superficial layers of the stratum corneum.

Such changes are more easily objectivable in African-American subjects in whom the skin takes a perfectly visible ashy appearance. It is also advisable not to systematically associate dry

skin with old skin even if in elder subjects (18), as in them we globally note a decrease in the hygroscopic quality of the stratum corneum and in the desquamation of corneocytes and the retention of keratin, contributing to give a drier and rougher appearance to the skin (19).

Oily Skin

Whereas dry skin reflects a functional change of different skin components, the oily skin results from an overactivity of the sebaceous glands, leading to an overproduction of sebum overflowing on the skin, giving it a characteristic oily and shiny appearance.

In fact, sebum results from the disintegration of specific cells, the sebocytes, a short time before they are secreted from the sebaceous gland. Once again it results from a cell differentiation. Originally, sebum contains squalene, waxes, triglycerides, and sterols. Under the effect of resident bacteria, one part of the triglycerides is immediately hydrolyzed, and the main part of the cholesterol is esterified, the sebum excreted containing a significant quantity of free fatty acids contributing to the acidity of the pH of the skin surface.

Then this sebum blends with epidermal lipids produced from the destruction of the desquamated horny cells that also contain triglycerides and cholesterol to form the surface lipidic film covering the stratum corneum.

Human beings have the particularity to have at their disposal sebaceous glands almost all over the body, but their activity is not the same on all the anatomical sites. The production of sebum is more important on head, face, neck, shoulders, and thorax, areas where a hyperseborrhea can be the conjunction of a high production of the glands and of a greater number of glands (20).

Sebum is a natural skin detergent that gives the skin an amphiphilic wettability through the presence of free fatty acids and wax (21). It also plays a part in the maintenance of the functional qualities of hairs, a fungistatic activity, while having a nutritional function for bacterial species useful for the organism, and finally, a protective function against excessive dehydration in a dry environment through its effect on the epidermal barrier function, even if the sebum is not known to have a dampening activity (22) and has no influence on the skin's hydration level (23).

The change of its rate of production depends on genetic, endocrinic, and environmental factors (24).

The opposite of oily skin would not be dry skin since they can coexist, for example, on face. Such a statement is currently supported by many workers (25).

Actually, young children fairly never have seborrheic outbreak before the age of seven years, when the first secretion of androgenic precursors starts to form. This production will progress to reach its maximum at adolescence and then decrease with age.

It is also worth noting the racial differences related to sex—men globally having an oilier skin than women (19). Finally, at cosmetological level, it must be retained that oily skin is sometimes erythrosic, easily irritable, and particularly fragile.

Mixed Skin

It corresponds to a complex skin where the different types previously described coexist on different areas of body or face. The characteristic example is the face, where solid and oily skin with well-dilated pores on the medio-facial area can coexist with a fragile skin with fine grains on cheeks.

Such a skin requires conjugating the particularities and sensitivities peculiar to normal, dry, and oily skins.

A Peculiar Case: The Sensitive Skin

Racial, individual, and intra-regional differences in the skin reactivity to a number of external stimuli have been widely documented during the last 20 years. Contradictory findings about sensitive skin have been reported. However, the general belief is that such a specific reactivity, more frequent in the populations with light skin, corresponds to the conjunction of a different aspect of the skin barrier and vascular response and to a heightened neurosensory input, all related to a genetic component (26–29).

BIOPHYSICAL CHARACTERISTICS OF THE SKIN

As the skin constitutes the external cover of the whole human body, its role has been reduced since a long time to play a protective part against external aggressions.

The intense multidisciplinary exploration of the skin carried out during the past 30 years progressively enabled to better determine the specific function of its components, the nature and importance of the exchanges with the surrounding organs, and finally, the vital function that the skin exerts on the organism, in addition to its main part in natural defense.

These progressive discoveries show that the skin's functionality and immunity must not be separated anymore and lead to the concept of a real neuro-immuno-cutaneous endocrine system—the NICS (30).

As a living organism, the skin is in constant renewal and undergoes at the same time a progressive aging with a parallel decrease in its functionality; moreover, today it still remains difficult to distinguish what depends on natural evolution from what is under external control, especially concerning the actinic one.

At external level, the renewal leads to a progressive change of the skin's surface state, a perceptible sign of the changes of both physiological functions and biophysical properties.

To measure the effects of aging and possibly to prevent its happening, it is important to identify analytical parameters, as realistically as possible, which correspond to the population concerned. It is particularly true for the analysis of biophysical data.

Beyond the interindividual variations or those that can result from the methodological approach or from the material of measurement used, many authors have tried to identify the influence of the race, sex, and age of the populations observed and even the anatomical site on which the observations are made by the results obtained. The results of these investigations are sometimes contradictory, but from now on, they enable us to emphasize some tendencies to be taken into consideration when conducting studies on the human being.

The good previous knowledge of these differences is notably essential to know the efficacy, acceptability, and even tolerance of products applied topically such as cosmetics or dermatological products.

Their impact shall completely differ according to the market they are intended to, not necessarily for being inefficient, but only for not being directly suitable for the targeted population; not necessarily for questions of habit and mode, but mainly because they do not correspond to the potential consumers' ethnological specificities.

This part will give a brief reminder of the incidence of race, age, sex, and exposure site on the most commonly explored biophysical characteristics of the skin.

Incidence of Race

It is useless to talk about the interracial morphological differences. They are obvious and never gives rise to confusion at the very risk to complicate the problem of ethnical integrations.

At macroscopical level, Caucasian, Hispanic, Asian, and African skins are very different at first sight as their color is enough to give them a well-distinct appearance.

This difference disappears at microscopical level as all the types of skin have the same qualitative structure. However, this similarity is lower at quantitative level. So, for example, the size and cytoplasmic dispersion of melanosomes are completely different for black and Caucasian skins (31–33), because they correspond to different needs of photoprotection (34).

In fact, important functional differences exist between races and correspond to their necessary adaptation to the environment they are meant to live in. There are also several consequences regarding the repairing between ethnic skins (35).

So, whereas the mean thickness of the horny layer is similar between the different races (36,37), the number of cell layers in the stratum corneum of the black skin is higher than that noted in Caucasian or Asian skins. Black skins therefore have a more compact stratum corneum with a greater cohesion between cells that makes them difficult to remove (38). However, the surface of corneocytes is identical for all the types of skin (39). In apparent contradiction to this greater cell cohesion, it is advisable to emphasize that the spontaneous surface desquamation is significantly more important in blacks than in Caucasians or in Asians (39).

These differences must be taken into account notably when the capacity of the products for acting on cell renewal or for reducing skin drying is studied.

Interracial differences also exist concerning the melanocytic system. Even if each type of skin basically has the same number of melanocytes per unit of surface, there is no similarity concerning their structure (31) and their functionality (38). Whereas the melanosomes are small and concentrated in the keratinocytes to be then degraded in the superficial layers of the epidermis of Caucasian skins, they are much bigger, widely scattered in all the layers of the keratinocytes and are not degraded when they arrive in the horny layer of black skins, giving them a characteristic color (40). Colorimetric and spectrophotometric studies have shown that the interindividual and intersexual differences of skin coloration in the different races are mainly related to the blood concentration in hemoglobin for the Caucasian subject, both to the hemoglobin and melatonic pigment content in the Asian subject, and only to the concentration in melanin in the black subject (41).

Racial differences concerning the functionality of the epidermal appendices also exist.

Contrary to a firmly fixed notion, the number of sweat glands is not different between the racial types, whatever the geographical site, as the variations depend more on exogenous than on genetic factors (42,43). Today, nothing explains the different interracial smells, probably depending on bacteria (38).

It even never has been possible to demonstrate a possible racial incidence on sebaceous secretion as some authors report a more important activity for black skins (44,45), whereas others report no substantial difference in sebaceous production between races in their comparative studies (46). A recent study showed a more important sebaceous production on the back in the white than in the black skin (47).

Thorough studies have explained the interracial differences in the hair shape (48,49) and in pilosity, but did not manage to objectivate the differences between their chemical components (50).

The advancement of knowledge enables today to retain the assumption that the genetic factors and the intrinsic differences between ethnical groups actually have less importance than their capacity for adaptation to the environment they live in. Many recent publications reinforce this concept (51–53).

This different adaptation according to the races can have significant repercussions according to the field investigated.

Skin Relief

Wrinkles result from distinct structural changes occurring in specific parts of the dermis and the subcutaneous tissue. They are part of the skin's aging process, which combines both intrinsic and extrinsic components (54–56).

There is little information concerning the possible racial differences as the intra-ethnical variations according to the age and possibly the site seem to have a much more important impact on the variability of the measurements. However, among people of same age, it has been shown that the number of wrinkles is the highest in Caucasians, followed at a same level by the Hispanic and black people, the smallest number of wrinkles is observed in Asian subjects (57). A comparative analysis of the number of wrinkles on 10 anatomical sites of Caucasian and black subjects of same ages shows that actually the difference only concerns the peri-auricular area (58).

Color

The interracial difference is obvious and mainly depends on the content, size, and distribution of the melanosomes (59,60). As said, the number of melanocytes per unit of surface is the same for all the races but their structure is different (33,61,62). The color of black skin is mainly related to the particular migration of the melanosomes that invade all the epidermal layers and reach the horny layer without undergoing degradation, a process that is completely different from what happens in the skin of Caucasians (34,63).

Pigmentation favors a better protection against sun radiations and therefore actinic aging. This can explain why, from this point of view, aging is quicker for the Asian skin (60). The racial differences in constitutive pigmentation are also directly related to the incidence of pigmentation disorders (64), the black skin being much more exposed to hyper-chromatic spots that appear under the effect of external aggressors, or to hypo-chromatic spots for lack of

sun exposure (63,65,66). An order of increasing sensitivity to these alterations of pigmentation has been established, classifying the black skin as the most exposed, followed by the white skin sensitive to hyperpigmentation spots, then to a lesser degree Hispanic and Asian skins (57,60,67).

Because of the difference between the carnations of the different ethnic groups, it was not possible to have a similar classification for all of them. If it remained possible to define in a similar way three types of complexion for Caucasians, African Americans, and Hispanic Americans (dark, medium, light), only the Japanese skin had to be identified according to a pink-ocher-beige color scale (67).

Concerning skin brightness measured from the parameter L* of the CIE L*a*b* system, the best improvement of skin brightness after sun exposure is noted in Caucasians, followed in decreasing order by the Asian skin, the Hispanic skin, and the black skin that mainly remains dull. Except the black skin that has a lower index of brightness, all the other types of skin have a similar index in absence of sun exposure (57).

pH

According to some authors, no interracial difference is observed concerning skin pH (57). Others report a slightly higher pH for the Caucasian, in comparison with the black race (68–70). These variations rather depend on the age of the population examined as the interracial deviations are mainly noted in people aged between 30 and 50 years. The apparent contradiction in the black skin could be explained by a higher cohesion of the keratinocytes in the stratum corneum associated with specific mechanisms in its formation and renewal (71).

Electrical Conduction

The measurement of electrical conductance on the skin superficial layers enabled to show that it is the highest for the black skin, lesser for the Hispanic and Asian skin, and the lowest for the Caucasian skin (47,68,69,72–74). This electrical resistance is reported to be twice as high in black as in white skins (69).

Another study (58) seems to demonstrate that on the contrary there would be no difference between the electrical conduction of the skins of Caucasian subjects and of white subjects. It enables to conclude that the racial criterion is not the only parameter to be taken into account in the study of the skin's electrical conductivity. So, the measurement of capacitance on different skin sites enables to show contradictory interracial differences in the same study (58).

It is worth noting that the black skin shows a higher epidermal water content, although no change of the TEWL is observed. This particularity is justified by the greater cell cohesion of the stratum corneum, previously evoked for this ethnical group (75).

Trans-Epidermal Water Loss

Many experimental results show no interracial difference concerning the basal level of TEWL (47,72,76). More advanced studies enabled to establish that these global results were only giving an apparent response as the TEWL of the subjects of black race is actually significantly higher than that notably of Caucasian subjects, this difference being made up for in vivo by a lesser vasodilatation of the black skin under the effect of external aggressors.

This demonstration initially carried out in vitro (77) has been confirmed in vivo later on (47) by using substances able to neutralize the microcirculation locally.

The interracial variation could be related to the skin content in creaminess, the TEWL being inversely proportional to their concentration (78).

Interracial differences in skin permeability and barrier effect have been demonstrated under the effect of vasodilative agents (79) that show under the same experimental conditions a lower TEWL in subjects of Caucasian race than in those of Asian and black races, which are comparable with each other. When the aggression is a stripping, it has been shown that the return to normal depends more on the phototype of the skin than on the race, the darkest skins having a quicker recovery (80).

Biomechanical Properties
Measurements of the immediate extensibility (Ue), viscoelastic deformation (Uv), and capacity for immediate recovery (Ur) of the skin of the forearms of subjects of Asian, Caucasian, and black races to a deformation created by the twistometer have shown significant interracial variations particularly between Caucasian and black skin, which go in one or the other direction, depending on whether the measurements are performed on sites protected from sun or not (72): For the three races, the extensibility is lower when the skin is used to sunshine in comparison with what it is on a nonexposed site, this difference being clearly more marked for the Caucasian skin (arbitrary values ranging from 34 ± 3 to 40 ± 3 for the black skin and from 49 ± 2 to 28 ± 2 for the Caucasian skin, respectively).

The variations in viscoelastic responses are not significant between protected site and exposed site for the black subjects but are significant for the Caucasians and Hispanics even if no interracial difference is noted.

Black skin has the same capacity for recovery on both sides of the forearm, whereas there are significant differences between the two sites to the detriment of exposed areas for the Hispanic and Caucasian skins.

The capacity for recovery of the black skin is higher than that of the Caucasian skin.

The calculation of the module of elasticity $\left(\frac{1}{\text{extensibility}} \times \text{skin thickness}\right)$ that takes into account the incidence of skin thickness on the site of measurement showed significant differences between the three races to the advantage of the black skin, whereas the deviation between exposed site and protected site was only significant for the white race (72).

The elasticity index, measured by the ratio of recovery to extensibility enabled to show no appreciable difference between races. These results were confirmed by other authors using other sites and other equipments (68). The best elasticity of the black skin in comparison with the white skin would result from its greater content in elastic fibers per unit of surface (81).

Seborrheic Production
Sebaceous secretion would be globally more important on the black skins, followed by the white skins, by the Hispanic skins, and to a lesser extent by the Asian skins (36,44). This variation is partly questioned by other authors who have found no substantial difference in sebaceous production between Caucasian subjects and black subjects (45). Here again, the anatomical site taken into account seems to be deciding. The black skin has a higher lipidic content than that of the other races (82). Concerning this point, a seasonal variation is noted, the black skin being more lipidic in the summer than in the winter, notably on face, apparent paradox of a skin both dry and shiny, result of the superposition of a constitutional xerosis on a protective film of surface, made up of a mixing of sweat and sebum (83).

Actinic Aging
The analysis of the penetration of light into the skin and of the effects it induces was reported by many authors (84–87) who particularly took into account the behavioral difference between the Caucasian skin and the black skin. In spite of structural differences in the stratum corneum, the total reflectance of light at its level is located between 4% and 7% for the Caucasian and the black people (84). On the contrary, there is a significant difference in the light transmission through the epidermis of the Caucasian skin especially at wavelengths corresponding to the ultra violet (UV) radiations, which results in a considerable decrease in the natural capacity for actinic protection of this ethnic group. This transmission is less important in the subjects with the Hispanic skin (87). Similar differences were noted with UVA.

On the whole sun spectrum, it results in a natural capacity for photoprotection of the Caucasian skin three to four times as low as the black skin (88,89). This difference is directly related to the distribution of melanosomes in all the epidermal layers of the black skin (90).

The physiological and morphological impact of aging may affect the ethnic populations in different ways. As an example, comparative studies have shown that furrows appear earlier in French than Japanese women even if grade severity is found higher in elderly Japanese women. On the contrary, visual features related to the skin pigmentation appear earlier and in a more accurate way in Japanese women (91,92).

The examination of the available data concerning racial variations enables to conclude that these differences affect a reduced number of parameters, that the variations noted have a limited incidence, and that the results published are often contradictory. As a consequence, the interracial studies on the biophysical properties of the skin have to be tackled cautiously as the deviations observed actually depend on several factors that can act in a synergic or antagonistic way. Therefore, each experimental result will have to be confirmed. In addition, the dispersion of the results obtained in this type of study must incite the experimenter to establish study protocols that involve an enlarged number of subjects correctly selected to avoid the fact that the variability of individual responses hides the reality of intergroup differences.

Incidence of Sex

Although the influence of sex on the results of biophysical measurements is often quoted in bibliography, little precise information is supplied, maybe because this criterion actually has little real influence on the results.

However, there are morphological differences in the skin according to the sexes. In fact, the skin thickness is greater in men on most of the sites usually used for biophysical measurements (90,93,94), whereas for women, the skin is thicker at dermal level (95).

Other authors reported no significant differences for the forearms (96–98). Observations made on male and female Asian subjects enabled to show no difference between sexes concerning the number of layers of coenocytes (99). The skin thickness would reduce more quickly with aging in women than in men (100).

Skin Relief

To our knowledge, no publication brings relevant data concerning the influence of sex on the state and evolution of the skin relief.

The friction coefficient is also independent of sex (101).

Color

As already said, colorimetric and spectrometric studies have shown that pigmentation is more important in men than in women (41). A study carried out with a colorimeter on a Caucasian population showed that the parameter a* is generally the highest but that actually there is an interaction between sex and age for each of the parameters L*, a*, and b* (102).

pH

Measurements performed on different skin sites confirmed the absence of any influence of sex on the skin pH (103).

Electrical Conduction

A great number of investigators have dealt with the electrical conduction to characterize the hydration level of the superficial layers of the skin, as it is a deciding factor in the study of the neurosis or of the functionality of cosmetic products.

Several research teams have tried to determine the influence of the sex on the variability of the results observed. Different parameters have been explored, some directly representative of the skin's electrical conductivity such as the capacitance and impedance and the others representative of the opposite effect, i.e., the resistivity to conduction, such as the measurement of resistance.

No difference between sexes was shown concerning the conductance (101) and impedance (58). The more controversial publications concern the capacitance as some experimenters report no difference between sexes (104), whereas others, on the contrary, report a more important resistance to conduction in women than in men, on the basis of measurements performed on several anatomical sites (96).

Trans-Epidermal Water Loss

Studies conducted by different authors on the TEWL have shown no variation between sexes (101,105,106). Other researchers have reported a more important water loss in men than in

women (96,107); one of them in a study performed on Asians has related this difference to a lower basal metabolism in women (108).

Biomechanical Properties

The incidence of the sexes on the measurements of the biomechanical properties of the skin depends on the parameters used. Its dispensability is reported to be higher in women, independently of the sites chosen (109). Noncomparative measurements between sites have shown, on the forehead of women, an initial skin tension higher than that of men. This elastic retraction is also reported to be relatively more important on the leg in women. The nonelasticity index is relatively more important in women than in men, but the absolute values of this index are clearly different according to the sites observed (90).

Finally, these authors report that there is no difference between sexes, whatever the sites concerning the Young's module (90) and the hysteretic curve (109) for values that, in absolute, considerably differ between sites (110,111).

Seborrhea Production

The literature reports little relevant information on the incidence of sexes on sebum production. The rare publications mention a significant difference as men generally have, on the various sites studied, a higher sebum rate than women (96). On the other hand, the extent of this variation would be low compared with the incidence of race (44). The production of sebum would decrease with age, more particularly in women (62).

Incidence of Age

Because of the continuous aging of the skin and its incidence on its structure and functionality, the age of the subjects included in a study is often the main element to obtain relevant results. As we will consider in this chapter, age has a direct impact on the evolution of most of the biophysical parameters of the skin.

Skin Relief

Many publications have shown the incidence of aging on the increase in its roughness, the evolution of the microdepressionary network of the skin (110), and the development of wrinkles whatever the ethnic group considered (57).

To simplify, roughness can be considered as submitted to external and internal influences such as the climatic environment, the sun exposure, and the effect of cosmetic products but also the water content of the skin's superficial layers (112–115). The destructuring of the skin micro-relief as the appearance of lines and then of wrinkles result from a deeper change of the proper skin structure, a characteristic that progressively becomes irreversible even if its term can be reduced by palliative care (55).

Many methods have been proposed to measure as accurately as possible the levels of skin roughness, its microdepressionary network, or its different wrinkles.

These methods, most of the time instrumental, resort to the use of microsensors, image analyzers, and photometric or echographic analyzers able to supply a very great number of parameters among which only a few have real relevance.

Aside from these methodologies, now on mostly traditional, there are new developments, among which the frictional and acoustic measurements, which allow a more precise information. As an example, it has been demonstrated that a significant increase of the sound level between children and adult skins is indicative of their different smoothness (116).

Independently of the methodologies used, some facts have been established: The length of the microdepressionary network decreases with age (110), and the depth of the folds grows hollow as the first wrinkles develop (58). A systematic echographic analysis of wrinkles enabled to establish a scale of values per ethnic group, according to the age and to the site observed (117); the best correlation has been established for the number of wrinkles of the periocular area (118).

All the bibliographical data show that the evolution of the microdepressionnary network is particularly sensitive beyond the age of 40 years as the main lines start to grow hollow progressively (119). The lines of secondary orientation progressively disappear between the

age of 50 and 80 years, and we observe monodirectional lines orientated in the direction of the skin deformation and the multiplication of great spaces whose folds are not visible microscopically (110,114).

Color

For all the races, there is a decrease in the hyperpigmentation spots related to the age of the subjects (57). The colorimetric examination enables to note a decrease in brightness of the skin in the Japanese and in the Caucasians (120) as measured by the parameter L* of the CIE L*a*b* system (102). Concurrently, there is no significant change of the colorimetric parameters a* and b* and of the parameter C, corresponding to the skin's saturation (121).

In practice, these variations can differ according to the site observed and the level of sun exposure (57).

In total, we can deduce from the bibliographical data that there is a decrease in the brightness of the skin with aging but also that this variation depends on the site where the measurement is performed.

pH

There are few available data on the subject. To our knowledge, the only explorations published underline the absence of any variation in the skin pH measured on several sites according to the age of the subjects taking part in the study (57).

Electrical Conduction

The conductance generally increases with the age in all the ethnic groups (57). The capacitance measured comparatively in young and old subjects appears significantly lower in old subjects (58). In practice, this evolution is not linear as the capacitance actually increases with age until 50 years and decreases later on (122).

However, these observations must be considered cautiously because a more detailed analysis that takes into account the measurements on several anatomical areas shows that actually the value of conductance and capacitance is also closely related to the measurement site (96,101,104,123).

The electrical impedance measured with the spectrometer also varies according to age as the values of the indexes of magnitude (MIX), real part (RIX), and imaginary part (IMIX) increase with age, whereas the index of phase (PIX) evolves in the opposite direction (107). The indexes MIX and IMIX are considered as the most representative of aging.

Trans-Epidermal Water Loss

The relation between TEWL and age is most often questioned as some authors conclude that there is no relation between these two parameters (124,125), whereas others found that this relation does exist but is very slight (118) or that this correlation varies according to the anatomical sites where the measurements are performed. An increase in the TEWL on the forehead is described (96,122). On the whole, the authors rather report a decrease in the TEWL according to age on most of the other sites examined (96,101,125).

These contradictory data incite to act with the maximum attention to measure this parameter, taking care to have an objective reference at disposal for each measurement.

Any correlation to the measurements of capacitance is strongly questioned (126–128).

Biomechanical Properties

Globally, a decrease in skin elasticity with age has been reported (110,129). This is the same for tonicity and extensibility.

Actinic Aging

In the adult person, epidermal proliferation rate decreases with age. It can be 10 times higher in younger (second decade) than in older (seventh decade) individuals, and for a given age, the decrease was demonstrated to be 10 times faster in sun-exposed areas than in unexposed ones. These constant reductions seem to be independent of the ethnic origin and season (130).

Incidence of Site

As previously seen, the racial criteria, age, and sex are not enough to define the skin's response to an aggression or to a possible restructuring effect. In fact, important variations exist in the subject considered separately according to the sites on which the measurements are performed, these variations being sufficiently important to invalidate the experimental results.

Without trying to be exhaustive, this last part of the analysis supplies many concrete examples meant to incite the experimenters to choose accurately the site of measurement, according to its specificity, to the exploration that must be undertaken and also according to the reference, which is taken into account for the appreciation of the significance of the effects observed. The spontaneous changes of the skin's state over time according to intercurrent factors that depend on physiological and hormonal variations and on its proper aging therefore imply that their incidence is systematically taken into account, such an approach can only be performed case by case.

The skin's thickness is not the same between anatomical sites as established in the publications of many authors through numbered data and different instrumental measurements. So, for example, the skin's thickness measured in the subject of Caucasian race is less on the forearm than on the forehead, of the order of 0.9 and 1.7 mm, respectively (90). These values are slightly higher than those described by other authors (93,131–133) but can be taken into account as the approach was performed through a more elaborated technique based on high-resolution scanning (90,100). In addition to the differences that exist between anatomical sites, there are great variations for the same area. This is the case, for example, between different areas of face (96), between the dorsal and volar area of the forearm (72), and between different locations of the forearm (134).

Measurements performed with a scanner on 22 anatomical sites of young male and female Caucasians enabled to note that the skin is all the more echogenic since it is thinner and that at acoustic level the response of the reticular dermis is denser than that of the papillary dermis. This acoustic density, also inversely proportional to the skin's thickness, is consequently variable according to the thickness of the anatomical sites measured (95).

It must be underlined that in spite of differences in the absolute values from site to site, the evolution of the response of a given site can be predictive for other sites in the same person. This is of most interest in clinical research. As an example, the volar forearm is considered as representative of the face for measuring the skin's hydration and biomechanical properties (135).

Skin Relief

As it has already been said, at basal state, skin relief is directly representative of the state of anisotropy of the local tensions, and the structural deformations or changes it undergoes are directly dependent on the constraints underwent (mechanical constraints and aging but also external aggressions) (136). This relief is therefore necessarily specific according to the sites observed as it can be shown by a simple visual examination of the structure and topography of the skin at different levels, for example, face, neck, limbs, and hands (137). Beyond the structural differences between anatomical sites, there are also differences in levels of roughness (58,138–140).

Color

There are important natural variations in the skin color between anatomical sites in absence of the additional effects on melanogenesis induced by sun exposure. Colorimetric measurements performed according to the CIE $L^*a^*b^*$ system on 18 different sites enabled to note in the subjects of Caucasian race of prototypes I and II a more important variation in the parameter a^*, directly connected to the redness of the skin (141).

A comparative analysis between cheeks, forehead, and volar side of the forearm, usually exposed to the sun, showed that the forearm is lighter than the sites on face, the values of the parameters a^* and b^* being significantly highest for the forehead (119,138). Important variations between the measurements performed on different site of a same anatomical area are also reported. Thus, for example, the variation in the values a^* and b^* is between distal and proximal forearm (141) and high and low part of the back (102). For a given race, the parameter L^* seems to be slightly influenced by the anatomical site where the measurement is performed (119,138,141).

The location of the site of measurement is therefore very important during a repeated colorimetric analysis of the skin. The interference that results from the variation in pigmentation according to its exposure to the sun's UV radiations is very important and can also induce higher deviations than those existing between anatomical locations.

All the experimental studies that resort to colorimetric measurements have to take the incidence of this interference into account on the results recorded.

pH

To our knowledge, few authors took an interest in the incidence of the site of measurement on the value of the skin pH, maybe only because the buffer function of the skin does not enable to note, for the same race, great variations between anatomical sites. However, in a work conducted on 574 Caucasian males and females of different ages, repeated measurements showed that the pH of cheek (4, 2–6, 0) would be significantly higher than that of forehead (4, 0–5, 6), which confirms the previous observations (103,142). Another worker reports no difference between repeated measurements of the pH on the cheek, arm, and calf (57).

Electrical Conduction

A very great number of research undertaken to have a better knowledge of the state of the skin hydration, notably through the study of its electrical conduction, quickly enabled to establish that it is not homogeneous on the whole human body. Most of the data refer to the anatomical sites most sensitive to skin drying, which are also the most exposed to the external aggressions and particularly to the sun.

The stability of the experimental results obtained depends for a great part on the choice of the methodology implemented. According to some experimenters, the equipment that measures the capacitance actually seems to supply the most stable data (58,96,104,138).

All the authors report significant differences between anatomical areas and generally consider the forehead as the site where capacitance (57,58,96,101,104) and impedance (107) are the highest, the different sites of the face seem to give fairly similar results (28,96,138).

Here again, some researchers have shown that the different sites of the same anatomical area, for example, the dorsal and volar sides of the forearm, which correspond to different morphologies, have unequal conduction. However, these differences also occur according to the race considered (72).

Here again, the location of the site of measurement is very important as it ensures that the analysis in the variation of electrical conduction over time remains relevant.

Trans-Epidermal Water Loss

The variation in TEWL according to the anatomical sites explored has been broadly demonstrated. On the whole, the comparative studies have shown a maximal water perspiration on palms followed by the sole of the foot, the back of the hand, and then by the different sites of face (28,96,101,107,138,143–145). However, there seems to be no significant deviation between proximal and distal sites of the same geographical area (72,134). On the other hand, measurements performed comparatively on five sites taken symmetrically on both the forearms of 16 subjects of Caucasian race showed the existence of significant deviations between symmetrical sites that do not enable to consider the contralateral site as equivalent, concerning its TEWL. This fact questions a traditional experimental concept and justifies the randomization of sites to take this dominance into account, related to the laterality of the subjects that take part in a study (146).

Biomechanical Properties

The variability of the skin's thickness and of its structure according to the geographical locations considered clearly has an influence on the biomechanical properties. The value of the Young's module is consequently significantly higher on the forehead that on the forearm. Conversely, the initial tension of the skin is higher on the forearm (90). The extensibility measured on 22 skin sites is the most important on the forehead and the lowest important on the foot. This is the same for hysteresis (109).

Tonicity, plasticity, and elasticity decrease with the age in different proportions between sites, the measurements performed over time on the forearms, giving the most stable results whatever the dimension of the probes used in an experimental model by extensometry (110).

The variations in extensibility, elastic recovery, elasticity, and viscoelasticity between sites of the same geographical area do not systematically vary in the same way according to the race considered. This is the case concerning the variations noted after measurements performed on the dorsal and volar sides of the forearms of Caucasian, Hispanic, and black subjects (72).

Seborrheic Production

The global sebum rate also varies according to the sites as they do not have the same concentration in active sebaceous glands. It is the most important on the forehead, chin area, and upper part of the plexus and back (147).

Actually there is no divergence concerning the sebum content of the different anatomical sites according to the authors who took an interest in this subject (57,96,138,148).

For many researchers, this inter-site difference would correspond to different quantities of lipids (148), which have, according to the authors, equivalent (97) or different (149) compositions. This apparent disagreement could be actually explained by the fact that the studies are carried out at different periods of the year as the seasonality influences the contents in lipidic components particularly in Caucasians (150).

CONCLUSION

The resort to biophysical methods to quantify the instantaneous state of the skin or its evolution under the effect of the aggressions of the environment or inversely under the effect of products able to prevent its evolution is justified only when the methodologies implemented enable to take into account its extraordinary structural and functional diversity.

In fact, to ensure its protective, moisturizing, thermoregulatory, and nutritional parts as well as its keratogenic, melanogenic, and reserve functions that are specific to the different layers it is made up of, the skin has, beyond the global specificities related to the race, age, and sex of the subjects, functional specificities that do not allow a global analysis.

The organ in charge of the main part of the relation of the whole organism with its external environment, the skin, has a permanent capacity for adaptability to interfere with the experimental data. Its incidence therefore has to be systematically taken into account.

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3 Functional Map and Age-Related Differences in the Human Face: Nonimmunologic Contact Urticaria Induced by Hexyl Nicotinate

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INTRODUCTION

Age-related and regional variation studies of the human skin reactivity to various irritants have been reported (1–5). Marked variation of the various areas of the face in reactivity to the hydrophilic substance, benzoic acid, has been documented by Shriner (6).

Hexyl nicotinate (HN) is a pale yellow lipophilic substance insoluble in water, but soluble in ethanol and methanol. It is the ester of hexyl alcohol and nicotinic acid. It is usually used in a concentration of 2% in the following product types: facial moisturizer, around eye cream, antiaging, mask, exfoliant, and sunscreen.

In the present study, HN was used to induce nonimmunologic contact urticaria (NICU) in the same sites documented by Shriner (6). Blood-flow changes were recorded to determine potential regional and age-related differences in cutaneous vascular reactivity to HN.

HN chemical structure

CLINICAL STUDY

Two age groups were studied: 10 healthy volunteers in the young group, aged 29.8 ± 3.9 years, ranging from 24 to 34 years, and 10 in the older group, aged 73.6 ± 17.4, ranging from 66 to 83 years.

Exclusion criteria were a history of atopy and current antihistaminic drug use.

Eight regions (forehead, nose, cheek, nasolabial and perioral areas, chin, neck, and volar forearm) were studied in terms of pharmacodynamic response to HN.

On the day of the experiment, the subjects were allowed to acclimate to the examination room for 15 minutes, then, baseline measurements were taken on the studied locations.

Baseline measurements of the cutaneous blood flow were taken using a laser Doppler flowmeter (LDF) (laser blood-flow monitor MBF3D®, Moor Instruments, England) (7). Blood flow was monitored at 1 measurement per second for 30 seconds and the values averaged.

Using a saturated absorbent filter paper disc (0.8-cm diameter) (Finn Chamber Epitest Ltd Oy, Finland), HN 5 mM in ethanol was applied on the eight skin areas for 15 seconds to elicit NICU. Then blood-flow measurements were taken every 10 minutes for 1 hour in order to detect the maximum vascular response of the skin to HN.

Room temperature and relative humidity were recorded each time a subject was studied. Room temperature during the young group study (20.3 ± 2.3°C) was significantly ($p = 0.042$) lower than in the older group study (22.1 ± 2.3°C).

Relative humidity during the young group study (52.6 ± 3.8) was significantly higher ($p = 0.009$) than in the older group study (46.5 ± 5.5).

To compare the measurements of the various skin sites within each group, the ANOVA test for analysis of variance was used. The two-tailed Student's t test for unpaired data was used to compare the differences between the two age groups.

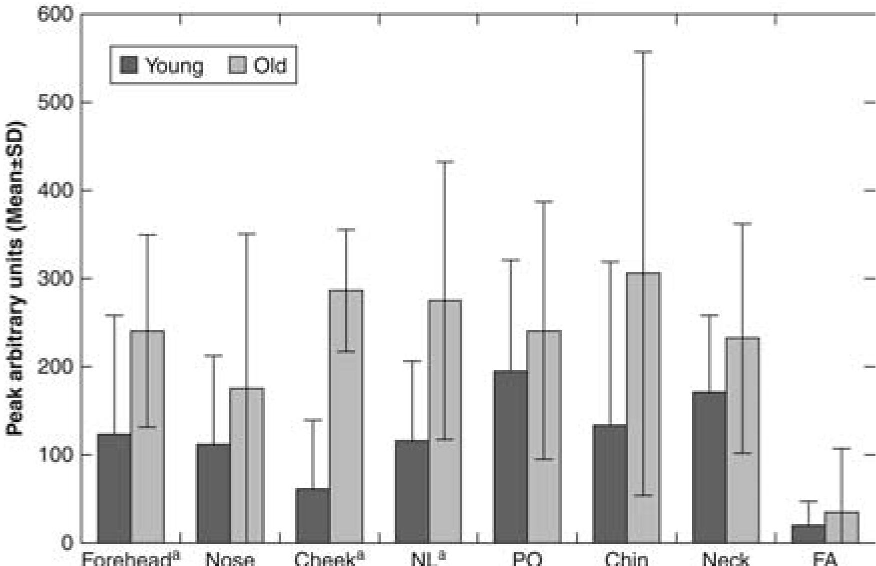

Figure 1 Baseline LDF to peak changes. Regional variation in the young and old-age groups and age-related differences. [a]The regions where the difference between the two age groups was significant ($p < 0.05$). *Abbreviations*: LDF, laser Doppler flowmeter; FH, forehead; NL, nasolabial area; PO, perioral area; FA, forearm.

COMPARISONS BETWEEN GROUPS AND SITES

Cutaneous reactivity to HN was assessed by the baseline to peak changes (peak = maximum; LDF – baseline; LDF). In some investigations, area under the curve was also considered to assess these changes (6,8,9), but since it was correlated to peak values (6), only the baseline to peak changes (peak) were considered in our study.

Comparison Between Regions

In the young group, the perioral area, followed by the neck, was the most sensitive to HN. The perioral and the nasolabial areas, the nose, the forehead, and the neck were more sensitive than the forearm ($p < 0.05$) (Fig. 1). The perioral area ($p = 0.012$) and the neck ($p = 0.009$) were more sensitive than the cheek.

In the older group, all the areas of the face were more sensitive than the forearm. The chin followed by the cheek and the nasolabial area was the most sensitive. However, no difference in reactivity to HN was found between the various areas of the face. The forearm was the less-sensitive area in both groups.

Comparison Between the Two Age Groups

Peak values were higher in the older group in three areas: forehead ($p = 0.047$), cheek ($p < 0.001$), and nasolabial area ($p = 0.012$) (Fig. 1).

In the young group, the highest vascular responses to HN were the perioral area and the neck. In the older group, the chin, cheek, and nasolabial area showed the highest skin reactivity to HN.

This difference between the two age groups might be partly explained by the enlargement of the sebaceous glands in the elderly (10), which could be induced by the long-term exposure to the sun. The UVA has been reported to induce sebaceous gland hyperplasia (11), which might lead to the enlargement of the sebaceous glands in the face when compared to other areas (12,13) and in the elderly when compared to the younger subjects (10,14).

Appendages may be an important factor in HN absorption, since the areas in the older group where peak values were significantly higher than the young group are known to have a high appendage density (15), and the enlargement of the sebaceous glands in the elderly (10) might explain that in the older group the absorption of HN seems to be higher where the appendage density increases.

Reviews and investigative studies that discuss the contribution of the various structures of the skin in the drug diffusion have been published. Some studies note that the contribution of the appendages in the skin permeability to chemicals should not be overlooked especially during the early phase of absorption (16–18). The appendageal route was reported to contribute to methyl nicotinate transport in the skin (5). Using normal and artificially damaged skin (without follicles and sebaceous glands), Hueber (19) demonstrated that the appendageal route accounts for the transport of hydrocortisone and testosterone, but is more important for this latter and more lipophilic compound. Illel et al. (20), studying rat skin, found that appendageal diffusion is a major pathway to the absorption of hydrocortisone, caffeine, niflumic acid, and *p*-aminobenzoic acid. Other studies (21,22), suggest that intercellular lipids composition is a major factor in barrier function.

However, one should keep in mind that skin reactivity to HN is probably not the expression of the sole transcutaneous penetration of the molecule, but also the manifestation of individual variability in the vascular response to HN and metabolic activity of the skin. Skin penetration and permeation of drug after topical administration depend on the physicochemical properties of the drug molecule, as well as the function of the skin as a transport barrier, and can be influenced by the applied formulation. These factors, along with skin first-pass metabolism and hemodynamic parameters of the cutaneous tissue, determine the bioavailability of topically applied drugs. The site of pharmacologic activity of HN was postulated to be the blood capillaries next to the epidermis–dermis junction. HN was reported to be metabolized to nicotinic acid during tissue permeation to an extent limited for the epidermis, but very pronounced for the dermis (23). The resulting metabolite has the same pharmacologic effect as the parent compound (24). Skin esterases were reported to be mostly located in the dermis and in skin-associated glands such as hair follicles (23). There was no esterase activity in stratum corneum. This metabolic aspect should be considered when biological activity of various topically applied drugs is studied, as well as the chronobiologic aspect, knowing that the vasodilatation of peripheral blood vessels after topical application of nicotinates follows a circadian rhythm, the maximal effect being observed during the day and the minimal at night (25).

CONCLUSION

Many factors certainly account for the percutaneous absorption of the drugs. Besides the various physical parameters used in our study, noninvasive methods for the study of the appendageal density (26) and the stratum corneum lipids composition (27) should be considered to evaluate the influence of these two parameters on percutaneous absorption of chemicals.

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4 The Baumann Skin-Type Indicator: A Novel Approach to Understanding Skin Type

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INTRODUCTION

Over the latter part of the last century, the dry, oily, combination, or sensitive skin-type classifications, which were identified in the early 1900s by cosmetics magnate Helena Rubinstein, have held sway in terms of characterizing the skin. While there have been significant innovations and even more substantial growth in the skin care product market during this time span, few notable advances have been made to further our understanding or ability to characterize skin types. Consequently, practitioners have had insufficient information to use in divining the most appropriate skin care product selections for their patients. The Baumann skin-type indicator (BSTI) is a novel approach to categorizing skin types, which greatly expands on the skin-type designations of Rubinstein and, in the process, provides assistance to practitioners and patients/consumers alike in making sense of the numerous available skin care formulations, many of which are now touted for particular skin types, as well as in selecting the most suitable products. The BSTI is based on the identification of skin type using four dichotomous parameters characterizing the skin: dry or oily, sensitive or resistant, pigmented or nonpigmented, and wrinkled or unwrinkled (tight). A four-letter skin-type designation is derived from the answers to a 64-item questionnaire and considers all the four skin parameters at once. Sixteen possible skin types, each delineated using the four-letter code denoting one end of each parameter, characterize the BSTI (Fig. 1). Ideally, patients will self-administer the BSTI to ascertain baseline skin type and reuse the questionnaire after significant life changes (e.g., moving to a different climate, pregnancy, menopause, andropause, chronic stress), which can induce modifications to skin type (1). This chapter focuses on the basic science underlying the four fundamental skin-type parameters and, in the process, characterizes in varying levels of depth the 16 skin types. In addition, some attention is paid to treatments, mainly topical and noninvasive, on the basis of the BSTI system.

SKIN HYDRATION

Oily (O) Vs. Dry (D)

"Dry skin," also known as xerosis, results from a complex, multifactorial etiology and is characterized by dull color (usually gray-white), rough texture, and an elevated number of ridges (2). The primary factors that regulate the level of skin hydration and that contribute to dry skin are the levels of stratum corneum (SC) lipids, natural moisturizing factor (NMF), sebum, hyaluronic acid (HA), and aquaporin. The role of the SC and its capacity to maintain skin hydration is the most important of these factors in terms of dry skin. The SC is composed primarily of ceramides, fatty acids, and cholesterol. These constituents help protect the skin and keep it watertight when they are present in the SC in the proper balance. SC equilibrium is also thought to be maintained via stimulation of keratinocyte lipid production and keratinocyte proliferation by primary cytokines (3).

When the primary components of the SC are not in proper balance, the skin's capacity to maintain water is decreased, and the skin becomes more susceptible to environmental factors. With the skin barrier thus impaired, transepidermal water loss (TEWL) increases and the skin is left dry and sensitive. This occurs because the enzymes essential for desmosome metabolism are inhibited by inadequate hydration, leading to the abnormal desquamation of corneocytes (4). At the same time, superficial SC desmoglein I levels remain high. The resultant compromised

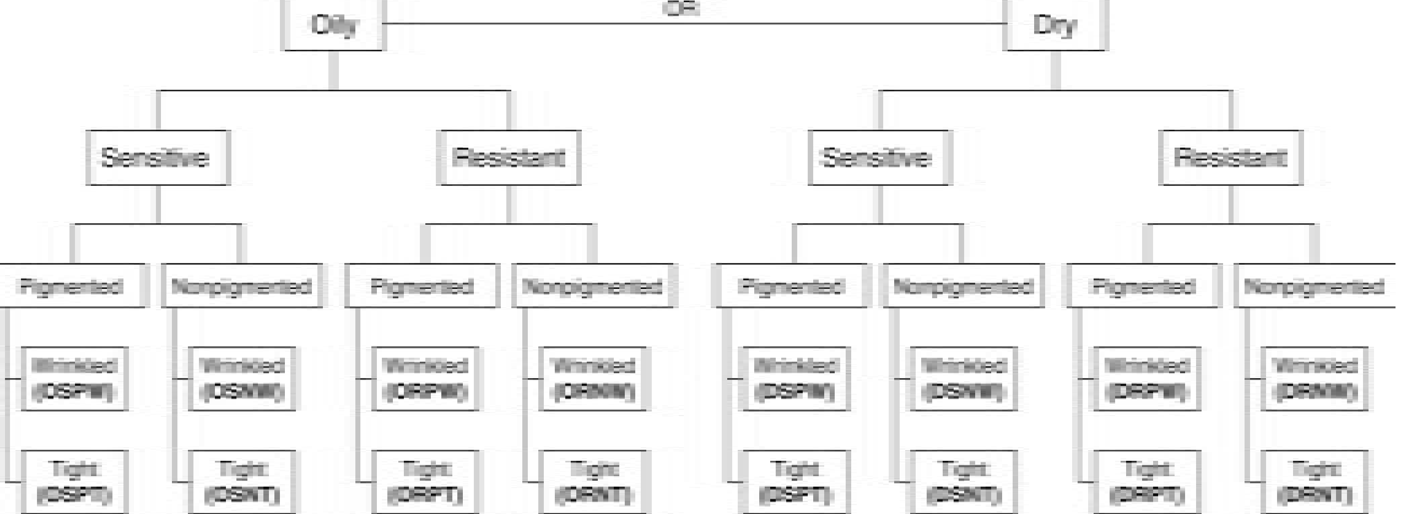

Figure 1 The BSTI skin types. The BSTI questionnaire can be located by registering online at http://www.SkinIQ.com. The Web site is frequently updated with the latest data as new questions are developed. The nonidentifying data collected on this Web site will be used to expand knowledge of skin-type prevalence around the world.

desquamation leads to a visible accrual of keratinocytes, leaving a rough and dry appearance to the skin (5). Dry skin has also been associated with a perturbation in the lipid bilayer of the SC as a result of elevated fatty acid levels and reduced ceramide levels (6). Exogenous factors, such as UV irradiation, acetone, chlorine, detergents, and protracted exposure to or immersion in water, can also affect and inhibit the lipid bilayer. In addition, recent studies have suggested that local pH fluctuations may account for the initial cohesion and ultimate desquamation of corneocytes from the SC surface. These alterations are thought to selectively activate numerous extracellular proteases in a pH-dependent manner (7).

NMF, derived from the breakdown of the protein filaggrin, is an intracellular, hygroscopic compound present only in the SC that is released by lamellar bodies and plays an integral role in maintaining water within skin cells. Filaggrin, which is composed of lactic acid, urea, citrate, and sugars, imparts structural support and strength to the lower layers of the SC. A cytosolic protease breaks it down into free amino acids, such as arginine, glutamine (glutamic acid), and histidine, in the stratum compactum, an outer SC layer (8). These water-soluble substances remain inside the keratinocytes and avidly cling to water molecules. Aspartate protease (cathepsin) initiates this chain of events and is believed to regulate the pace of filaggrin decomposition into NMF as well as the level of NMF (9). It is important to note that external humidity levels can affect cathepsin, resulting in changes in NMF production. After an individual enters a low-humidity environment, the pace of NMF production typically increases over the course of several days of getting acclimated (10). Notably, xerosis and icthyosis vulgaris are associated with low NMF levels. In addition, UV irradiation and surfactants can inhibit NMF production. However, NMF production cannot yet be artificially regulated through the use of any products or procedures.

HA can bind 1000 times its weight in water, and its presence in the dermis assists the skin in retaining water. HA is also found in the epidermal intercellular spaces, particularly the middle spinous layer, but is not present in the SC or stratum granulosum (11). Produced primarily by fibroblasts and keratinocytes, HA has an estimated turnover rate of 2 to 4.5 days in mammalians (12). Although the role of HA in skin hydration has not been fully elucidated, aged skin, which is less plump than youthful skin, is characterized by decreased levels of HA. Significantly, topically applied HA does not penetrate the skin (13). Nevertheless, several manufacturers include HA in topical skin care products and claim that they are effective.

Aquaporin-3 (AQP3) is a member of a family of homologous integral membrane proteins and a subclass of aquaporins called aquaglyceroporins that facilitate water transport and small neutral solutes, including glycerol and urea, across biological membranes (14). Present in the urinary, respiratory, and digestive tracts as well as the kidney collecting ducts and, notably, epidermis, AQP3 was shown recently to be expressed copiously in the plasma membrane of epidermal keratinocytes in human skin (15). The water conduction function in the skin is thought to occur along an osmotic gradient below the SC, where high AQP3-mediated water permeability is manifested. In this context, AQP3 water clamps viable epidermal layers to promote the hydration of cutaneous layers beneath the SC. A high concentration of solutes (Na^+, K^+, and Cl^-) and a low concentration of water (13–35%) have been shown to exist in the superficial SC that produce in the steady-state gradients of solutes and water from the skin surface to the viable epidermal keratinocytes (16–19). Nevertheless, the relationship between keratinocyte fluid transport and SC hydration as well as the molecular mechanisms of fluid transport across epidermal keratinocyte layers remains poorly understood. It is thought though that AQP3 enhances transepidermal water permeability to protect the SC from water evaporating from the skin surface and/or to spread water gradients throughout the layer of epidermal keratinocytes (15). In a study evaluating the functional expression of AQP3 in human skin, researchers observed that the water permeability of human epidermal keratinocytes was inhibited by mercurials and low pH, which was consistent with AQP3 involvement (15). Some of the same investigators considered skin phenotype in transgenic mice lacking AQP3 and discovered substantially decreased water and glycerol permeability in AQP3 null mice, supporting earlier evidence that AQP3 functions as a plasma membrane water/glycerol transporter in the epidermis (20). In most areas of the skin, conductance measurements revealed significantly diminished SC water content in the AQP3 null mice. Epidermal cell water permeability is not an important determinant of SC hydration, however, because water movement across AQP3 is slower in skin than in other tissues (21). Currently, only extracts of

the herb Ajuga turkestanica have been demonstrated to exert an influence in regulating AQP3 (22). Ajuga turkestanica is included as an ingredient in a high-end line of skin care products. Eventually, pharmacological manipulation of AQP3 may lead to its use in treating skin conditions caused by excess or reduced hydration.

Sebum, the oily secretion of the sebaceous glands containing wax esters, sterol esters, cholesterol, di- and triglycerides, and squalene, imparts an oily quality to the skin and is well known to play an important role in acne development (23). A significant source of vitamin E, sebum is also believed to confer cutaneous protection from exogenous elements and, perhaps, when production is decreased, contribute to dry skin (24). The xerosis aspect of this theory has not received much support though, as low sebaceous activity has not been found to foster dry skin. In fact, a more complex role for sebum production in the causal pathway of xerosis has been expounded. It has been previously assumed that sebum does not alter epidermal permeability barrier function because skin with few sebaceous glands, such as that in prepubertal children, manifests normal basal barrier function (25). Indeed, prepubertal children (aged 2–9 years) often present with eczematous patches (pityriasis alba) on the face and trunk, which are not associated with sebaceous gland activity. In addition, the pharmacological involution of sebaceous glands with supraphysiological doses of isotretinoin has no impact on barrier function or SC lamellar membranes (26–28).

Although sebum levels do not alter barrier function, sebum may still play a role in the etiology of xerosis in people with dry, resistant skin (DR in the BSTI system). Lipids from meibomian glands, which are modified sebaceous glands found in the eyes, act against dryness by preventing tear evaporation (29,30). TEWL is prevented in a similar fashion, as sebum-derived fats form a lipid film over the skin surface. This theory received support from a recent study that assessed permeability barrier homeostasis and SC hydration in asebia J1 mice that demonstrated sebaceous gland hypoplasia (31). Investigators observed normal barrier function in these sebum-deficient mice, which they ascribed to unaltered levels of the three primary barrier lipids—ceramides, free sterols, and free fatty acids—and the persistence of normal SC extracellular membranes. The mice did exhibit reduced SC hydration, however, suggesting that an intact intercellular membrane bilayer system, although sufficient for permeability barrier homeostasis, does not necessarily imply normal SC hydration. It is worth noting that normal SC hydration levels were restored with the topical application of glycerol. Sebaceous gland-derived triglycerides are hydrolyzed to glycerol before they are transported to the skin surface in normal skin. In individuals with low sebum production, replacing this glycerol may be an effective way to ease their xerosis. Using glycerol has also been demonstrated to be successful in accelerating SC recovery (32).

Patients rarely, if ever, complain about reduced sebum production, but elevated sebum production, yielding oily skin that can be a precursor to acne, is a common complaint. Several factors are known to influence sebum production. Age, in particular, has a significant and well-known impact, as sebum levels are usually low in childhood, rise in the middle-to-late teen years, and remain stable into the seventh and eighth decades until endogenous androgen synthesis dwindles (33). Sebum production is also affected by one's genetic background, diet, stress, and hormone levels. In a study of 20 pairs each of identical and nonidentical like-sex twins, nearly equivalent sebum excretion rates with significantly differing acne severity were observed in the identical twins, but a significant divergence was seen in both parameters among the nonidentical twins, suggesting that acne development is influenced by genetic and exogenous factors (34). Using oral retinoids to reduce sebaceous glands is a well-established approach, but this capacity has not been demonstrated in topical retinoids. No topical products have been shown to lower sebum production.

Skin Care for the O–D Parameter

An intact SC and barrier, normal NMF and HA levels, normal AQP3 expression, and balanced sebum secretion are qualities of the skin that fall in the middle of the oily–dry spectrum. Increased sebum secretion, regardless of whether it contributes to acne development, is typically the reason that the skin may be described as falling on the oily side of this continuum. Oily skin that is also prone to acne would be characterized as oily, sensitive (OS within the BSTI framework), as acne-infiltrated skin is distinguished by heightened sensitivity (see section "Acne Type"). Treatment for individuals with OS skin should concentrate on lowering sebum levels using retinoids, reducing or eliminating cutaneous bacteria with antibiotics,

benzoyl peroxide, or other antimicrobials, and complementing with anti-inflammatory agents. Individuals with oily skin but no acne (the OR type within the BSTI) should be treated only to decrease sebum production, unless other skin-type parameters dictate otherwise (e.g., hyperpigmentation or wrinkling). Sebum secretion has been shown to be effectively reduced using oral ketoconazole as well as oral retinoids, but no topical products have yet shown such success (35,36). Further, unwanted sebum in OR skin can be camouflaged using sebum-absorbing polymers and talc.

Treatment of dry skin starts with the identification of factors contributing to dryness. The other BSTI skin parameters can provide clues. The skin barrier is likely impaired in a patient whose skin is dry and sensitive (DS in the BSTI system). To treat such skin, products that repair the skin barrier (i.e., formulations that include fatty acids, cholesterol, ceramides, or glycerol) should be used. In a patient with dry photodamaged skin (with a high score on the W vs. T parameter), lower HA levels likely account, at least in part, for the dryness. Skin care products that include HA are useless in this context as topically applied HA is not absorbed into the skin. Recent studies have suggested that HA levels may be boosted through the use of glucosamine supplements (37). The role of glucosamine has not been established though, as one small single-blind study demonstrated wrinkle enhancement but no improvement in skin hydration (38). Dry skin that is habitually exposed to the sun likely exhibits an impaired skin barrier and diminished NMF. Treatment for such skin should concentrate on repairing the barrier and reducing or avoiding sun exposure. If sun exposure cannot be avoided, adequate sun protection is necessary, of course.

Harsh foaming detergents, which remove hydrating lipids and NMF from the skin, should be avoided by all patients with dry skin. Such detergents are found in body and facial cleansers as well as in laundry and dish cleansers. All patients with dry skin should also abstain from bathing for prolonged periods, especially in hot or chlorinated water. Humidifiers are recommended for people with very dry skin who live in low-humidity environments, as application of moisturizers is recommended two to three times daily and after bathing. Several over-the-counter (OTC) moisturizers (e.g., occlusives, humectants, and emollients) are effective in hydrating the skin and serve as worthy adjuncts to the aforementioned pharmacological and behavioral approaches to treating dry skin. Indeed, moisturizers are the third most often recommended type of OTC topical skin product (39). Moisturizers are typically formulated as water-in-oil emulsions (e.g., hand creams) and oil-in-water emulsions (e.g., creams and lotions).

SKIN SENSITIVITY

Sensitive (S) Vs. Resistant (R)

A potent SC that provides especially reliable protection to the skin, rendering harmless allergens and numerous irritating exogenous substances, characterizes resistant skin. Individuals with such skin are unlikely to experience erythema (unless overexposed to the sun) or acne (though stress or hormonal fluctuations could lead to a breakout). Such skin also confers an interesting set of advantages and disadvantages. On the positive side, resistant skin allows for the use of most skin care formulations with an extremely low probability of incurring adverse reactions (e.g., acne, rashes, or a stinging sensation). However, resistant skin also renders many skin care products ineffective, with individuals with such skin experiencing difficulty in detecting differences among cosmetic formulations and exhibiting an exceedingly high threshold for product penetration and efficacy.

Sensitive skin is more complex than resistant skin in terms of characterization, presentation, diagnosis, and treatment. Nevertheless, the diagnosis of sensitive skin is increasingly common (40). The majority of people that complain to a dermatologist about sensitive skin are healthy women of childbearing age. On an individual basis, sensitive skin incidence diminishes with age, fortunately. The prevalence of sensitive skin continues to increase, though. While numerous skin care products are increasingly touted as suitable for sensitive skin, such skin remains challenging to treat. Variations in the qualities of sensitive skin and poor self-diagnosis account for this difficulty. Indeed, four discrete subtypes of sensitive skin have been identified: acne type, rosacea type, stinging type, and allergic type. Consequently, the products marketed for sensitive skin are not necessarily suitable for all sensitive skin subtypes, which is

a phenomenon that presents some unusual treatment challenges. All four sensitive skin subtypes do share a significant feature, though: inflammation. The treatment approach to any kind of sensitive skin understandably begins with a focus on alleviating and eliminating inflammation. Treatment for patients with more than one sensitive skin subtype, which is not uncommon, is, of course, more complicated.

Acne Type

This is the most common subtype of sensitive skin because of the prevalence of acne, which is by far the most common skin disease. Individuals with such sensitivity are prone to developing acne, black heads, or white heads. Acne typically affects adolescent and young adults, equally by sex, between 11 to 25 years old. Most of the remainder of the millions of those suffering from acne are adult women, who display a hormonal aspect to their acne. The complex interplay of four primary factors is at the heart of acne pathogenesis: an increase in sebum production, clogging of pores (which results from dead keratinocytes inside the hair follicles clinging more strongly than in people without acne and can also result from elevated sebum production), presence of the bacteria *Propionibacterium acnes*, and inflammation. Significantly, acne can occur as a result of various causal pathways or in idiopathic presentations, but the sine qua non of the condition is the amassing and adherence of dead keratinocytes in the hair follicles due to elevated sebum production, leading to clogged follicles and appearance of a papule or pustule. This is followed by the migration of *P. acnes* into the hair follicle, where the combination of the bacteria, sebum, and dead keratinocytes stimulates the release of cytokines and other inflammatory factors. In turn, an inflammatory response is provoked that manifests in the formation of redness and pus. Indeed, in chronic inflammatory conditions such as acne, high levels of primary cytokines, chemokines, and other inflammatory markers are typically present (3). To treat acne, the therapeutic intention is to target the four main etiological factors. This translates to decreasing sebum production (using retinoids, oral contraceptives, and/or stress reduction), unclogging pores (using retinoids, α-hydroxy acids, or β-hydroxy acid), eliminating bacteria (using benzoyl peroxide, sulfur, antibiotics, or azelaic acid), and reducing inflammation (using any of a wide array of anti-inflammatory products).

Rosacea Type

The acneiform condition rosacea affects 14 million people in the United States, typically adults aged between 25 and 60 years, according to the National Rosacea Society (41). Those with the rosacea subtype of sensitive skin exhibit a tendency toward recurrent flushing, facial redness, and experiencing hot sensations. The etiology of rosacea remains elusive, but this condition shares the aforementioned symptoms with acne, along with papules, but is distinguished by the formation of salient telangiectases. Avoiding the triggers that exacerbate symptoms is, of course, recommended for rosacea treatment, as is using anti-inflammatory ingredients to reduce the dilation of the blood vessels. Eosinophils, which are versatile leukocytes, contribute to the initiation and promotion of various inflammatory responses (42,43). The aim of rosacea therapy is to inhibit eosinophilic activity, decrease vascular reactivity, neutralize free radicals, and hinder immune function, the arachidonic acid pathway, and degranulation of mast cells (which frequently migrate to areas of eosinophil-mediated disease). Several anti-inflammatory medications are available for the treatment of rosacea, including antibiotics, immune modulators, and steroids. The most effective anti-inflammatory ingredients (many of which are botanically derived) in the copious supply of topical rosacea therapeutic agents include aloe vera, arnica, chamomile, colloidal oatmeal, cucumber extract, feverfew, licochalcone, niacinamide, quadrinone, salicylic acid, sulfacetamide, sulfur, witch hazel, and zinc (44).

Stinging Type

People with this particular subset of sensitive skin exhibit a predilection to experiencing stinging or burning sensations in response to various factors and triggers. This tendency is best characterized as a nonallergic neural sensitivity. "Stingers" or the stinging tendency can be identified through the use of numerous tests. The lactic acid stinging test is the best-regarded, standard way to assess patients who complain of invisible and subjective cutaneous irritation (45). This test has, in fact, been used to show that individuals with "sensitive skin" experienced a much stronger stinging sensation than those in a healthy

control group (46). It is worth noting that erythema does not necessarily accompany the stinging sensation, as many patients report stinging without experiencing redness or irritation (47). Nevertheless, exposure to lactic acid is more likely to elicit stinging in patients with rosacea distinguished by facial flushing (48). Topical products that contain α-hydroxy acids (particularly glycolic acid), benzoic acid, bronopol, cinnamic acid compounds, Dowicel 200, formaldehyde, lactic acid, propylene glycol, quaternary ammonium compounds, sodium lauryl sulfate, sorbic acid, urea, or vitamin C should be avoided by patients that are confirmed to have the stinging subtype of sensitive skin.

Allergic Type

Over the course of a year, the use of personal care products, including deodorants, perfumes, nail cosmetics, as well as skin and hair care products, elicit adverse reactions in 23% of women and 13.8% of men, according to a recent epidemiological survey in the United Kingdom (49). Individuals with the allergic subtype of sensitive skin are more prone to exhibit erythema, pruritus, and skin flaking. Patients tested for allergies to cosmetic ingredients are typically patch tested for 20 to 100 ingredients, with erythema or edema in the tested area indicating an allergy to the particular ingredient. Several studies have demonstrated that approximately 10% of dermatological patients who were patch tested were found to have an allergy to at least one ingredient common in cosmetic products (50). Fragrances and preservatives are the most common allergens, and most reactions, approximately 80%, arise in women aged 20 to 60 years (50). Overexposure to common allergens, by using several skin care products, raises the risk of inducing allergic reactions. In particular, individuals with the D skin type (within the BSTI system) who have an impaired SC manifested by xerosis are more likely to exhibit an increased incidence of allergic reactions to topically applied allergens (51).

On the basis of the guidelines of the BSTI, oil control is necessary for those with OS skin. An acne or rosacea regimen would also likely be necessary for the OS type. Treatment to repair the SC is indicated for people with DS skin. Therapy to ameliorate wrinkles and to prevent the development of new ones is recommended for individuals with sensitive, wrinkled (SW) skin. Frequently, people with sensitive, pigmented (SP) skin request procedures or topical applications to reduce or remove hyperpigmentation and therapy to lessen the likelihood of developing new dyschromias.

SKIN PIGMENTATION: PIGMENTED (P) VS. NONPIGMENTED (N)

This skin-type parameter refers to the proclivity to develop unwanted hyperpigmentations on the face or chest. Within the BSTI framework, the focus is on the pigmentary changes or conditions that can be ameliorated with topical skin care products or minor dermatological procedures. In this context, melasma, solar lentigos, ephelides, and postinflammatory hyperpigmentation are representative conditions for the pigmented skin type. Considerable anxiety is often associated with the presentation of these skin lesions, and patients often pay substantial sums in the attempt to treat these conditions. To best treat these pigmentary problems, it is incumbent upon the physician to understand the source of pigmentation. In addition, the practitioner can be well served in terms of making suitable product selections for patients to place such knowledge within the context of other aspects of an individual patient's full (BSTI) skin type.

The enzymatic breakdown of tyrosine into dihydrophenylalanine (DOPA) and then dopaquinone leads to the synthesis of two types of skin pigment (melanin), eumelanin and pheomelanin (52). These skin pigments (of which eumelanin is the more abundant and which regularly correlates with the visual phenotype) are produced by melanocytes, which use melanosomes to transport the pigments to keratinocytes (53). One melanocyte is typically attached to approximately 30 keratinocytes. Melanosomes are surrounded by keratinocytes, which absorb the melanin after activation of the protease-activated receptor (PAR)-2 (54). Expressed in keratinocytes but not melanocytes, PAR-2 is a seven transmembrane G-protein-coupled trypsin/tryptase receptor activated by a serine protease cleavage. PAR-2 is believed to regulate pigmentation via exchanges between keratinocytes and melanocytes (55). Notably, melanogenesis can also be initiated by UV irradiation. Under these conditions, melanogenesis is a defensive manifestation to protect the skin and is characterized by accelerated melanin

synthesis and transfer to keratinocytes, leading to darkening of the skin in the exposed areas (56). Melanocytes synthesize more melanin in darker-skinned people, and their larger melanosomes accommodate this comparatively greater abundance of melanin and consequently break down more slowly than in lighter-skinned people (55).

Inhibiting tyrosinase, thus preventing melanin formation, and blocking the transfer of melanin into keratinocytes represent the two main pathways through which the development of skin pigmentation can be hindered. Hydroquinone, vitamin C, kojic acid, arbutin, mulberry extract, and licorice extract are the most effective tyrosinase inhibitors. Skin pigmentation is also thought to be inhibited by two small proteins contained in soy—soybean trypsin inhibitor (STI) and Bowman–Birk inhibitor (BBI). Both STI and BBI have been shown in vitro and in vivo to exhibit depigmenting activity and to prevent UV-induced pigmentation by inhibiting the cleavage of PAR-2 (57). Consequently, STI and BBI are thought to influence melanosome transfer into keratinocytes, thereby exerting an effect on pigmentation. Niacinamide, a vitamin B_3 derivative, has also been demonstrated to hinder the melanosome transfer from melanocytes to keratinocytes (58). Soy and niacinamide, the most effective PAR-2 blockers, are the main agents for preventing this transfer.

There are three classes of topical agents used within the two pathways of inhibiting melanin formation. In addition to the inhibitors of tyrosinase and PAR-2, exfoliating products (e.g., α-hydroxy acids, β-hydroxy acid, retinoids) have the capacity to increase cell turnover to outpace the rate of melanin production. Such exfoliation can also be achieved through microdermabrasion and the use of facial scrubs. Broad-spectrum sunscreens should also be employed in any skin care program intended to reduce or eliminate undesired pigmentation. The most effective way of preventing pigmentary alterations remains the avoidance of chronic sun exposure. Within the BSTI framework, a person with a penchant for developing unwanted dyspigmentations has "P" type skin, or, otherwise, "N" type skin.

SKIN AGING: WRINKLED (W) VS. TIGHT (T)

Cutaneous aging is a complex multifactorial phenomenon described in terms of endogenous and exogenous influences that ultimately manifest in alterations to the outward appearance of the skin. Endogenous aging—known as natural, chronological, or intrinsic aging in this case—is a function of heredity or cellular programming. The aging-related manifestations of such forces that occur over time are, therefore, considered inevitable and beyond human volition. Exogenous aging—known typically as extrinsic aging—is driven by chronic exposure to the sun and other deleterious environmental elements (e.g., cigarette smoke, poor nutrition) and, therefore, can be avoided, though not always easily. While these etiological strains appear, and have been typically evaluated, as discrete processes, recent findings suggest that UV irradiation—the leading cause of extrinsic aging—may also alter the normal course of chronological aging. Therefore, it is possible that there is a significant overlap in the processes of intrinsic and extrinsic aging. For the purposes of this discussion, however, intrinsic and extrinsic aging will be considered separately.

Cellular or intrinsic aging is currently best understood with reference to telomeres, specialized structures that shield the ends of chromosomes. Telomere length shortens with age, and this erosion is considered an internal aging clock as well as the source for one of the currently espoused theories on chronological aging (59). The enzyme telomerase, which lengthens telomeres and imparts stability, is expressed in approximately 90% of all tumors and in the epidermis, but is absent in several somatic tissues (59,60). This suggests that most cancer cells, as opposed to normal healthy cells, are not programed for apoptosis or cell death. For this reason, cancer and aging are thought to represent opposite sides of the same coin. Current knowledge regarding telomeres and telomerase has not yet been harnessed for any viable antiaging therapies, primarily because little is known regarding the safety of artificially increasing telomere length.

As implied in the definition, extrinsic aging is a premature aging of the skin that is the result of the interplay of external factors and human behaviors resulting in the chronic exposure to such factors, and thus falls within the realm of human control. By far, exposure to UV irradiation is the leading cause of extrinsic aging; indeed, such premature aging is often referred to as photoaging. Of course, other factors such as smoking, other pollution, poor

nutrition, excessive alcohol consumption, and protracted stress among additional exogenous influences can contribute to accelerating cutaneous aging. Significantly, photodamage precedes photoaging, and this evolves through several mechanisms, including the formation of sunburn cells, thymine and pyrimidine dimers, production of collagenase, and induction of an inflammatory response. In addition, photodamage and aging have been associated with signaling through the p53 pathway subsequent to UV-induced (especially by UVB) telomere disturbance (61,62). The best-known deleterious effects of UV (UVA, 320–400 nm, in particular) include photoaging, photoimmunosuppression, and photocarcinogenesis, but much has yet to be discovered regarding the mechanisms through which UV irradiation engenders such extensive harm (63). Nevertheless, as the aforementioned theory implies, intrinsic aging can be thought to be impacted by the primary source of extrinsic aging, as chronic UV exposure can damage DNA and accelerate the diminution of telomeres, which is known to play a role in chronological aging.

Cutaneous aging is evidenced, first and foremost, by the formation of rhytides, which develop in the dermis. Because few topical skin care products can actually penetrate to this layer of the skin to affect wrinkles, the dermatological approach to antiaging skin care concentrates on preventing the formation of wrinkles (64). This translates to a focus on replenishing or maintaining the three primary structural constituents of the skin, collagen, elastin, and HA, which are known to degrade with age. Despite the inadequacy of most topical formulations to deliver active ingredients that alter these components, some products have been shown to exert such an impact on collagen and HA. Specifically, collagen synthesis has been shown to be spurred by topical retinoids, vitamin C, and copper peptide as well as oral vitamin C (65–67). The synthesis of HA and elastin has been demonstrated in animal models to be stimulated by retinoids (68,69). In addition, HA levels are thought to be enhanced through glucosamine supplementation (37). However, no products have yet been demonstrated or approved for inducing the production of elastin.

Collagen, elastin, and HA can also be broken down by inflammation; therefore, targeting ways to reduce inflammation represents another significant approach to preventing or mitigating cutaneous aging. Skin inflammation can result from reactive oxygen species (ROS) or free radicals acting directly on growth factor and cytokine receptors in keratinocytes and dermal cells. Although their effects on cutaneous aging are not fully understood, growth factors and cytokines are known to act synergistically in a complex process involving several types of growth factors and cytokines (70). Antioxidants protect the skin from ROS via various mechanisms not yet fully explained. However, the events through which ROS directly impact the aging process are known. UV exposure is thought to induce a chain of events, acting on growth factors and cytokine receptors in keratinocytes and dermal cells. This yields downstream signal transduction from the activation of mitogen-activated protein (MAP) kinase pathways, which accrue in the cell nuclei, developing into cFos/cJun complexes of transcription factor activator protein 1, in turn leading to the breakdown of cutaneous collagen as a result of the induction of matrix metalloproteinases, including collagenase, stromelysin, and 92-kDa gelatinase (71,72). The use of antioxidants is thought to delay or act against photoaging in this context by preventing these pathways from synthesizing collagenase. Kang et al. demonstrated that production of the UV-induced cJun-driven enzyme collagenase was inhibited by the pretreatment of human skin with the antioxidants genistein and *N*-acetyl cysteine.

Numerous antioxidants, such as vitamins C and E, and coenzyme Q10, as well as botanically derived ingredients (e.g., caffeine, coffeeberry, ferulic acid, feverfew, grape seed extract, green tea, idebenone, mushrooms, polypodium leucotomos, pomegranate, pycnogenol, resveratrol, rosemary, silymarin) are found in skin care products. Despite compelling evidence in the literature substantiating the potency of these antioxidant ingredients, there is a paucity of data demonstrating their efficacy in topical formulations. Research is ongoing to harness their potential in such products, however. Research and development might also yield technological advances in tissue engineering and gene therapy that result in innovative therapeutic applications of growth factors, cytokines, and, perhaps, telomerase (73). Currently, the best approaches to combat cutaneous aging remain behavioral—avoiding sun exposure (particularly between 10 a.m. and 4 p.m.); using broad-spectrum sunscreen daily; avoiding cigarette smoke, pollution, and excessive consumption of alcohol; reducing stress; eating a diet high in fruits and vegetables; taking oral antioxidant supplements or topical antioxidant formulations; and regularly using prescription retinoids.

CONCLUSION

The four traditional expressions used to describe skin type have remained prominent and largely unchallenged over the last century. However, the terms "dry," "oily," "combination," and "sensitive" as characterizations of the skin have been found to be inadequate guides or gauges for finding the most suitable formulations among the ever-burgeoning supply of skin care products. The BSTI proposes that four fundamental skin parameters, covering the spectra from dry to oily, sensitive to resistant, pigmented to nonpigmented, and wrinkled to tight, can be used to better understand and more accurately depict the nature of human skin and identify an individual's skin type among the 16 possible permutations. Because the skin qualities described in the BSTI are not mutually exclusive, all four parameters must be considered when identifying skin type. A four-letter BSTI code is derived from answers to a 64-item self-administered questionnaire, with each letter corresponding to the end of the spectrum of each parameter that an individual favors. With this code, consumers and physicians can more readily select the most suitable OTC skin products, and practitioners may be assisted in treating various skin conditions with the topical formulations most appropriate for a patient's skin type.

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5 Ethnic Differences in Skin Properties: The Objective Data

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INTRODUCTION

Clinical differences in dermatologic disorders may be influenced by ethnic variation in skin properties. Previous investigations by objective methods have provided evidence of ethnic differences in skin properties, but the data have often been contradictory (1). Although, it remains difficult to establish clinically applicable ethnic trends, recent investigations have further emphasized the need for distinct research on disease processes and treatment responses in ethnic skin when defining appropriate clinical management.

We explore and attempt to clarify recent objective data that have become available in the context of transepidermal water loss (TEWL), water content (WC) (via conductance, capacitance, resistance, and impedance), blood vessel reactivity (BVR), pH gradient, microtopography, sebaceous function, vellus hair follicle distribution, morphology and distribution of melanosomes, and resistance to photodamage to differentiate skin properties of different ethnic groups. In addition, as objective definitions of skin color are yet to be established, we introduce certain objective differences that have been established to date. We searched MEDLINE®, MD Consult, Science Citations Index, the Melvyl Catalogue (CDL-Hosted Database of University of California, San Fransicsco, California, U.S.), and standard dermatology textbooks from 2002 to August 2006. Keywords in searches included words pertaining to race (i.e., race, ethnicity, black, African, white, Caucasian, Asian, Hispanic) and dermatology (i.e., skin, skin physiology, skin function) (1).

TRANSEPIDERMAL WATER LOSS

TEWL is a method of measuring the skin's barrier function and is currently defined as the total amount of water vapor loss through the skin and appendages, under nonsweating conditions (2). Measured in various studies, both at baseline and after topical application of irritants, it is the most studied objective measure in defining differences between the skin of different ethnicities (1).

In 1988, Wilson et al. (3) demonstrated higher in vitro TEWL values in black compared with white cadaver skin matched for age and gender. While Sugino et al. (4) (abstract only) similarly found in vivo baseline TEWL to be blacks > Caucasians > Hispanics > Asians, Berardesca et al. (5) found no significant difference in vivo in baseline TEWL between race and anatomic site for blacks, whites, and Hispanics. Warrier et al. (6) tried to clarify such discrepancies in data and found TEWL to be significantly lower on the cheeks and legs in blacks compared with whites. However, on the basis of a study of Caucasian subjects showing that TEWL values vary by anatomic location, it is difficult to compare differences in TEWL between the sites examined by Warrier et al. (6) (cheeks and lower legs) to those of other studies (forearm, inner thigh, and back) (1,3,5,7–10).

Additionally, in vivo studies have observed ethnic variation in TEWL in response to topical irritants and/or tape stripping. In two earlier studies, Berardesca and Maibach (7,9) found that blacks showed higher TEWL levels than whites after topical application of sodium lauryl sulfate (SLS), suggesting an increased susceptibility of blacks to irritation, but found no significant differences between Hispanic and white skin. Kompaore et al. (10) also showed significantly higher TEWL values in blacks and Asians compared with whites with topical methyl nicotinate (MN), before and after tape stripping; TEWL values were highest in Asians with increased tape stripping. In contrast, Aramaki et al. (11) later found no significant differences in TEWL between Japanese and German women before or after SLS stress; these

findings were further supported by another study (unpublished data) (12) on Asian skin that found no statistically significant differences between Asians and Caucasians before or after tape stripping.

Comparing TEWL on the basis of degree of skin pigmentation rather than ethnicity, Reed et al. (13) found that subjects with skin type V/VI required more tape strippings than skin type II/III to achieve the same TEWL; thus, skin type V/VI had increased barrier strength. Furthermore, the water barrier function, measured by TEWL, in skin type V/VI was shown to recover more quickly. Berardesca et al. (14), examining differences in TEWL between women of skin type I/II and skin type VI, also demonstrated that recovery of water barrier function was greater in skin type VI, but the difference was not statistically significant. Additionally, unlike finding by Reed et al. (13), Berardesca et al. (14) found that skin type VI had a higher TEWL at baseline and after each tape stripping, though TEWL increased for both groups with each tape stripping.

Recently, additional studies on TEWL have contributed to evidence of ethnic skin differences. Tagami (15) continued investigation of Asian skin by comparing TEWL between Japanese and French women under similar environmental conditions. The research team measured TEWL on cheeks and mid-flexor surface of forearms of all subjects and, similar to finding by Sugino et al. (4), found that TEWL was lower in Japanese women, but the data were not statistically significant. Of note, skin type or ethnicities were not specified within the French group.

Hicks et al. (16) grouped patients on the basis of skin color, as in a study by Reed et al. (13), while studying the difference between susceptibility of black (skin types V/VI) and white skin (skin types II/III) to irritant contact dermatitis (ICD). After exposure to 4% SLS, changes in TEWL and stratum corneum (SC) thickness of the skin on the volar forearm were negatively correlated in both groups. White participants showed a trend toward greater mean increases in TEWL after SLS exposure than black participants, supporting the possibility that the barrier function in black skin is more durable than white skin, but the differences were not statistically significant. Overall, results from all methods of evaluation suggested reduced susceptibility of black skin to ICD. However, while there was no significant difference between SC thickness of control sites in both groups [consistent with the 1974 study by Weigand et al. (17)], the SC thickness was significantly less in blacks as compared to whites after exposure to 4% SLS at 48 hours. This pattern of SC thinning seems to contradict the findings of reduced susceptibility of black skin to ICD. A larger sample size may be necessary to clarify this discrepancy and achieve a statistically significant trend in TEWL changes.

In another evaluation of differences between African-American and white skin, Grimes et al. (18) did not find significant differences in TEWL in vivo. Methods of evaluation included clinical evaluation and instrumental measurements of sebum level, pH, moisture content, and TEWL. Although there were differences in visual assessment of photoaging and hyperpigmentation, the baseline instrumental findings from all methods indicated no significant differences between African-American and white skin. In a subset of subjects participating in a chemical challenge of 5% SLS, though there was an early significant change in TEWL in white participants, TEWL was similar in both groups after 24 hours. The overall findings support the postulation that, objectively, there is little difference between African-American and white skin. However, again on the basis of small sample size, it is difficult to make definitive conclusions based on the data.

Pershing et al. (19) found a significant difference in TEWL between Caucasians and Asians with topical application of capsaicinoids. The study measured TEWL after application of capsaicinoid analogs at various concentrations on volar forearms. Increasing concentrations of total capsaicinoid were not associated with a proportional change in TEWL in either Caucasians or Asians. However, a capsaicinoid concentration of 16 mg/mL produced statistically less TEWL in Asians than Caucasians ($p < 0.05$); specifically, there was an increase of the mean TEWL in Caucasians but a decrease in Asians. The investigators concluded that changes in TEWL between Caucasians and Asians with capsaicinoids, but not irritants [e.g., SLS in a study by Aramaki et al. (11)], may reflect the effect of vehicle composition (isopropyl alcohol for capsaicin vs. water for irritants) or other physiologic skin functions (e.g., cutaneous blood flow) in determining TEWL.

Astner et al. (20) evaluated ethnic variability in skin response to a household irritant (ivory dishwashing liquid) with graded concentrations of the irritant to the anterior forearms

of Caucasian and African-American subjects. The investigators observed significantly higher mean values for TEWL in Caucasians compared with African-Americans ($p \leq 0.005$), as previously observed in study by Warrier et al. There was a positive, dose-dependent correlation between TEWL values and irritant concentration in all groups. However, not only was the mean TEWL higher in Caucasians, but the relative increment of increase in response to the graded irritant concentrations were also higher in Caucasians when compared with African-Americans ($p \leq 0.005$).

Overall, the data regarding TEWL (recent studies summarized in Table 1) continue to be inconsistent. Unlike the majority of previous studies, findings by Berardesca et al. (5), Hicks et al. (16), and Grimes et al. (18) do not support a statistically significant difference in TEWL between black and Caucasian skin. Most studies have shown a greater TEWL in blacks compared with whites (3,4,7,10,13,14); however, Warrier et al. (6) and Astner et al. (20) (after irritant stress) found TEWL to be less in blacks than whites. Additionally, TEWL measurements with regards to Asian skin remain inconclusive as previous studies observed baseline measurements in Asian skin to be equal to black skin and greater than Caucasian skin (10), less than all other ethnic groups (4), or no different than other ethnic groups (11,12); while, more recently, Tagami (15) did not find any statistically significant difference between Asian and French skin. Also recently, Pershing et al. (19) found an increase in TEWL of Caucasians but a decrease in TEWL of Asians in response to high-potency capsaicinoids, the results of which are difficult to categorize. Further clarification of both baseline and post-irritant TEWL in different ethnic groups will be valuable in determining whether ethnic differences in barrier function could influence varying susceptibility to dermatologic disorders and response to topical therapy.

WATER CONTENT

WC or hydration of the skin is measured by skin capacitance, conductance, impedance, or resistance based on the increased sensitivity of hydrated SC to an electrical field (21). Of note, possible sources of error or variation in measurement include sweat production, filling of the sweat gland ducts, the number of hair follicles, and the electrolyte content of the SC (22).

An early study by Johnson and Corah (23) found that blacks had higher levels of skin resistance at baseline than whites; as a higher resistance indicates a lower WC, these findings implied black skin as having a lower WC (1). Later, when comparing WC by capacitance before and after topical SLS, Berardesca and Maibach (7) found no significant differences in WC between blacks and whites at baseline or after SLS stress. In a similar study comparing Hispanics and whites, they found a higher WC in Hispanics at baseline, but the difference was not statistically significant (9). However, a study by Berardesca et al. (5), using conductance, demonstrated a greater baseline WC in blacks and Hispanics compared with whites on the dorsal arm and a greater WC in Hispanics than blacks and whites on the volar forearm.

Warrier et al. (6) examined WC by capacitance and found black women to have a significantly higher WC on the cheeks than white women, but there were no significant differences at baseline on the forearms and the legs of the two ethnic groups, suggesting that anatomic location could influence measurements. Manuskiatti et al. (24), also measuring WC of black and white women by capacitance, found no ethnic differences in WC on nine different anatomic locations. In contrast, Sugino et al. (4) included Asians in their study and, by measuring WC with impedance, found that WC was highest in Asians compared with Caucasians, blacks, and Hispanics.

Recently, Sivamani et al. (25) (study summarized in Table 2) compared differences in impedance between Caucasian, African-American, Hispanic, and Asian subjects. In addition to measuring baseline differences, the researchers assessed differences in response to polyvinylidene chloride occlusion, topical petrolatum, and topical glycerin applied to the volar forearm. Baseline measurements showed no significant differences in impedance between age, gender, or ethnicity. Notably, although there were no significant differences between right and left forearms, significant baseline variation was found between the distal and proximal volar forearms; the proximal forearms showed lower impedance than the distal forearms ($p < 0.001$). We can infer baseline differences in WC among anatomic sites from this study [as suggested by findings from Warrier et al. (6)]. Additionally, all interventions showed

Table 1 Transepidermal Water Loss (TEWL)[a]

Study	Technique	Subjects	Site	Results
Tagami (15)	In vivo	Japanese women 120 French women 322 (ages 20–70 yr, all)	Cheeks and mid-flexor forearm	• TEWL Japanese $<$ whites but not statistically significant
Hicks et al. (16)	In vivo—topical application of 1% and 4% SLS (irritant)	White: Skin type II 6 Skin type III 2 Black: Skin type V 5 Skin type VI 1 (ages 18–40 yr, all)	Volar forearm	• TEWL Whites $>$ blacks but not statistically significant
Grimes et al. (18)	In vivo—topical application of 5% SLS (irritant)	African-American 18 White 19 (ages 35–65 yr, women, all) African-American 3 White 5	Inner forearm	• Baseline: No significant difference • After SLS stress: immediate increase in TEWL of white subjects, but increase no longer evident after 24 hr and found to be similar to African-Americans (not statistically significant)
Pershing et al. (19)	In vivo—topical application of capsaicinoid analogs	Caucasians: Male 3 Female 3 Asians: Male 3 Female 3 (ages 19–63 yr, all)	Volar forearm	• Increasing concentrations of total capsaicinoid not associated with proportional change in TEWL, in all subjects • Capsaicinoid concentration of 16 mg/mL produced ↑ mean TEWL in Caucasians, ↓ mean TEWL in Asians ($p < 0.05$)
Astner et al. (20)	In vivo—topical application of ivory soap (irritant)	Caucasians 15 (Skin type II/III) African-Americans 15 (Skin type V/VI) [ages 18–49 yr, all]	Anterior forearm	• Positive dose-dependent correlation between TEWL and irritant concentration: Mean TEWL Caucasians $>$ African-Americans ($p \leq 0.005$) • Relative increment of increase in TEWL after irritant: Caucasians $>$ African-Americans ($p \leq 0.005$)

[a]All of the evidence supports TEWL blacks > whites, except for studies by Berardesca et al. (5), Hicks et al. (16), and Grimes et al. (18), which found no significant difference, and Warrier et al. (6) and Astner et al. (20), which found blacks < whites. TEWL measurements of Asian skin are inconclusive, as they have been found to be equal to black skin and greater than Caucasian skin [Kompaore et al. (10)], equal to Caucasian skin [Aramaki et al. (11), and Tagami (15)], and less than all other ethnic groups [Sugino et al. (4)]. Pershing et al. (19) found an increase in TEWL of Caucasians but a decrease in TEWL of Asians in response to high concentrations of topical capsaicinoids.

Abbreviations: SLS, sodium lauryl sulfate; yr, years.

Table 2 Water Content[a]

Study	Technique	Subjects	Site	Results
Sivamani et al. (25)	In vivo—impedance, topical application of petrolatum and glycerin	White 22 African-American 14 Hispanic 14 Asian 9 (ages 18–60 yr, all)	Volar forearm	• Baseline: no significant differences in electrical impedance between age, gender, or ethnicity; impedance of proximal $<$ distal forearm ($p < 0.001$) • After topical interventions: all interventions produced decrease in impedance; degree of decrease varied by intervention. No significant differences between age, gender, or ethnicity.
Grimes et al. (18)	In vivo—capacitance	African-American 18 White 19 (ages 35–65 yr, women, all)	Inner forearm	• Baseline: African-Americans $<$ whites, but not statistically significant

[a]Ethnic differences in water content, as measured by resistance, capacitance, conductance, and impedance are inconclusive.
Abbreviations: mo, months; SLS, sodium lauryl sulfate; yr, years.

decreases in impedance from baseline (degree of decrease varied by intervention), but no significant differences between age, gender, or ethnicity. The authors concluded that there is little variation in volar forearm skin across gender, age, and ethnicity, providing an adequate site for testing of skin and cosmetic products.

Grimes et al. (18) (study summarized in Table 2) measured baseline moisture content on the inner forearms of African-American and white women on the basis of capacitance. Similar to study by Sivamani et al. (25), this study found no significant variation in baseline moisture content between African-American and white subject inner forearms.

The findings by Johnson and Corah (23) implied ethnic variance in WC. However, the SLS-induced irritation studies by Berardesca and Maibach (7,9) revealed no significant differences in WC between the races at baseline or after SLS stress, and Manuskiatti et al. (24) found no baseline difference in WC between blacks and whites. Berardesca et al. (5), Warrier et al. (6), and Sugino et al. (4) later demonstrated ethnic variability in WC, but the values varied by anatomic site. In contrast, Sivamani et al. (25) and Grimes et al. (18) recently reported no significant ethnic variation in WC, baseline and after various topical interventions, further supporting studies by Berardesca and Maibach (7,9) and Manuskiatti et al. (24). Sivamani et al. (25) also demonstrated variation of WC between different anatomic sites and with specific interventions. Of note, impedance, as used in the studies by Sugino et al. (4) and Sivamani et al. (25), is less widely used than capacitance and conductance and has been shown to be more sensitive to environmental and technical factors that affect the SC (21); this makes it difficult to compare the results presented by these latter two studies to other studies.

BLOOD VESSEL REACTIVITY

Measurements of cutaneous blood flow facilitate the objective evaluation of skin physiology, pathology, irritation, and response to treatment (26). Objective techniques for the estimation of blood flow include laser Doppler velocimetry (LDV) and photoplethysmography (PPG). LDV is a noninvasive method based on measurement of the Doppler frequency shift in monochromatic laser light backscattered from moving red blood cells (26,27). PPG works by recording the backscattered radiation of infrared light that is not absorbed by hemoglobin as a measure of the amount of hemoglobin in the skin (26).

In 1985, Guy et al. (28) used both techniques to study the response to topical MN in healthy black and white subjects and observed a similarity in BVR. However, Gean et al. (29), also using different concentrations of topical MN while measuring LDV, observed that blacks

had a greater BVR to all concentrations and Asians had a greater BVR to higher doses in comparison with Caucasians.

Berardesca and Maibach (7,9) later found no significant differences in LDV between black and white skin or between Hispanic and white skin, at baseline or after topical SLS. However, a subsequent study by Berardesca and Maibach (30) measured LDV in response to corticosteroid application, finding a decrease in BVR of blacks compared with whites.

Kompaore et al. (10) added a different element of physical stress by evaluating LDV before and after tape stripping in black, Caucasian, and Asian subjects. After application of MN, but before tape stripping, there was no difference between the groups in basal perfusion flow, but lag time before vasodilatation was greater in blacks (decreased BVR) and less in Asians (increased BVR) compared with Caucasians. After 8 and 12 tape strips, though BVR increased in all three groups, it increased significantly more in Asians. This response in BVR to tape stripping confirmed the importance of the SC in barrier function. Aramaki et al. (11) also examined Asian skin, but found no difference in LDV at baseline or after SLS-induced irritation between Japanese and German women.

Recently, an investigation done by Hicks et al. (16) demonstrated no significant difference in BVR, measured by LDV, between black and white participants with topical SLS. The results obtained are in conflict with several previous studies that have suggested differences between black and white skin (10,28–30). However, the investigators expressed doubt in the validity of the LDV measurements because of technical difficulties in using the flowmeter.

The results of the recent study on BVR are summarized in Table 3. Since studies on BVR have administered different vasoactive substances, they cannot be objectively compared (1,31). Additionally, measurements may differ according to anatomic sites and, as noted by Hicks et al. (16), it has been previously reported that small changes in position of the measuring probe can produce significant changes in measurements and may result in decreased reliability of results.

MICROTOPOGRAPHY

Skin microrelief reflects the three-dimensional organization of the deeper layers and functional status of the skin (32). Research has been performed relating changes in skin microtopography to age and, more recently, relating changes to ethnic origin (Table 3). Guehenneux et al. (32) studied changes in microrelief with age in Caucasian and Japanese women, simultaneously during winter in Paris and Sendai. Both Caucasian and Japanese women showed an increase in the density of lines measuring >60 μm in depth and a decrease in the density of lines measuring <60 μm with increasing age. However, this change was found to be more pronounced and occur at a younger age in Caucasian women. In addition, although no changes in orientation of lines with age were found in Japanese women, changes correlating with an increase in skin anisotropy with age were found in Caucasian women. Note, it is difficult to assess the reliability of ethnic comparison in this study as the subjects were studied in two distinct geographical locations where environmental exposures may differ.

Diridollou et al. (33) compared skin topography among women of African-American, Caucasian, Asian, and Hispanic descent. Skin microrelief of the dorsal and ventral forearms was investigated in terms of the density of line intersections, in which a higher density of the intersection indicated smoother skin, and line orientation, in which a smaller angle difference between the two main directions of the lines indicated higher anisotropy. On the ventral forearms, the data supported the fact that the roughness and anisotropy of the skin increased with age in all four ethnic groups; the density of intersection decreased, and angle between lines of different orientation became smaller. The same results were produced by the dorsal forearms, a sun-exposed site, but changes were significantly less pronounced for the African-American subjects, indicating a possible resistance to photoaging in this group. Overall, the density of the intersections was less for Caucasians and Hispanics than for Asians and African-Americans. In addition, the anisotropy was higher for Caucasians than for Hispanics or Asians, and significantly higher than African-Americans.

Diridollou et al. (33) concluded that roughness and anisotropy are more pronounced in Caucasian skin than in Hispanic, Asian, and African-American skin. Guehenneux et al. (32) also found more pronounced changes of topography and higher anisotropy in Caucasian skin

Table 3

Study	Technique	Subjects	Site	Results
a. Blood vessel reactivity[a]				
Hicks et al. (16)	Topically administered SLS (irritant); LDV	White 7 Black 6 (ages 18–40 yr, all)	Volar forearm	• SLS stress: no significant difference in LDV response between groups
b. Microtopography[b]				
Guehenneux et al. (32)	In vivo—skin replicas and interferometry	Caucasian 356 Japanese 120 (ages 20–80 yr, women, all)	Volar forearm	• ↑ in the density of lines > 60 μm and ↓ in the density of lines < 60 μm in depth with increasing age in both; change in Caucasians > Japanese and at earlier age in Caucasians. • Anisotropy: ↑ with age in Caucasians, no change in Japanese
Diridollou et al. (33)	In vivo–SkinChip	310 women (ages 18–61 yr, all; African-American, Caucasian, Asian, Hispanic)	Dorsal and ventral forearms	• Roughness and anisotropy ↑ with age on both dorsal and ventral forearms in all groups; Caucasians > Hispanic and Asians and African-Americans. • Density of the line intersections: Caucasians and Hispanics < Asians and African-Americans.
c. pH gradient[c]				
Grimes et al. (18)		African-American 18 White 19 (ages 35–65 yr, women, all)	Above left eyebrow	• Baseline: African-Americans < whites, but not statistically significant
d. Sebaceous function[d]				
Aramaki et al. (11)	In vivo—sebumeter; topical application of SLS (irritant)	Japanese women 22 (mean age 25.84 yr) German women 22 (mean age 26.94 yr)	Forearm	• Baseline sebum levels: Japanese < whites ($p < 0.05$) • After SLS stress: Japanese > whites ($p < 0.05$)
Grimes et al. (18)	In vivo—sebumeter	African-American 18 White 19 (ages 35–65 yr, women, all)	Forehead	• Baseline sebum levels: African-Americans < whites, but not statistically significant
De Rigal et al. (35)	In vivo—sebumeter; sebutape	387 women (ages 18–70 yr, all; African-American, Hispanic, Caucasian, Chinese)	Forehead and cheeks	• Mean sebum excretion rate: same across all ethnic groups. • Number of sebaceous glands: Chinese and Hispanics < Caucasians and African-Americans. • Sebum level decrease with age: linear in Chinese; sudden ↓ around age 50 yr for other 3 groups.

(*Continued*)

Table 3 (*Continued*)

Study	Technique	Subjects	Site	Results
e. Vellus hair follicles[e]				
Mangelsdorf et al. (36)	In vivo—skin surface biopsies	Asian 10 African-American 10 (ages 25–50 yr, males, all)	Forehead, back, thorax, upper arm, forearm, thigh, calf	• Distribution of follicle density for different body sites same in all groups: highest on forehead, lowest on calf. • Follicle density on forehead: Caucasians > African-Americans > Asians ($p < 0.01$); no significant differences on other sites. • Calf and thigh: Asians and African-Americans—smaller values for volume ($p < 0.01$, both), potential penetration surface ($p < 0.01$, both), follicular orifice ($p < 0.01$ and $p < 0.05$, respectively), and hair shaft diameter ($p < 0.01$, both). [results compared to Caucasians studied in Otberg et al. (37)]

[a]Each study, except for the study by Berardesca and Maibach (30) comparing Hispanics and whites, Aramaki et al. (11) comparing Japanese and German women, and Hicks et al. (16) comparing blacks and whites, reveals some degree of ethnic variation in blood vessel reactivity.
[b]Difficult to compare two studies because of different techniques. However, both studies demonstrate an increase in anisotropy with age in Caucasians.
[c]Evidence supports that pH of black skin is less than white skin. However, Berardesca et al. (14) demonstrate this difference after superficial tape stripping of the volar forearm, but not at baseline; while Warrier et al. (6) demonstrate the difference at baseline on the cheeks but not on the legs; and the results from Grimes et al. (18) did not reach statistical significance.
[d]Ethnic differences in sebaceous function are inconclusive.
[e]Unable to draw conclusions regarding ethnic differences in vellus hair follicle distribution and morphology because of insufficient evidence.
Abbreviations: EM, electron microscopy; LDV, laser Doppler velocimetry; PLS, parallel-linear striations; SC, stratum corneum; SLS, sodium lauryl sulfate; yr, years.

as compared with Asian skin, and at an earlier age. However, the results of both studies cannot be compared or integrated as they used different tools of investigation and different evaluation parameters.

pH Gradient

Ethnic differences in pH of the skin have also been investigated to evaluate variation in skin physiology. In examining differences in pH between Caucasian (skin types I/II) and African-American (skin type VI) women at baseline and after tape strippings, Berardesca et al. (16) found no significant differences at baseline. After tape stripping, the pH in both ethnic groups decreased with more tape strippings. However, they found a significantly lower pH in blacks compared with whites in the superficial layers of the SC, but not in the deeper layers. Warrier et al. (6) also found a lower pH on the cheeks and legs of blacks compared with whites, but the pH difference on the legs did not reach statistical significance.

Since these earlier studies, similar results were produced in the study by Grimes et al. (18). The skin pH, measured above the left eyebrow, was found to be lower in African-American women than white women, but the results did not reach statistical significance.

Thus, the skin pH has been found to be lower in blacks compared with whites in three different studies, but in different anatomic locations and with varying statistical significance. It can be implied from these studies that there may be some difference between whites and blacks in SC pH, but the the confounding factors remain to be explored (Table 3) (1).

SEBACEOUS FUNCTION

Sebum is a semisolid secreted onto the skin surface by glands attached to the hair follicle by a duct (34). The functions of sebum include protection from friction, reduction of water loss, and protection from infection. Sebum levels have been confirmed to decline with age; however, there are few studies on the effect of race on baseline sebum secretion. Grimes et al. (18) used a sebumeter to measure sebum levels on the foreheads of African-American and white women. The results showed lower levels of sebum on African-American skin than on white skin, but differences were not statistically significant.

A study by de Rigal et al. (35) investigated the sebaceous function of women of African-American, Hispanic, Caucasian, or Chinese descents. Measurements were performed using a sebumeter and sebutape on the forehead and cheeks to compare sebum excretion rate and number of sebaceous glands according to ethnicity and age. The mean gland excretion was the same across ethnic groups. However, the number of sebaceous glands was lower in Chinese and Hispanic groups as compared with Caucasian and African-American groups. In addition, the pattern of normal sebum decreased with age differed in each population; the decrease was linear in the Chinese group, but the other three groups exhibited a sudden decrease around age 50 years.

Aramaki et al. (11) assessed sebum secretion as a part of their study investigating skin reaction to SLS at concentrations of 0.25% and 0.5%. Before and after application of SLS to the forearms of each subject, sebum levels were determined by a sebumeter. The baseline sebum levels were lower in Japanese women than in white women. However, after SLS 0.25% and 0.5% application, sebum levels were higher in the Japanese women ($p < 0.05$).

The latter two studies suggest that significant differences exists between sebum levels according to ethnicity. The de Rigal et al. (35) study found that although the mean sebum excretion was the same across ethnic groups, the number of sebaceous glands and the normal sebum decrease with age varied between groups. This may indicate a difference in distribution of sebum independent of sebum levels among ethnic groups. Aramaki et al. (11) determined sebum levels to be lower in Japanese women as compared with white women at baseline, but Japanese women expressed an increase in sebum levels in response to irritant stress. This irritant response may represent a physiologic attempt to increase barrier defense. Further studies will be useful to elucidate whether differences in barrier defense between ethnic groups are based on varying baseline sebum levels or varying sebaceous response to physical stress (Table 3).

VELLUS HAIR FOLLICLES

As follicular morphology and distribution may affect penetration of topical medications and consequent treatment response, Mangelsdorf et al. (36) investigated vellus hair follicle size and distribution in Asians and African-Americans as compared to whites (Table 3). Skin surface biopsies were taken from seven body sites of Asians and African-Americans, with body sites matched to locations described by Otberg et al. (37) in their study on Caucasians. In comparing the results of the three ethnic groups, the distribution of follicle density at different body sites was the same; the highest average density was on the forehead and the lowest on the calf for all groups. However, follicular density on the forehead was significantly lower in Asians and African-Americans ($p < 0.001$). The Asians and African-Americans also exhibited smaller values for potential pentration surface ($p < 0.01$, both groups), follicular orifice ($p < 0.01$ and $p < 0.05$, respectively), and hair shaft diameter ($p < 0.01$, both groups) on the thigh and calf regions. The authors concluded that the significant ethnic differences in follicle structure and pattern of distribution, especially in calf and forehead regions, emphasize the need for skin absorption experiments on different skin types to develop effective skin treatments.

MELANOSOMES

Ethnic differences in number of melanocytes, number of melanosomes, and morphology of melanosomes has been of great interest in working toward the development of objective definitions of skin color (Table 4). The biosynthesis of melanin, a cutaneous pigment, occurs in a melanosome, a metabolic unit within the melanocyte; melanosomes are then transported via melanocyte dendrites to adjacent keratinocytes (38).

In 1969, Szabo et al. (39) examined Caucasoids, American-Indians, Mongoloids (from Japan and China), and Negroids to observe melanosome groupings. The melanosomes in keratinocytes of Caucasoids and Mongoloids were found to be grouped together with a surrounding membrane. In contrast, the Negroid keratinocytes showed numerous melanosomes, longer and wider than in other racial groups, and mostly individually dispersed. Additionally, they observed an increase in melanosomes of keratinocytes of all races after irradiation, with grouping of melanosomes maintained in Caucasoids and Mongoloids. The authors concluded that individually dispersed melanosomes give a more uniform and dense color than the grouping found in fair skin.

In 1973, Konrad et al. (40) studied melanosome distribution patterns in hyperpigmented white skin alone and found that when comparing hyperpigmented lesions to control areas, there were no uniform differences in the distribution patterns of melanosomes. In addition, the degree of clinical hyperpigementation was not associated with specific distribution patterns. However, they did note an important relationship between melanosome size and distribution: the percentage of melanosomes dispersed singly increased with increasing melanosome size. The authors also reported findings with experimental pigment donation, showing that large melanosomes are taken up individually by keratinocytes and dispersed singly within their cytoplasm, while small melanosomes are incorporated and maintained as aggregates. These data suggested melanosome size differences as the basis for skin color differences.

More recently, Thong et al. (41) quantified variation in melanosome size and distribution pattern on volar forearms of Asian (phototypes IV/V), Caucasian (phototype II), and African-American (phototype VI) skin. The proportions of individual and clustered melanosomes were compared for each ethnic group and showed statistically significant differences ($p < 0.05$). Melanosomes in Caucasian skin were distributed as 15.5% individual versus 84.5% clustered. Meanwhile, in African-Americans, the melanosomes were distributed as 88.9% individual versus 11.1% clustered. The Asian melanosome distribution was intermediate between the latter two groups, as 62.6% individual versus 37.4% clustered. The investigators also determined the mean $\pm$ standard deviation (SD) size of melanosomes distributed individually to be larger in comparison with those distributed in clusters for each ethnic group. The mean $\pm$ SD of random melanosomes in each group differed as African-American skin showed significantly larger melanosome size than Caucasian skin, and Asian skin showed melanosome size as intermediate between the two other groups. Thus, there was a trend of progressive increase in melanosome size when moving from Caucasian to African-American skin that

Table 4 Melanosomes[a]

Study	Technique	Subjects	Site	Results
Szabo et al. (39)	In vivo—EM	Caucasoid 5 American-Indian 6 Mongoloid 3 Negroid 7 (age not reported)	Not reported	• Caucasoids and Mongoloids: grouped melanosomes • Negroids: longer and wider melanosomes, predominantly individually dispersed.
Alaluf et al. (42)	In vivo—EM; alkali solubility of melanin	European 10 Chinese 8 Mexican 10 Indian 10 African 10	Dorsal forearm and volar upper arm	• Average melanosome size: dorsal forearm > volar upper arm, in all ethnic groups ($p < 0.001$); African > Indian > Mexican > Chinese > European • Melanosome size ~ total melanin content ($p < 0.0001$) • Light melanin fraction: African < (Mexican and Chinese) < Indian < European • Dark melanin fraction: African and Indian > (Mexican and Chinese) > European • Total amount of melanin: African and Indian > Mexican and Chinese and European ($p < 0.001$)
Thong et al. (41)	In vivo—EM	Chinese 15 (Skin type IV/V, ages 10–73 yr) Caucasian 3 (Skin type II, ages 22–49 yr) African-American 3 (Skin type VI, ages 18–52 yr)	Volar forearm	• Proportion of individually distributed to clustered melanosomes: African-Americans > Asians > Caucasians ($p < 0.05$) • Mean ± SD size of melanosomes distributed individually > clustered, in all ethnic groups. • Mean ± SD size of random melanosomes: African-Americans > Asians > Caucasians ($p < 0.05$)

[a]Darker skin has more individually dispersed melanosomes in comparison with lighter skin; individually dispersed melanosomes tend to be larger in size than clustered melanosomes.
Abbreviations: EM, electron micrograph; SD, standard deviation; yr, years.

corresponded with the progression from clustered to predominantly individual melanosome distribution. In addition, degradation patterns of melanosomes in the upper levels of epidermis varied by ethnic group. As keratinocytes became terminally differentiated and migrated to the SC, melanosomes were completely degraded and absent in the SC of light skin, while intact melanosomes could be seen in the SC of dark skin. Asian skin showed an intermediate pattern where few melanosomes remained in the corneocytes; interestingly, the remaining melanosomes were predominantly individual, indicating that clustered melanosomes may be degraded more efficiently during this process.

Alaluf et al. (42) examined the morphology, size, and melanin content of melanosomes on the volar upper arms and dorsal forearms of European, Chinese, Mexican, Indian, and African subjects living in South Africa. The melanosome size of dorsal forearm (photoexposed) skin was observed as approximately 1.1 times larger than melanosome size of volar upper arm (photoprotected) skin ($p < 0.001$) when data were pooled from all ethnic groups; each ethnic group separately showed a similar trend, but lacked statistical significance. In addition, a progressive and statistically significant increase in average melanosome size was observed when moving from European (light) to African (dark) skin types. The melanosome size was directly correlated with total melanin content in the epidermis of all subjects ($p < 0.0001$). When comparing the epidermal melanin content among ethnic groups, the investigators found a downward trend in the amount of alkali-soluble melanin (light-colored pheomelanin and DHICA-enriched eumelanin) in epidermis as the skin type became progressively darker; African skin contained the lowest amount ($p < 0.02$). Indian skin presented an exception to this trend with higher concentrations of light melanin fractions than both Mexican and Chinese skin ($p < 0.05$). However, both African and Indian skin showed about two times more of the alkali insoluble melanin (dark-colored DHI-enriched eumelanins) than the Mexican, Chinese, and European skin types ($p < 0.001$). Overall, the melanin composition showed a trend toward higher fractions of alkali-soluble melanins while moving from darker (African) skin to lighter (European) skin. In addition, African and Indian skin revealed the highest total amount of melanin ($p < 0.001$) and did not differ significantly from each other.

Despite the data showing differences in number and distribution of melanosomes, recent studies find no evidence of differences in numbers of melanocytes among ethnic groups (38). For example, Alaluf et al. (43) found no significant difference in melanocyte number between African, Indian, Mexican, or Chinese skin types using immunohistochemical methods. They did consistently find 60% to 80% more melanocytes in European skin than all other skin types ($p < 0.01$), but the authors felt a larger sample size would be necessary to confirm this observation. Tadokoro et al. (44) also found approximately equal densities of melanocytes in unirradiated skin of Asian, black, and white subjects ranging from 12.2 to 12.8 melanocytes per mm.

Thus, it is generally accepted that differences in skin color are supported more by differences in melanosome distribution, size, and content rather than melanocyte number. Szabo et al. (39) observed larger and more individually dispersed melanosomes in Negroid keratinocytes and concluded that individually dispersed melanosomes may contribute to a more dense skin color. Konrad et al. (40) further noted that the number of singly dispersed melanosomes increased as melanosome size increased. Thong et al. (41) quantified the ethnic differences in melanosome size and distribution, finding a gradient in relative proportion of individual versus clustered melanosomes that corresponded with size of melanosomses. At one extreme, African-American skin showed larger melanosomes that were predominantly individually dispersed; and with Asian skin displaying intermediate results, Caucasian skin was at the other extreme, showing smaller melanosomes that were predominantly clustered. Alaluf et al. (42) also revealed a progressive increase in melanosome size as ethnic skin went from lighter to darker. Furthermore, dark skin contained more total melanin and a larger fraction of DHI-enriched (dark colored) eumelanin than light skin.

ANTIMICROBIAL PROPERTIES

In 2001, Mackintosh (45) reviewed evidence discussing the role of melanization of skin in the innate immune defense system. He reported that a major function of melanocytes, melanosomes, and melanin in skin is to inhibit the proliferation of bacterial, fungal, and other parasitic infections in the dermis and epidermis. Numerous studies are cited showing

evidence that melanocytes and melanosomes exhibit antimicrobial activity and are regulated by known mediators of inflammatory response. The review aims to support the hypothesis that immunity and melanization are genetically and functionally linked. The author notes that previous reports have implied a reduced susceptibility of dark-skinned individuals to skin disease. In addition, it is postulated that the evolution of black skin could represent high pressures from infection, especially in tropical regions. In five out of six recent investigations, people of African descent have been shown to be less susceptible to scabies, fungal dermatophytosis, cutaneous *Candida albicans* infections, and bacterial pyodermas than whites. Additionally, although Rebora and Guarrera (46) demonstrated increased skin microflora in blacks, they found that the severity of dermatitis in black subjects was significantly less ($p < 0.01$), suggesting the possibility of increased barrier defense. This evidence may explain that the existence of melanocytes and melanization in different parts of the body is independent of sun exposure, as in the genitalia, as well as the latitudinal gradient in skin melanization. The presented evolutionary data are compelling and indicates a necessity for controlled studies to clarify whether the number of melanocytes, size of melanosomes, or type of melanin can affect the antimicrobial properties of skin.

PHOTODAMAGE

Although there is evidence for objective differences in skin color, it remains unclear what role these differences in melanin and melanosomes play in dermatologic disorders. Section IX ("Ethics and Regulations") of this article introduced the potential role of melanosomes in antimicrobial defense. The most extensively studied function of darker skin color, however, has been in resistance to photodamage from UV radiation. End effects of photodamage include skin cancer, which are well documented as affecting lighter-skinned individuals more than those with darker skin.

In determining a relationship between melanosome groupings and sun exposure, studies have observed that dark-skinned whites, when exposed to sunlight, have nonaggregated melanosomes, in contrast to light-skinned, unexposed whites who have aggregated melanosomes. Similarly, there are predominantly nonaggregated melanosomes in sunlight-exposed Asian skin, and primarily aggregated melanosomes in unexposed Asian skin (38,47).

Alaluf et al. (42) noted an increase in melanosome size in photoexposed skin versus photoprotected skin in all ethnic groups; the melanosome size was directly correlated with epidermal melanin content, suggesting increased melanogenesis in photoexposed areas. Van Nieuwpoort et al. (48) demonstrated that with increased melanogenesis, light-skin melanosomes showed elongation and reduction in width with no significant change in surface area, while dark-skin melanosomes enlarged in both length and width with an increase in volume. On the basis of these data, although all skin types show an increase in epidermal melanin with sun exposure, both distribution and morphology may influence unequal filtering between light- and dark-skin types.

In another study, Rijken et al. (49) investigated response to solar-simulating radiation (SSR) among white (phototype I–III) and black skin (phototype VI). In each white volunteer, SSR caused DNA damage in epidermal and dermal cells, an influx of neutrophils, active photoaging-associated proteoytic enzymes, and keratinocyte activation. Also, some white volunteers showed IL-10-producing neutrophils in the epidermis; IL-10-producing cells have been postulated to be involved in skin carcinogenesis. In black-skinned individuals, aside from DNA damage in the suprabasal epidermis, there were no other changes found; basal keratinocytes and dermal cells were not damaged. The authors concluded that these results were best explained by difference in skin pigmentation and that melanin functions as a barrier to protect basal keratinocytes and the dermis from photodamage.

Other studies have suggested that filter properties of melanin alone do not provide sufficient protection against DNA damage in underlying cells. Tadokoro et al. (50) investigated the relationship between melanin and DNA damage after UV exposure in subjects of five ethnic origins (black, white, Asian, others not specified), Fitzpatrick phototypes I through VI. They found measurable damage to DNA in all groups, and DNA damage was maximal immediately after irradiation, gradually returning to baseline over time. The immediate DNA damage levels were higher in whites and Asians in comparison with blacks and Hispanics. In

addition, the whites and Asians showed lower constitutive levels of melanin content. However, the kinetics of DNA damage removal differed among individual subjects, showing no association between melanin content or ethnic group and DNA repair rates. The authors noted that though melanin affords significant protection against initial DNA damage, other properties of melanin, such as antioxidant properties and radical scavenging properties, may play roles in minimizing the ultimate level of UV damage. Ethnic differences in expression of receptors involved in melanosome uptake and melanocyte-specific proteins, both before and after UV exposure, are also being investigated.

The studies by Rijken et al. (49) and Tadokoro et al. (50) indicate that differences in patterns and kinetics of DNA damage in response to UV radiation exist between ethnic groups. Additionally, there is evidence of differences between photoexposed skin and photoprotected skin in melanosome aggregation patterns, melanosome size, melanosome shape, and melanogenesis (38,42,47,48); it is yet unclear how these results relate to differences in melanization and resistance to photodamage between ethnic groups.

CONCLUSION

The U.S. Census Bureau estimates that the population is composed of 12.1% black or African-American, 13.9% Hispanic, or Latino, and 11.9% other nonwhites (51). It has been predicted that people with colored skin will constitute a majority of the United States and international populations in the 21st century (52). In light of these statistics, objective investigation of relationships between ethnicity and differences in structure and function of skin becomes important for developing appropriate treatment protocols. The Food and Drug Administration (FDA) currently recommends inclusion of more ethnic groups in dermatologic trials, citing evidence that physiologic differences in skin structure between races can result in varying efficacies of dermatologic and topical treatments (53). However, data on ethnic differences in skin, physiology, and function are few; the studies that do exist consist of typically small patient populations. Consequently, few definitive conclusions can be made.

Notably, it is sometimes difficult to interpret studies on ethnic differences as each study may use different definitions of race or ethnicity. Race seems to emcompass genetic variations on the basis of natural selection, which include, but are not limited to, pigmentation (53); pigmentation appears to be based mainly on erythema, melanin, and the skin's response to physiologic insult. Anthropologists divide racial groups into Caucasoid (e.g., Europeans, Arabs, Indians), Mongoloid (e.g., Asians), Australoid (e.g., Australian aborigines), Congoid or Negroid (e.g., most African tribes and descendants), and Capoid (e.g., the Kung San African tribe) with the idea that racial variations were selected to facilitate adaptations to a particular environment (54). Some reject the relevence of any genetic basis for race, stating that 90% to 95% of genetic variation occurs within geographic populations rather than across racial groups (53).

Ethnicity, on the other hand, is a more general term, defined as how one sees oneself and how one is seen by others as part of a group on the basis of presumed ancestry and sharing a common destiny, often with commonalities in skin color, religion, language, customs, ancestry, and/or occupation or region (54). Thus, ethnicity overlaps with race but also depends on more subjective and cultural factors, while race seems to encompass genetic variations based on natural selection (1). Nevertheless, studies have been able to show differences on the basis of various ethnic categorizations.

On the basis of the data collected during our review, there exists reasonable evidence (Table 5) to support that black skin has a higher TEWL, variable BVR, lower skin surface pH, and larger melanosomes with more individual distribution when compared with white skin by means of objective measurements; the role of differences in melanization in the antimicrobial properties of skin and resistance to photodamage remain uncertain. Although some deductions have been made about Asian and Hispanic skin, the results are contradictory, and further evaluation is necessary (1). Ethnic differences in WC remain inconclusive as the prior data are contradictory and recent data have not shown statistically significant differences. Differences in sebaceous function, although statistically significant, are inconclusive. In addition, there is insufficient evidence at this time to draw any conclusions about differences in microtopography and follicular morphology and distribution.

Table 5 Summary

Evidence supports	Insufficient evidence for	Inconclusive
• TEWL black > white skin • Variable ethnic blood vessel reactivity • pH black < white skin • Darker skin has more individually dispersed melanosomes; individually dispersed melanosomes larger than clustered melanosomes.	• Deductions regarding Asian and Hispanic skin Ethnic differences in:[a] • Microtopography • Vellus hair follicle morphology/distribution	Ethnic differences in: • Water content • Sebaceous function

[a]Microtopography and vellus hair follicle morphology/distribution were labeled as "insufficient evidence for" ethnic differences rather than "inconclusive" because only two studies or less have examined these variables.
Abbreviation: TEWL, transepidermal water loss.

Objective data on differences in skin properties between ethnic groups not only emphasize the value of investigation of disease processes and treatment responses in ethnic skin but also highlight the growing list of physiogic variables involved. Future studies could be strengthened by detailing definitions of how subjects are designated to a particular race or ethnic group in addition to skin phototype and would enable more reliable comparisons of results from different studies.

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6 Sensitive Skin: Sensory, Clinical, and Physiological Factors[a]

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INTRODUCTION

Certain individuals experience more intense and frequent adverse sensory effects than the so-called normal population after topical use of personal care products, a phenomenon known in popular usage as sensitive skin. Consumer reports of sensitive skin are self-diagnosed and often not verifiable by objective signs of physical irritation. Manufacturers of cosmetic and personal care products are challenged to provide safe products to consumers with vast differences in skin type, culture, and habits. This review examines the still incomplete understanding of this phenomenon with respect to etiology, diagnosis, appropriate testing methods, possible contributing host factors (e.g., gender, ethnicity, age, anatomical site, cultural and environmental factors), and the future directions needed for research.

The term "sensitive skin"—of lay origin (1)—commonly refers to an exaggerated and unpleasant sensitivity of the skin to frequent or prolonged use of everyday products such as cosmetics or toiletries. Epidemiological surveys reveal a high prevalence of sensitive skin. A telephone survey of 800 ethnically diverse women in the United States found that 52% professed sensitive skin, with no statistical difference between ethnic groups (2). A U.K. mail survey of 2058 men and women found that 51.5% of the women and 38.2% of the men reported sensitive skin, as well (3).

Researchers have largely ignored consumer reports of sensory irritation because they are both difficult to quantify and frequently (50%) unaccompanied by visible signs (4). The reactions, however, are ubiquitous and globally dispersed and demand a clinically satisfactory understanding. The question is not merely academic; before introducing any new product into the marketplace, manufacturers perform both skin safety testing and risk assessment to ensure skin compatibility under a variety of potential exposure conditions (5). Consumer-perceived skin sensitivity is critical commercially as well, even though it is largely sensory without obvious physical effect, it strongly influences consumer choice (6). In fact, 78% of consumers who profess sensitive skin report avoid some products because of unpleasant sensory effects associated with their use (2).

DEFINITION AND CLASSIFICATION OF SENSITIVE SKIN

The term "sensitive skin" needs to be defined precisely. A tenuous consensus in the literature is that sensitive skin is characterized by subjective complaints of discomfort without predictable classical visible signs of irritation (7) and without an immunological response (7,8). Although transient redness, dryness, or tenderness may accompany adverse sensations (8), and sensitive skin may be less supple or hydrated (9), subjects often experience sensory effects only (8). Subjective irritation (9), invisible irritation (4), nonimmunological adverse skin reactions (1), nonimmunological inflammation, and self-estimated enhanced skin sensitivity (SEESS) (10) have been proposed as more clinically meaningful terms.

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[a]Adapted from Farage MA, Katsarou A, Maibach H. Sensory, clinical and physiology factors in sensitive skin: A review. Contact Dermatitis 2006, 55:1–14; with kind permission from Blackwell publishing group.

It is believed that some subjects report greater incidence of adverse reactions to certain products because of higher sensitivity (1–3,5,9). Some individuals possess exaggerated sensitivity to specific individual irritants (11). Despite the fact that some studies have shown that sensitive skin patients are capable of distinguishing products on the basis of blinded sensory endpoints (1,12), a clinically satisfactory description of observed sensitivities is still out of reach.

Progress in defining sensitive skin has been hampered by various issues. The condition is typically self-diagnosed (7), and there is no agreement, beyond heightened sensitivity, on its symptoms (1). Its presentations include stinging, itching, burning, dryness, erythema, desquamation, papules, wheals, and scaling (1,13), which occur over a wide range of intensities (8). To further complicate the diagnosis, cutaneous irritation is a syndrome with multiple potential factors (7) such as age, genetics, hormonal factors, skin dryness, race, skin pigmentation, anatomical region, preexisting diseases, cultural factors, and environmental factors (4).

Another challenge in the proper identification of the appropriate target population is finding the best testing methods. Many people who profess sensitive skin do not predictably experience visible signs of the sensations reported, while some who describe themselves as nonsensitive react strongly to tests of objective irritation (14). In one study, an irritant dose that was completely tolerable by 99 subjects caused pronounced irritation in the 100th one. Another study tested a three-irritant panel in 200 subjects and found that 197 subjects reacted to at least one of three irritants, while three subjects did not respond at all (15). In addition, the severity of individual responses to irritants tested varied tremendously (16), even among chemicals with similar modes of action (1).

Testing has revealed sizeable variation within the same individual at different anatomical sites (16) and even at the same anatomical site on the contralateral limb. An aluminum patch test of irritant response to the surfactant sodium lauryl sulfate (SLS) found that the right and left arms differed significantly in 47% of individuals tested (17). Furthermore, the methodology used may introduce additional variability: a similar SLS patch test using a Finn chamber resulted in 84% of the subjects testing identically on the right and left arms (17). Most methods have focused on objective assessment of physical effects to the skin rather than on the sensory effects reported (12), and most test protocols have relied on exaggerated exposure (5) of uncertain relationship to actual consumer use (1). In addition, most actual testing has included very few subjects, while few have restricted subjects to those with demonstrated sensitivity (5).

It is likely that the phenomenon of sensitive skin, when unraveled, will prove to be an umbrella classification comprised of distinct subgroups of clinical sensitivities. Pons-Guiraud (7) proposed three subgroups: (*i*) *very sensitive* skin, reactive to a wide variety of both endogenous and exogenous factors with both acute and chronic symptoms and a strong psychological component; (*ii*) *environmentally sensitive* skin, comprised of clear, dry, thin skin with a tendency to blush or flush and reactive to primarily environmental factors; and (*iii*) *cosmetically sensitive* skin, transiently reactive to specific and definable cosmetic products (7). Muizzuddin et al. (18) defined three subgroups somewhat differently. Their classification includes *delicate skin*, characterized by easily disrupted barrier function not accompanied by a rapid or intense inflammatory response; *reactive skin*, characterized by a strong inflammatory response without a significant increase in permeability; and *stingers*, characterized by a heightened neurosensory perception to minor cutaneous stimulation (18).

METHODS APPLIED IN CLINICAL STUDIES

Researching sensitive skin has employed a variety of methodological approaches. Chemical and mechanical irritants of numerous types and mechanisms have been employed, and numerous methods of assessing reactivity have been devised. Methods can largely, however, be broken down into those that assess neurosensory response (sensory reactivity tests), those that assess visible cutaneous signs of irritation (irritant reactivity tests), and those that measure structural and physiological parameters of the skin for indications of irritant effect (dermal function tests) (Table 1).

Sensory Reactivity Tests

Sensory reactivity tests focus on the neurosensory component of the sensitive skin response. The most popular has been the sting test (19), in which lactic acid is applied to the skin [other

Table 1 Methods for Evaluating Sensitive Skin

Objective of test	Parameter measured and most common irritant	Assessment methodology	Advantages	Disadvantages
		Sensory reactivity tests		
To provide measure of sensory perception of pain in absence of visible irritation	Stinging sensation with lactic acid as most common irritant	Sensory perception questionnaire typically utilizing point scale	• Very quick, easy, and inexpensive • Requires no instrumentation	• Often nonreproducible • Lacks objective criteria • Relationship to objective measures of irritation undefined
		Irritant reactivity tests		
To provide measure of visible sequelae to irritant exposure (typically dryness or erythema)	Objective cutaneous irritation with SLS as most common irritant	Visual scoring (measures skin irritation by visual inspection)	• Relatively inexpensive and quick • Requires no instrumentation	• Often nonreproducible • Not objective • Relationship to neurosensory perception undefined
		LDV (measures skin irritation by blood flow)	• Noninvasive • Objective • Quantitative • Biomechanical assessment	• Requires expensive instrumentation • Indirect measure, less sensitive than TEWL • Defines negative and positive reactions, but does not quantitate differences in positive reactions well • Some irritants (NaOH, dithanol) do not cause measurable response
		Color reflectance (measures skin irritation by minute color change)	• Noninvasive • Objective • Accurate • Reproducible • Allows quantitative comparison of erythema both within and between individuals	• Requires expensive instrumentation • Indirect measure • Less sensitive than TEWL
To provide evaluation of water loss in skin not attributable to sweating	TEWL (barrier integrity) with SLS as most common irritant	Evaporimeter (closed chamber, open chamber, and ventilated chamber)	• Quantitative • Best measure of skin damage	• Requires expensive instrumentation • Requires stringent conditions • Easily confounded by temperature, humidity, host factors
To provide measure of water content of the skin by assessment of a dielectric constant	Skin hydration with SLS as most common irritant	Electrical capacitance, Corneometer®	• Quantitative • Relatively quick	• Defines arbitrary units, difficult to standardize, assumes ceteris paribus • Confounded by skin surface features and salt content • Little correlation with irritant testing

(Continued)

Table 1 Methods for Evaluating Sensitive Skin (*Continued*)

Objective of test	Parameter measured and most common irritant	Assessment methodology	Advantages	Disadvantages
		Structural sensitivity tests		
To provide evaluation of structural alteration of skin as a result of exposure to cutaneous irritants	Skin thickness with SLS as most common irritant	Ultrasound Confocal light microscopy Light microscopy	• Quantitative, relatively quick • Highly accurate • Noninvasive, suitable for any anatomical site • Quantitative • Requires no specialized equipment • Highly accurate • Quantitative, accurate • Allows direct measurement on unmodified skin • Allows assessment of skin beyond surface depth	• Requires expensive instrumentation • Labor intensive • Histological preparations subject to artifacts • Invasive, not suitable for all anatomical sites • Requires specialized expensive equipment
To provide assessment of alteration of skin permeability as a result of exposure to cutaneous irritants	Skin penetrability with SLS as most common irritant	UV light	• Correlates well with skin sensitivity	• Requires specialized expensive equipment

Abbreviations: LDV, laser Doppler velocimetry; NaOH, sodium hydroxide; SLS, sodium lauryl sulfate; TEWL, transepidermal water loss; UV, ultraviolet.

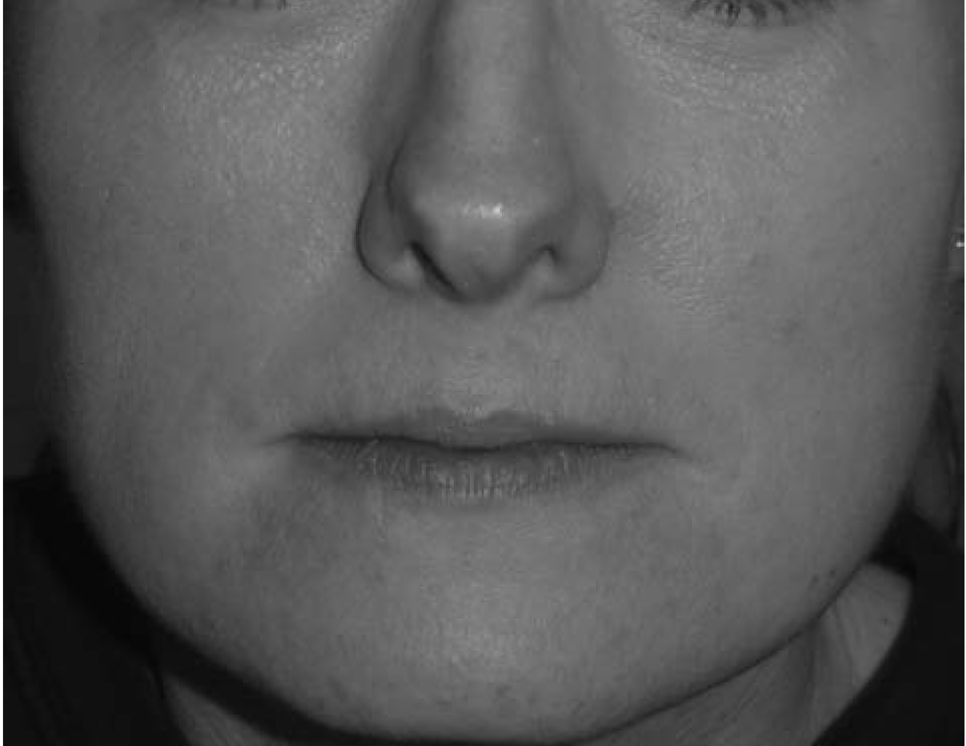

Figure 1 The nasolabial fold, an area considered highly sensitive because of a permeable horny layer, a high density of sweat glands and hair follicles, and rich innervations.

agents, including capsaicin, ethanol, menthol (1), sorbic acid, and benzoid acid (9), have also been employed]. Tape stripping, a procedure that removes the stratum corneum, is sometimes performed before irritants are applied (20).

Typically, the irritant is applied to the nasolabial fold, an area considered highly sensitive because of a permeable horny layer, a high density of sweat glands and hair follicles, and rich innervations (Fig. 1) (8,21). Sensory feedback is collected and typically quantified by a labeled magnitude scale (13). Although the sting test is considered to be the best approach to defining a potentially susceptible population (1), it has not proven predictive of sensitivity to other irritants (21). It does have the advantage of being simple, quick, and relatively inexpensive to perform, producing a mild, transient response without visible effect.

Although reports in the literature are relatively few, the innervation of the dermis and epidermis has also been evaluated for physiological differences that could explain heightened sensitivity, typically by staining neural tissue with compounds that illuminate specific components of the neurosensory network (22).

Irritant Reactivity Tests

Irritant reactivity tests attempt to measure objective signs of irritation. The SLS method has been the most common. A common ingredient of many cosmetics and other personal care products, SLS is an anionic emulsifier with an irritant potential at a concentration of greater than 1% or less (17). SLS modulates surface tension, alters the stratum corneum, increases blood flow, and enhances skin permeability (17). It is a primary irritant that damages skin by direct cytotoxic action, without prior sensitization (17).

SLS as well as other potential irritants have been applied in patch tests (1), including chamber-facilitated patch tests (5,10,17,18), repeat insult patch tests (14,18,23), open application tests (17,18), soak or wash tests (17), and plastic occlusion stress tests (POST) (17).

Irritant testing has often employed exaggerated exposure (4,13), with a demonstrated capability of achieving product differentiation (4). Newer versions of the approach exaggerate effects by adding a frictional component (13). These protocols, however, are not applicable to

paper or tissue products, and many modern products produce few effects even under exaggerated conditions. Interpretative caution must be exercised as well. Even physiological saline can cause irritation with extended occlusive application (23), and real-life exposure is typically short term, not occluded, and cumulative (17).

Other irritants employed have included dimethyl sulfoxide (DMSO) (1), benzoic acid, *trans*-cinnamic acid (1), acetic acid (5), octanoic acid (5), decanol (5), and vasodilators (24). Mechanical irritation testing has evaluated facial tissue (20) and sanitary pad surfaces (23).

Frequently, reactivity to SLS and other irritants is scored visually to obtain clinically graded assessments of erythema and edema (8,25). Erythema has also been measured by cutaneous blood flow (10,26), plethysmography (26), and color reflectance (9). Laser Doppler velocimetry (LDV) measures cutaneous blood flow, indirectly evaluating penetration of vasoactive substances as a measure of permeability (26). Color reflectance measures slight changes in color within three values of hue (17). A correlation between skin color by this method and SLS dose has been demonstrated (9,17), although one author reported no correlation (17) as well as a correlation with visual erythema scoring (17). Both techniques offer a noninvasive (27) objective assessment of a subclinical skin process without external visible effects (13). When testing the irritant potential of vasodilators, however, LDV and color reflectance are an indirect measure dependent on vasodilation as the final endpoint of a five-step physiological process (27).

Visual scoring of irritancy in the vulva has demonstrated that the area reacts less intensively and recovers faster than does exposed tissue (28). Objective assessment by LDV, unfortunately, has been demonstrated to be less sensitive in that anatomical area (27). Available bioengineering techniques for quantifying irritation have, in general, proven less suitable in the vulvar area than in other body regions (28).

Dermal Function Tests

Structural sensitivity tests measure structural or physiological changes that may be associated with the neurosensory responses in sensitive skin. Transepidermal water loss (TEWL) measures skin surface water loss (29) as a determinant of the integrity of barrier function (30) and, therefore, quantifies skin damage (16). TEWL is considered an indicator of the functional state of the stratum corneum (29) and has proven to be a better measure of irritant susceptibility than clinical visual scoring (16). It is considered the single best measure of skin sensitivity; a high baseline TEWL was defined by one author as "the" diagnostic criteria (17). TEWL measurement has demonstrated a positive SLS dose-response curve for skin response (17), and TEWL baseline measurements have proven to be correlated with sensitivity to SLS (31). When compared with LDV, ultrasound, and color reflectance, TEWL was found to correlate best with SLS exposure (17).

TEWL measurement is often accompanied by tape stripping, a procedure that does not guarantee removal of the stratum corneum and that, when successful (13), no longer tests the effect on normal skin (13). TEWL is also easily affected by endogenous factors such as cutaneous blood flow, diurnal rhythm, and eccrine and sweat gland density (29), and it requires temperature and humidity control for meaningful results (13).

Skin hydration, typically assessed by electrical capacitance, is characterized by significant individual variation (17) and is heavily confounded by skin surface texture or density of hair (32). Results have not correlated well with irritant patch testing (17). Hydration can be assessed with a Corneometer® (10) and is also sometimes expressed by desquamation index (33).

Skin thickness has been measured by ultrasound (17). Ultrasound measurements after SLS exposure correlate well with TEWL assessment of barrier function (17). Light microscopy with cyropreservation, however, is a more accurate assessment of epidermal thickness (34). Skin penetration by ultraviolet (UV) light is dependent on both thickness and the structural composition of the skin. Cutaneous sensitivity to UV light was found to have positive correlation with skin sensitivity to a seven-irritant panel, especially as compared with traditional classification of skin type, which was less reliable (17).

Future Needs in Method Development

The usefulness of any particular technique depends on the relative and actual degree of changes present (28). Effective methodology could be defined as that in which sensitive skin subjects successfully and consistently discriminate between products (35). Traditional testing

has not achieved that goal or the ability to predict universal sensitivity (13). Useful methods will need to be cost effective, reproducible, and minimally invasive (13). Instrumental enhancement of visual scoring through polarized light and assessment of cytokine levels as a measure of subclinical tissue damage are being planned (13).

RELATIONSHIP BETWEEN IRRITANT STIMULATION AND SENSORY RESPONSE

A subgroup of sensitive subjects, termed "stingers," displays stronger sensory irritation to chemical probes for stinging and burning, and some subjects have higher erythematous responses to applied irritants (11).

Although initial studies observed an increased susceptibility to general irritation among stingers (19), most subsequent research found no correlation (1,8). Strong reactivity to one nonimmunological urticant has also failed to predict response to other urticants (1). There is significant disparity, in fact, between the severity of self-reported symptoms and the presence and strength of any objective signs (12), and few reports show correlation between sensory effects and objective endpoints (12).

Two studies that evaluated the relationship between neurosensory responses and objective clinical irritation and included only subjects that demonstrated sensory sensitivity showed a correlation between sensory and objective signs. A study of sensitivity to facial tissue (which did not exclude nonsensitive individuals) found that sensory effects were the most reliable measure of product differences (20).

Although no predictive value was demonstrated for any individual sensitivity when subjects were tested with a seven-irritant panel, a weak association between tests was demonstrated by statistical analysis of binomial probability (1). However, studies that evaluated the association of barrier function and sensitivity have yielded arguably the most conclusive results. A high baseline TEWL was associated with increased susceptibility to numerous cutaneous irritants by numerous studies and a variety of assessment methods (17).

HOST FACTORS AFFECTING SKIN SENSITIVITY

Numerous potential host factors (Table 2) undoubtedly play a role in experimental variability observed in sensitive skin. Basic differences are evident from epidemiological studies. This section summarizes the effects of gender, race, age, anatomical site, culture, environment, and other possible host factors on skin sensitivity.

Gender

In general, women seem to complain of sensitive skin more often than men do (6), although no gender differences were observed with respect to reactivity to 11 different tested irritants, including SLS (16). The thickness of the epidermis was observed to be greater in males than in females ($p < 0.0001$) (34), and hormonal differences, which may produce increased inflammatory sensitivity in females, have also been demonstrated (17,48).

Ethnicity

Racial differences, with regard to skin susceptibility to irritants, are among the fundamental questions in dermatotoxicology (5). Two large epidemiological studies reported no observed racial differences in reporting product sensitivity (2,3). Most testing, however, has focused on Caucasian females (5).

Differences have been observed in sensory perceptions, although substantive conclusions are hard to provide. Asians have been reported to complain of unpleasant sensory responses more often than Caucasians (37), supported by the observation that a higher incidence of dropouts in a Japanese clinical study was due to adverse skin effects as compared to those in Caucasian studies (37). There have also been reports of an increased sensory response as well as speed of response in Asian subjects versus Caucasian in sensory testing (37). Another study, however, found that fair-skinned subjects who are prone to sunburn had higher sensory responses to chemical probes than those with darker skin tones (11). No racial differences in innervation on an architectural or biochemical level have been observed (1).

Table 2 Host Factors Thought to Promote Sensitive Skin

Factor	Reference
Female gender	Willis et al., 2001 (3)
Youth	Cua, et al., 1990 (16)
Hormonal Status	Britz et al., 1980 (36)
Cultural expectations in technologically advanced countries	Loffler et al., 2001 (10)
Fair skin that is susceptible to sunburn	Agner, 1991 (11)
Susceptibility to blushing and/or flushing	Willis et al., 2001 (3)
Skin pigmentation	Berardesca and Maibach, 1996 (32) Robinson 2000 (5) Aramaki et al., 2002 (37)
Thin stratum corneum	Freeman et al., 1962 (38) Thomson, 1955 (39) Pons-Guiraud, 2004 (7)
Decreased hydration of stratum corneum	Johnson and Corah, 1963 (40) Corcuff et al., 1991 (41)
Disruption of stratum corneum	Loffler and Effendy, 1999 (30) Pons-Guiraud, 2004 (7)
Increased epidermal innervation	Marriott et al., 2003 (42)
Increased sweat glands	Aramaki et al., 2002 (37)
Increased neutral lipids and decreased sphingolipids	Lampe et al., 1983 (43)
Decreased lipids	Seidenari et al., 1998 (9) Reinertson and Wheatley, 1959 (44) Brod, 1991 (45) Elias and Menon, 1991 (46) Schwarzendruber et al., 1989 (47)
High baseline TEWL	Lee and Maibach, 1995 (17)

Abbreviation: TEWL, transepidermal water loss.

Studies of racial differences with regard to irritants have yielded conflicting evidence. Although black skin was demonstrated to have greater potential for irritant susceptibility than white skin (16), another study found blacks to be less reactive than Caucasians (15). Asians seemed to be more reactive than Caucasians in some studies and less reactive in others, even within studies conducted by the same investigator and under the same protocol (5). Tristimulus colorimeter assessment of skin reflectance observed that skin pigmentation was inversely associated with susceptibility to irritation (17), supported by the finding that irritant susceptibility to SLS is decreased after UVB exposure (tanning) (17).

Methyl nicotinate assessment of vasoactive response suggests that there may be genuine racial differences in permeability (26). Increased percutaneous absorption of benzoic acid, caffeine, and acetylsalicylic acid was demonstrated in Asians when compared with Caucasians, and decreased percutaneous absorption was observed in blacks (37,11).

Some structural differences with the potential to influence permeability have also been observed. Epidermal thickness was found to correlate with pigmentation ($p = 0.0008$) but not classical skin type (34). Tendencies to blush or flush are associated with both fair skin and a tendency to skin sensitivity, implying barrier impairment and increased vascular reactivity (3).

Blacks and Asians were shown to have higher baseline TEWL values than Caucasians (26). Although no significant differences in barrier function (Asian vs. Caucasian) were observed (37), differences in ceramides between races have been observed (32,37), as has a difference in the buoyant density of the stratum corneum (7). The number of sweat glands in the skin has been proposed as an influencing factor in permeability, and a huge variation in distribution and size of apocrine glands among races has been observed (37). Melanosomes of blacks have also been observed to be dispersed, while in Caucasians and Asians, they are membrane-bound aggregates (32).

Skin hydration has been observed to be higher in Black, Asian, and Hispanic subjects than in Caucasians (22). There has been some association observed in blacks between sweat gland activity and conductance (37), which may be because of the chemical composition of sweat (5). The increased electrical resistance observed in blacks implies increased cohesion or thickness of stratum corneum (32).

Human skin is individually variable, thus, the results of studies conducted in separate populations (often with different methods) are difficult to interpret (5). Parallel studies are needed to define genuine racial differences (5). A summary of racial differences between black and Caucasian skin is shown in Table 3.

Table 3 Racial Differences in Skin Properties

A comparison between the black and Caucasian rarces		
Skin property	Comparison results	References
Stratum corneum thickness	Equal in blacks and caucasians	Freeman et al., 1962 (38) Thomson, 1955 (39)
Number of cell layers in stratum corneum	Higher in blacks	Weigand et al., 1974 (49)
Stratum corneum resistance to stripping	Higher in blacks	Weigand et al., 1974 (49)
Lipid content in stratum corneum	Higher in blacks	Reinertson and Wheatley, 1959 (44)
Electrical resistance of stratum corneum	Higher in blacks (twofold)	Johnson and Corah, 1963 (40)
Desquamation of stratum corneum	Higher in blacks (twofold)	Corcuff et al., 1991 (41)
Corneocyte size	Equal	Corcuff et al., 1991 (41)
Amount of ceramides in stratum corneum	Lower in blacks	Sugino et al., 1993 (50)
Variability of structural parameters of stratum corneum	Increased in blacks	Weigand et al., 1974 (49)
Spectral remittance	Lower in blacks (above 300 nm—2- to 3-fold)	Anderson and Parrish, 1981 (51)
UV protection factor of epidermis	Higher in blacks (3- to 4-fold—13.4 vs. 3.4)	Kaidbey et al., 1979 (52)
UV protection factor stratum corneum	Higher in blacks (3.3 vs. 2.1)	Kaidbey et al., 1979 (52)
UVB transmission through epidermis	Lower in blacks (4-fold, 7.4 vs. 29.4)	Kaidbey et al., 1979 (52)
Stratum corneum UVB transmission	Lower in blacks (30.0 vs. 47.6)	Kaidbey et al., 1979 (52)
In vitro penetration of fluocinolone acetonide	Lower in blacks	Berardesca and Maibach, 1996 (32)
In vitro penetration of water	No difference Differences	Berardesca and Maibach, 1996 (32) Bronaugh et al., 1986 (53)
Topical application of anesthetic mixture	Less efficacy in blacks	Hymes and Spraker, 1986 (54)
In vivo penetration of C-labeled dipyrithione	Lower in blacks (34% lower)	Agner, 1991 (11)
In vivo penetration of cosmetic vehicle	Lower in blacks	Agner, 1991 (11)
Methylnicotinate-induced vasodilation	Time to peak response equal	Guy et al., 1985 (55)
	Slower in blacks	Kompaore et al., 1993 (26) Berardesca and Maibach, 1990 (56)
Baseline TEWL	Higher in blacks Higher in blacks (in vitro)	Kompaore et al., 1993 (26) Wilson et al., 1988 (57)
Reactivity to SLS (measured by TEWL)	Higher in blacks	Wilson et al., 1988 (57)
Reactivity to dichlorethylsulfide (1%)	Lower in blacks (measured by erythema, 15% vs. 58%)	Marshall et al., 1919 (58)
Reactivity to 0-chlorobenaylidene malonitrile	Lower, longer time to response in blacks	Weigand and Mershon, 1970 (59)
Reactivity to dinitrochlorobenzene	Lower in blacks, but trend toward equalization after removal of stratum corneum	Weigand and Gaylor 1974 (60)
Stinging response	Lower in blacks Equal	Frosch and Kligman, 1981 (61) Grove et al., 1984 (62)

Abbreviations: SLS, sodium lauryl sulfate; TEWL, transepidermal water loss; UV, ultraviolet; UVB, ultraviolet B.

Age

Studies on age differences in skin sensitivity are rare and not collectively conclusive (16). No differences in potential irritancy have been observed in subjects aged between 18 and 50 years (17), although the skin of younger adults was demonstrated to be more sensitive than that of elderly subjects (16). Interestingly, however, while tactile sensitivity has been shown to decrease with age (22), pain sensation is preserved (22). Studies in elderly subjects have demonstrated both decreased sensory nerve function and decreased skin innervation (22). The potential for visible irritation also decreased with advancing age (16). Although less reaction to an irritant stimulus was observed in elderly subjects, aged subjects took longer to heal (17).

Assessment of barrier function in the elderly compared with younger adults demonstrated a decreased difference in TEWL measurements after SLS exposure in the elderly (16). Although the thickness and number of layers in the stratum corneum do not change, turnover time in the elderly did increase (16). Elderly patients were also shown to have decreased sweat function, capability of inflammation and repair, skin hydration, and peripheral microcirculation (63).

Although the number of personal care products aimed specifically at children continues to expand, reports in the medical literature on skin sensitivity in children are almost nonexistent. Children, however, have a higher surface area to body mass ratio and therefore receive higher systemic exposure from dermal use of products (64).

Anatomical Site

Assessment of neurosensory and physiological differences in the skin at different anatomical sites has been performed using sensory stimulators, irritants, and various methodologies that evaluated structural components of the epidermis. Differences in skin sensitivity between anatomical regions have been observed.

Exposed Skin

The nasolabial fold has been reported to be the most sensitive region of the facial area, followed by the malar eminence, chin, forehead, and upper lip (42). Conflicting evidence regarding sensitivity has been reported with regard to arms, legs, and torso (16). SLS-sensitivity testing found that sensitivity increased from the wrist to the cubital fossa area (17).

Analysis of structural differences found that stratum corneum density varies tremendously by anatomical site: palms and soles are the thickest, while the genital area is the thinnest (65). The rate of turnover in the stratum corneum (37), 10 days in facial areas, is longer elsewhere (65). Stratum corneum thickness yielded inconsistent results (34). TEWL following SLS exposure was found to be greater at the wrist than other sites on the forearm (17).

Vulva

The vulva differs substantively from exposed skin in numerous characteristics likely to affect vulvar susceptibility to topically applied agents (14); a summary is presented in Table 4. The outer mons pubis and labia majora are keratinized and stratified, much like the skin in other areas (48). The vulva, however, is also characterized by a frictional component, occlusion, increased hydration (48), increased hair follicles and sweat glands, and increased blood flow (14). The labia minor (inner one-third) through the vestibule, which is increasingly hydrated, is thinner, not keratinized or clearly stratified, and absent of hair follicles and sweat glands (14).

Safety-testing protocols are typically designed to be done on exposed or partially occluded skin, and routine testing of potential irritants on the vulva itself are not logistically feasible (14). The elevated hydration of the vulvar area makes measurements difficult (29). Developed methods are, in general, less suitable to the vulvar area, and observed changes are less dramatic (28).

Permeability testing done in keratinized vulvar skin indicates that the vulva may be more permeable than other keratinized skin (48), although evidence is somewhat conflicting (14). The discrepancy may be related to the specific chemical tested and its postulated mechanism of tissue penetration. Polar molecules, surfactants, and steroids, known to have different polarities and therefore different penetration characteristics, have demonstrated sensitivity differences predicted by their chemical structures (14).

Table 4 Differences Between Keratinized Vulvar Skin and Other Regions of the Body

Characteristic	Difference in vulvar skin	Reference
Occlusion	Increased	Farage and Maibach, 2004 (14)
Permeability	Increased	Lesch et al., 1989 (66) van der Bijl et al., 1997 (67)
Friction	Increased	Elsner et al., 1990 (68)
Heterogeneity	Markedly increased	Elsner et al., 1990 (68)
Hydration	Increased	Elsner et al., 1990 (68) Erickson and Montagna, 1972 (69)
Number of hair follicles	Increased	Elsner et al., 1990 (68) Britz and Maibach, 1985 (70) Elsner and Maibach 1990 (71)
Number of sweat glands	Increased	Elsner et al., 1990 (68) Elsner and Maibach, 1990 (71)
Blood flow	Increased	Elsner et al., 1990 (68)
Innervation	Increased	Elsner et al., 1990 (72)
Capacitance	Increased	Marren et al., 1992 (73)
Baseline TEWL	Increased	Marren et al., 1992 (73)
Hydrocortisone absorption	Increased[a]	Britz et al., 1980 (36) Elsner and Maibach 1991 (27)
Reactivity to BKC	Increased[a]	Britz and Maibach, 1979 (74)
Reactivity to maleic Acid	Increased[a]	Britz and Maibach, 1979 (74)
Reactivity to SLS (low concentration)	Decreased	Elsner et al., 1991 (75)

[a]Compared specifically to forearm.
Abbreviations: BKC, benzalkonium chloride; SLS, sodium lauryl sulfate; TEWL, transepidermal water loss.

Nonkeratinized vulvar skin exhibits clearly increased permeability related to the absence of keratin and loosely packed, less-structured lipid barrier (14). In addition, the inner epithelia are thinner, representing a shorter distance to penetrate (14). Buccal tissue is often employed in a surrogate model for vulvar testing, as it has very similar structure and biochemistry (14). Buccal skin has been demonstrated to be 10 times more permeable than keratinized skin (48).

An association between facial skin reddening as a result of topical product use and the likelihood of vulvar erythema was shown in a recent study (76). The results of this study showed that individuals who presented with vulvar erythema at study enrolment reported statistically higher frequency of observable facial skin reddening with use of topical products.

Although the vulvar area may be particularly susceptible to cutaneous irritation (77), little objective published data exist on the relationship between feminine hygiene products and sensitive skin (78,79). Irritant reactions to feminine care products have been reported (73), with a few feminine products that contain chemicals known to be irritants in certain doses (20,73). However, the potential for heightened vulvar susceptibility to topical agents is not widely reported in literature (14). The contribution to irritation by topical agents though is substantial (14,48) and often underestimated (48). In fact, 29% of patients with chronic vulvar irritation were demonstrated to have contact hypersensitivity, and 94% of those were determined to have developed secondary sensitization to topical medications (73). Thus, reported sensitivity in the vulvar area often may be related to underlying contact hypersensitivity because of excessive use of topical hygienic and medicinal preparations (80).

Available bioengineering techniques are, in general, less suited for quantification of irritation in the vulvar area (28). TEWL, hydration by electrical capacitance, and pH—all invisible skin surface changes—are less sensitive in the well-hydrated environment of the vulva (28). Methods measuring inflammatory reactions are more sensitive in general than those measuring other sensitivity parameters (28) and are better used in combination than alone. The authors suggest that blood flow, pH, and color reflectance used in combination were found to be the best approach to measuring sensitivity to irritation in the vulvar area, with increased sensitivity and specificity compared with any individual assessment (28).

Safety testing must consider the potential for heightened permeability of skin in the vulvar area and increased secondary sensitization (14). Modification of risk assessment is also required, possibly by the insertion of uncertainty factors into the quantitative risk assessment (QRA)

system of risk calculation (14). Factors in range of 1 to 10 for keratinized vulvar skin and 1 to 20 for mucosal tissues have been proposed on the basis of permeability (14).

Cultural Factors

The first question that must be asked is whether a subgroup of people who have broad reactivity to personal care products genuinely exists. It has been proposed in both the popular media (81) and the medical literature (10) that the increasing incidence of sensitivity represents a "princess and the pea" effect, wherein it has become culturally fashionable to claim sensitive skin. A reported prevalence of greater than 50% in women on two separate continents (2,3) defies its perception as a minority complaint and tends to support a psychosocial component. The phenomenon is recorded in all industrial nations (2), however, and the prevalence reported in women from two continents was virtually identical [52% (2) and 51.5% (3)], lending credibility to consumer complaints supported by the observation that avoidance of products containing potential irritants can eliminate hypersensitivity (18).

Cultural factors may play a role as well. Hygiene practices are the most common cause of vulvar irritation (48). Fastidious cleansing routines (with douches, perfumes, medication, antifungal medications, and contraceptives), which often precede irritation (48), undoubtedly have some cultural component.

Environmental Factors

A majority of sensitive skin sufferers report unpleasant sensory responses to cold temperatures, wind, sun, pollution, and heat (2,7). An increased susceptibility to SLS was observed in the winter compared with the summer (17); it is known that low temperatures and humidity characteristic of winter cause lower water content in the stratum corneum (17).

Other Host Factors

Numerous other host factors that could influence skin include unusual occupational or leisure exposures to chemicals and home climate control measure (10). Long-term or excessive use (7) of personal care products can also create sensitivities. Daily topical use of corticosteroids has been demonstrated to produce fragile skin (7), and excessive use of topical medications has been demonstrated to be the source of up to 29% of vulvar dermatitis. Drug-induced sensitivity is also possible, although no reports on that issue were uncovered. Interestingly, one study found the thickness of the epidermis to be inversely proportional to the number of years that the subject had smoked ($p = 0.0001$) (34).

Another important consideration is the relationship of sensitive skin to other dermatological conditions. Atopic dermatitis (AD) is considered by many to be a possible predisposing condition (3,7). A positive relationship has been demonstrated between atopic dermatitis and stinging (7), and the density of cutaneous nerves has been demonstrated to be higher in atopic skin than in normal skin (82). Also, baseline TEWL in uninvolved skin in AD patients, which is higher than that of normal subjects (31), was shown to predict susceptibility to irritants in other sites (31). Atopy in general has been linked by some authors to the phenomenon of sensitive skin (31). Patients with respiratory atopy and active rhinoconjunctivitis were found to have increased skin susceptibility to irritants (30). It has been conjectured that alloallergens may disrupt barrier function, thereby increasing skin susceptibility (30). An association between sensitive skin and rosacea has also been postulated. In one study of rosacea patients, 64% were also found to be stinger-positive (82). Pulsed dye laser treatment of rosacea was demonstrated to result in decreased stinging (82).

DISCUSSION

The goal of premarket safety testing is to avoid unexpected consumer effects to marketed products (20). Skin testing is typically conducted tier-wise with increasing robustness (14); such testing combined with judicious product formulation lends confidence to market release (14). Recently, however, we have seen that safety-testing methods may not be robust enough (13). Consumers discriminated between products on the basis of how they felt during use, basing product preferences on perceived effects not predicted by premarket testing (13).

Methods capable of detecting very subtle skin benefits or potential for adverse effects are needed. Testing has been conducted primarily on normal subjects, bringing into question the need to focus on examining populations that may be inherently more sensitive to irritant effects (14). Limited studies that enrolled only subjects shown to have sensitive skin did find better correlation (78,79).

Few studies have been performed in parallel and fewer still with a multiple-irritant panel. Effective testing will require multiple regimes to identify truly sensitive people (1). A sensitive skin panel must be approached with great caution (8), however, and must define relevant exposures, limit confounding factors, and include irritants of different mechanisms. Correlation between sensory and objective data may be associated primarily with higher levels of exposure (4). In addition, current differences reported in SLS response may be related to the fact that two different forms of different irritant potentials have been employed (17).

At present, associations between observed reactivities are weak (10) and underlying pathophysiological factors poorly understood (18). Although it is clear that specific individuals have heightened sensitivity to different kinds of sensory and physical irritants, observed reactions are not predictive of generalized sensitivity, and the relationship between observed sensitivities is cloudy (8,18). Recent evidence suggests that sensitive skin may not be a single condition, but one that encompasses different categories of subjects and sensitivities on the basis of different mechanisms (9).

Sensory differences may be related to innervation (42). Dermal nerve fibers extend throughout viable epidermis as free nerve endings, but the epidermal component of this network is still poorly characterized (42). Epidermal nerve density variation could explain the different sensitivity thresholds in various anatomical sites (22). Although no differences in innervation have yet been observed (42), little research on this mechanism has been performed.

Barrier function has been shown to be a critical component of skin discomfort (11,18). The permeability barrier in the stratum corneum requires the presence of well-organized intracellular lipids (7,18) and depends highly on lipid composition (16). Increased neutral lipids and decreased sphingolipids are associated with superior barrier properties (16). Irritation results from the abnormal penetration in the skin of potentially irritating substances and a resulting decrease in the skin tolerance threshold (7). A weak barrier inadequately protects nerve endings and facilitates access to antigen-presenting cells, a mechanism that would support an association with atopic conditions (18).

The lipid content of the stratum corneum has been shown to be a more accurate predictor of skin permeability than stratum corneum thickness or cell number (16). Alterations of baseline capacitance values imply barrier impairment and support the view that hyper-reactivity to water-soluble irritants results from increased absorption (9).

Subclinical irritation may be the key to understanding sensitive skin (4). Sensations elicited by treatment with different products are generally discerned before observable differences (4). Visual irritation tests by definition measure lasting effects, while sensory effects are immediate (15). There is also indication that the skin has been damaged histologically before visible signs of inflammation or skin dryness. TEWL levels have been shown to increase without objective irritation (4), as has the release of inflammatory mediators (4,37). These findings have led to the hypothesis that clinical signs occur only when the threshold level of irritation is exceeded (4).

CONCLUSION AND RECOMMENDATIONS

Global marketing seeks to provide safe and useful products to an audience with tremendous potential differences in race, age, sex, skin type, culture, habits, and practical use of marketed product (5). It has become evident recently that sensory effects not predicted by current premarket testing are the main purchasing criteria of the consumer. An objective for improved testing would be the identification of a sensitive skin panel in which subjective data consistently correlated with objective data (1) and which includes irritants of different mechanisms and receptor types. Larger study populations are needed to overcome individual variability to obtain reproducible results (14). Tools to further exaggerate exposures, enhance ability to clinical score irritation (visual or via instrumentation), and identify new objective endpoints for subjective sensory effects are also needed (13). The challenge of the future is to

clarify the still murky correlation between self-perceived consumer sensory irritation and objective indications of clinical irritation, a correlation that is to date absent from the published literature (1).

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7 Neurophysiology of Self-Perceived Sensitive-Skin Subjects by Functional Magnetic Resonance Imaging

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INTRODUCTION

The diagnosis of sensitive skin is defined by neurosensory hyperreactivity of the skin and is essentially based on self-perceived sensations of people who report facial skin discomfort as stinging, burning, and itching when their skin is exposed to some environmental factors (wind, sun, or pollution) or after application of topical products (hard water, soap, or cosmetics) (1–3). Epidemiological studies performed on large populations have shown that about 50% of women declare that they have self-perceived sensitive skin (SPSS), and 10% fall into the category "very sensitive" (4). Similar percentages have been obtained in different populations: African Americans, Asians, Caucasians, or Hispanics (5). SPSS is lower in the male population (30%) and tends to decrease with age (4,6).

Even if reported, adverse reactions could be the very first symptoms of an irritant contact dermatitis (7), sensitive skin is not a pathological disorder (8).

This chapter will first present a short review of the different approaches for assessing sensitive skin. Then we will present in detail a new approach based on the analysis of the pattern of brain activation in self-assessed sensitive-skin subjects compared with nonsensitive-skin subjects using functional magnetic resonance imaging (fMRI).

TESTS AVAILABLE: A REVIEW

Psychophysical tests were proposed to measure the chemosensory response of the skin after application of lactic acid or capsaicin, for instance (9–11). With constant stimulation (for instance, a 10% lactic acid product as the stimulus), it has been shown that there was a statistically significant difference in the global degree of discomfort combining the sensations of stinging, burning, and itching, allowing two populations of subjects to be defined. A first group, characterized by low scores can be classified as subjects with nonsensitive skin, while a second group, characterized by high scores, can be classified as subjects with sensitive skin. However, these psychophysical tests are still based on the subject's self-perceived response.

A slightly modified procedure to the lactic acid stinging test proposed in 1977 (8) is nowadays the most widely used. However, it has been reported that it does not fully render the complexity of self-assessed sensitive skin, as illustrated by the discrepancy between lactic acid response and self-perception of sensitive skin (12–14). In 2000, this difference was taken into account for the recommendation to include "stingers" with a concomitant self-declared sensitive skin as panelist for safety testing (13).

Owing to the great similarity of symptoms induced by topically applied capsaicin to those associated with sensitive skin (10), a new elicitation test using a 0.075% emulsion of a pungent component extracted from chili peppers was proposed (11,15). Topical application of capsaicin leads to a short release of neuropeptides (substance P, CGRP) from peripheral nerve endings and causes the appearance of uncomfortable sensations. Authors reported that unpleasant reactions are more intense as also more frequent in SPSS subjects.

All these provocative tests are based on the quantification of the degree of discomfort in response to a defined stimulation (10% lactic acid or 0.075 capsaicin). In psychophysics, an

alternative method is based on detection threshold. This procedure has been tested recently (16) and consisted in attaining the detection threshold of topically applied capsaicin. Five capsaicin concentrations were used in 10% ethanol aqueous solution (3.16×10^{-5}%, 1.0×10^{-5}%, 3.16×10^{-4}%, 1.0×10^{-4}%, and 3.16×10^{-3}%). This new test of skin neurosensitivity which is easy, quick, and painless, appears to be promising for the diagnosis of sensitive skin; and could also provide a basis for the assessment of modulators of skin neurosensitivity.

In 1998, another psychophysiological test based on the assessment of peripheral sensitivity to thermal stimuli was suggested as a possible diagnosis of sensitive skin (17). Two recent studies reported contradictory results, which could indicate that differences in thermal sensitivity were too weak to consider this thermal indicator as an accurate predictive indicator of sensitive skin (16,18).

As both epidemiological surveys and psychological tests are partly subjective as these approaches are based on the verbal response of the volunteers, some authors have used noninvasive methods to analyze skin properties such as transepidermal water loss, skin hydration, or skin color. Instrumental measurements do not show large differences between subjects with sensitive skin and those with nonsensitive skin, even if some alteration of the barrier function in people with sensitive skin has been reported by some authors (14,19,20).

BRAIN PATTERN ANALYSIS OF SENSITIVE-SKIN SUBJECTS BY fMRI

Rationale

Our knowledge on sensitive skin shows us that it is not easy to assess because it mainly lacks visible, physical, or histological measurable signs, and such phenomenon has even led some authors to question the reality of this skin condition (21). However, when people report the subjective perception of discomfort or low painful sensations, it should be informative to study the responses of those with sensitive skin and those with nonsensitive skin during the final step of integration of the information, which takes place in the central nervous system. Regarding this topic, most studies have concerned the processes in the central nervous system of nociceptive information, such as pain perception, to describe the neural bases of pain intensity. More recently, some studies have analyzed a more subjective aspect of pain perception, including feelings of unpleasantness and emotions associated with future implications, termed "secondary affect" (22,23). Some authors have studied less severe sensations than pain such as itch, and reported activation of some similar structures as described for pain (24–26).

The aim of the study, detailed in the next paragraphs, was to assess brain activation during a provocation test involving very slightly painful stimulation and a feeling of discomfort, in two groups of subjects classified as sensitive skin or nonsensitive skin.

Materials and Methods

Subjects

After informed consent, 18 healthy young women (mean age: 33 ± 9 years) participated in this study, which was approved by the hospital ethics committee. The main inclusion criteria were absence of dermatological, neurological, or vascular condition affecting the face, nonuse of topical or systemic treatments that might interfere with the results of the test, and no contraindications to MRI.

Nine of them were classified as having sensitive skin and nine as having nonsensitive skin, based on their responses to the questionnaire described in the following section.

Questionnaire

To maximize differences between the two groups, subjects were required to have a response profile highly characteristic of sensitive skin on the questionnaire (Table 1). Sensitive skin was characterized by the cutaneous reaction to topical applications and to environmental factors.

Answers to the 13 questions were actually used to allocate groups. The following subjects were considered as having sensitive skin: those answering "yes" to two of the first three questions (sensitive skin, reactive skin, and irritable skin), yes to three of the four questions on skin reaction to cosmetics (questions 4–7), and yes to three of the six questions on the

Table 1 Sensitive-Skin Questionnaire with Frequencies of Positive Responses for Both Groups

Questionnaire	Sensitive skin ($n = 9$)	Nonsensitive skin ($n = 9$)
1. Do you regard yourself as having a sensitive facial skin?	100%	0%
2. Do you consider yourself as having a facial skin prone to irritation?	89%	0%
3. Do you consider yourself as having a reactive[a] facial skin?	100%	0%
4. Do you avoid certain cosmetics, which you feel may cause your facial skin to react[a]?	100%	0%
5. Do you consider that your facial skin reacts[a] readily to cosmetics or toiletries?	89%	0%
6. Do some cosmetics or toiletry products make your facial skin itch, sting, or burn?	100%	0%
7. Have you ever experienced an adverse reaction on your face to a cosmetic or toiletry product?	100%	0%
8. Does the expression "does not tolerate cold weather or a cold environment" apply to your facial skin?	89%	0%
9. Does the expression "does not tolerate hot weather or a hot environment" apply to your facial skin?	78%	0%
10. Does the expression "does not tolerate fast changes in temperature" (e.g., going into a warm shop from a cold street) apply to your facial skin?	100%	0%
11. Does going out in the wind cause your facial skin to itch, burn, or sting?	56%	0%
12. Does going out in the sun cause your facial skin to itch, burn, or sting?	67%	0%
13. Does your facial skin react[a] to air pollution?	56%	0%

[a]Stinging, burning, and/or itching sensations with or without redness.

environment (questions 8–13). In contrast, subjects who answered no to the 13 questions were classed as having nonsensitive skin. Table 1 shows the frequency of yes answers to the 13 questions in both groups. The table shows that the two groups were very different with regard to the auto-evaluation of skin sensitivity.

Task

Before the MR examination, it was clearly explained to the volunteers what would happen in the scanner and what they would be asked to do. It consisted of simultaneous application to the face of two products described as "likely to induce discomfort." Volunteers did not know that the lactic acid product was applied on the right side of their face (single-blind protocol).

During the MR acquisition, whenever they saw an arrow on the screen, subjects were asked to press the 4-position keyboard to report the level of discomfort perceived on the left side of the face when the arrow was pointing to the left and on the right side of the face when the arrow was pointing to the right. Particular attention was taken to check that all the subjects had the same understanding of the global degree of discomfort corresponding to the cumulative effect of stinging, burning, and itching.

A 4-level rating system was used:

1. 0: no or very slight discomfort
2. 1: slight discomfort
3. 2: moderate discomfort
4. 3: severe discomfort

fMRI protocol

Three-dimensional MR images were first acquired to have the exact brain anatomy for each subject. Then products A and B were applied simultaneously on the nasolabial folds for

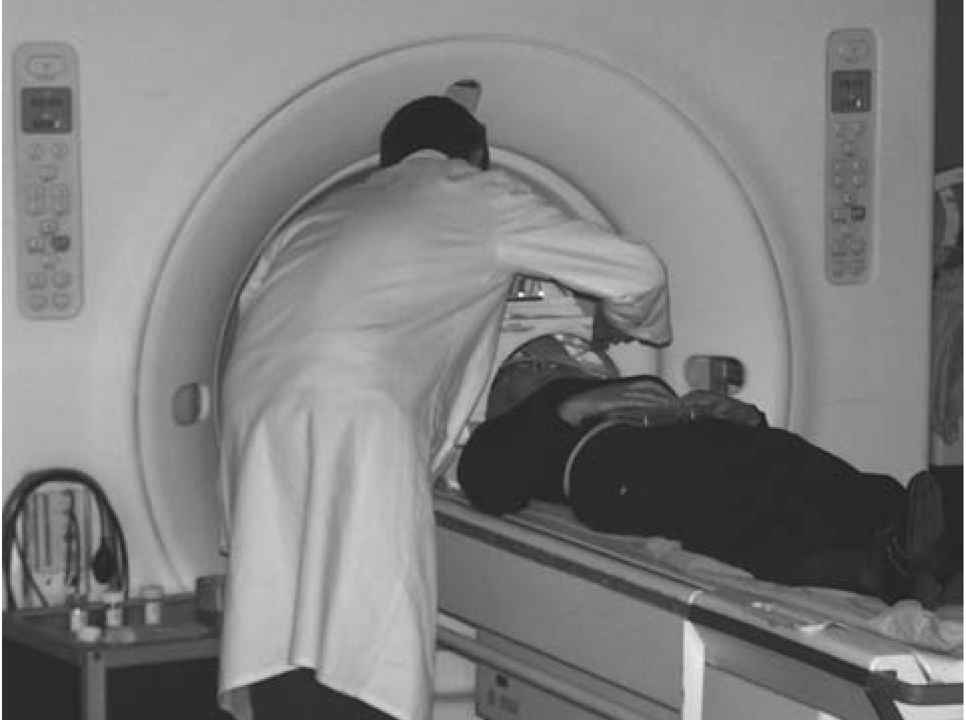

Figure 1 Lactic acid and saline solution as control were simultaneously applied to the nasolabial areas with a cotton wool bud. The subject's hand was on the 4-position keyboard to quantify the degree of discomfort induced by the products during the MR acquisitions. *Abbreviation:* MR, magnetic resonance.

10 seconds (Fig. 1), and fMRI acquisition (echo-planar imaging sequence) started immediately and consisted of following brain activation every 3 seconds during 10 minutes.

Results

Self-Assessment Results

A mean cumulative degree of discomfort was calculated for each group and each product and confirmed a statistically significant increase of discomfort on the side where the lactic acid was applied compared with the saline-solution side. The difference was greater in the sensitive-skin group.

We report (Fig. 2) the mean kinetic curve of discomfort for each condition.

The time intervals between 0 and 80 seconds and from 480 to 640 seconds were classified as a low- or null-discomfort period, while the phase between 80 and 480 seconds was classified as a medium- or high-discomfort period.

We used these results to construct the fMRI time contrast, as fMRI can only analyze brain activation by varying only one condition, which is in this protocol: the degree of discomfort.

fMRI Results

Brain activation when the arrow was pointing to the control side (saline solution). Figures 3A and B present mean activation maps for both groups corresponding to periods of time when subjects responded looking at the arrow pointing to the left (saline solution). It can be seen on the 3-D images that no activation was detected in any part of the brain. However, at least the visual cortex should have been activated as subjects received visual stimuli (the arrow projected on the screen), and the motor cortex should have been activated as subjects pressed the keyboard to rate the degree of discomfort. As the central phase was compared with the beginning and end phases of the time period, activation was stable over time, so that no difference was detected related to time for the visual and motor tasks, which were constant during the acquisition time.

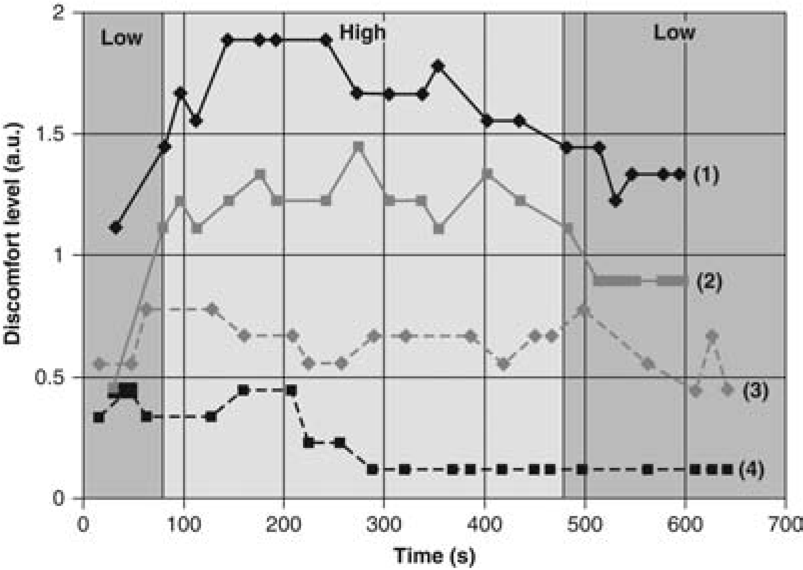

Figure 2 Kinetics of discomfort for both groups and for the two products. These curves were used to construct the fMRI contrast by differentiating a phase from 80 to 480 seconds corresponding to a high degree of discomfort, a phase from 0 to 80 seconds and a phase from 480 to 640 seconds corresponding to a low degree of discomfort. (1) Lactic acid (10%) on sensitive-skin subjects; (2) Lactic acid (10%) on nonsensitive-skin subjects; (3) Saline solution on sensitive-skin subjects; (4) Saline solution on nonsensitive-skin subjects. *Abbreviation:* fMRI, functional magnetic resonance imaging.

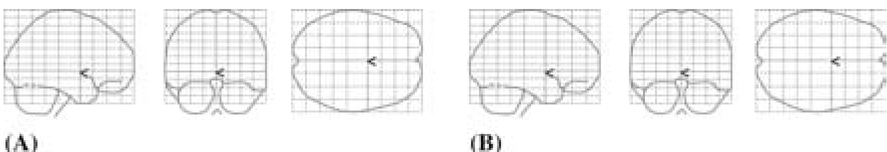

Figure 3 Brain activation maps obtained by fMRI. Saline solution as control. (**A**) Subjects with nonsensitive skin; (**B**) Subjects with sensitive skin. No changes in brain activation were observed as a function of time. Visual and motor stimuli were stable during the acquisition time. *Abbreviation:* fMRI, functional magnetic resonance imaging.

Brain activation when the arrow was pointing to the stimulated side (lactic acid solution). Figures 4A and B present the mean activation maps for both groups during periods pointing to the right (the lactic acid solution). In the nonsensitive skin group, most of the activated pixels were located in the left primary area of the sensory cortex (first step of the cortical pathway). Other small areas of activation can be seen in associated areas.

In the sensitive-skin group, the mean activated maps were very different. There was considerable activation in the left primary sensory area, and considerable bilateral activation in the sensory cortex and in the prefrontal cortex, as well as some activation in deeper structures located in the limbic system (Fig. 4B-inset).

Discussion

The results of subjective data (self-perceived clinical signs) from the lactic acid test in a limited number of subjects were consistent with the results in the literature obtained in a greater number of subjects (27,28). In both groups, the discomfort rating was higher in subjects with sensitive skin, and the kinetics were comparable over about 10 minutes, with rapid onset of discomfort and a perceptible decrease after 7 to 8 minutes. It is also important to relate this to the capacity to lateralize the discomfort perceived in the two facial zones, which were only separated by a few centimeters.

During responses concerning the control saline solution applied to the left side of the face (Fig. 3), no cerebral activation changing with time was observed in either group. However, throughout the acquisition, subjects saw the luminous arrow, which activated areas of the visual cortex and had to press the keyboard to give their responses, which activated areas of

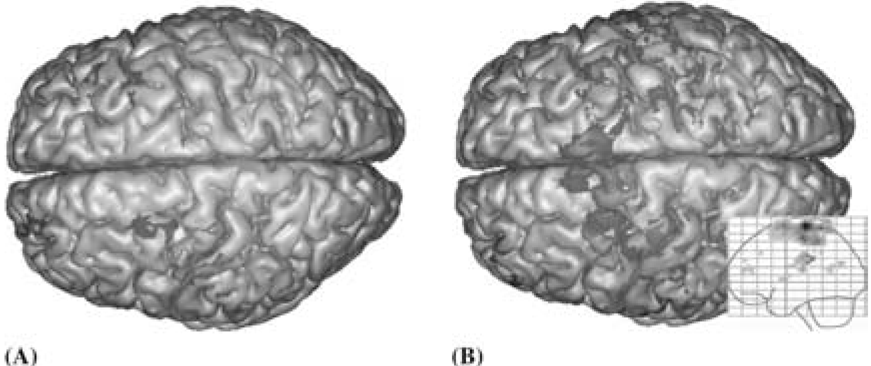

Figure 4 Brain activation maps obtained by fMRI. Lactic acid as a provocation test. (**A**) Subjects with nonsensitive skin; (**B**) Subjects with sensitive skin. Nonspecific activation was recorded in both groups in the primary contralateral sensory cortex, which can be considered as the first cortical pathway of this type of sensory perception. Bilateral extensions in the sensory cortex and the prefrontal cortex. Inset activation in internal structures, such as in cingulate cortex, was specific to the sensitive-skin group. *Abbreviation:* fMRI, functional magnetic resonance imaging.

the motor cortex. It can clearly be seen that there was no difference during the two phases chosen, since these stimuli were constant during the recording. The control recording demonstrates that the activation maps corresponding to perception of discomfort with lactic acid can be interpreted with confidence, based on the only stimulation changing over time in the protocol: the degree of discomfort.

In the group with nonsensitive skin, cerebral activation was essentially located in the left primary somatosensory area of the cortex. Since the afferent nerve fibers cross in the spinal cord, controlateral activation corresponds to the first step in neural treatment of the stimulation. Other activations, in very small areas, are more difficult to interpret. In the group of subjects with sensitive skin, cerebral activation maps present a very different pattern. As the first step of cortical integration, there was considerable activation of the primary area of the left sensory cortex, as in the group with nonsensitive skin. Bilateral extensions in the sensory cortex and the prefrontal cortex, together with activation of the subcortical areas (the cingular cortex) showed multidimensional perception of the sensation. These activations may be interpreted as the consequence of attention, emotion, and possibly planning the action in response to the unpleasant sensation induced by the stimulation particularly felt by subjects with sensitive skin.

As a consequence, these fMRI results contribute to reinforcing the confidence in self-assessment results, since groups differentiated on the basis of the questionnaire present different cerebral activation maps, and the contrast needed for the fMRI to compare two situations (presence/absence of discomfort) was based on the subjects' feelings in the MRI scanner and measured using a keyboard.

CONCLUSION

Although fRMI could not be considered as a tool to evaluate efficiency in routine products on SPSS subjects, the results we have reported here are of great interest in this field. The different brain activation observed with fMRI, between high SPSS subjects and none, is clearly reinforcing the neural pattern for this disorder.

In addition, it is of importance to observe that with the questionnaire we have developed all along the study we have conducted, we can select subjects with different neurophysiologic patterns as demonstrated by fRMI. Consequently, with this very simple mean we could get pertinent phenotypes regarding sensitive skin.

Finally, we also have to underline that the activated brain areas are those that are usually involved in the painful process. Everything occurs on SPSS subjects as if the threshold to feel discomfort of the skin is lower than the one for SPSS subject. The origin of this low threshold could be linked to specific central nervous system patterns, to peripheric neural patterns, or also to both. New studies are still needed to answer these questions.

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8 Tests for Sensitive Skin

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INTRODUCTION

Sensitive skin is a condition of subjective cutaneous hyperreactivity to environmental factors or topically applied products. The skin of subjects experiencing this condition reacts more easily to cosmetics, soaps, and sunscreens and often enhance worsening after exposure to dry and cold climate.

Sensitive skin and subjective irritation are widespread since the use of cosmetics is increasing in economically advanced countries.

The frequent use of preservatives, perfumes, emulsifiers, and plant extracts enhance the risk of adverse local reactions.

Signs of discomfort as itching, burning, stinging, and a tight sensation are commonly present, associated or not associated with erythema and scaling.

Generally, substances that are not commonly considered irritants are involved in this abnormal response. They include many ingredients of cosmetics such as dimethyl sulfoxide, benzoyl peroxide preparations, salycilic acid, propylene glycol, amyldimethylaminobenzoic acid, and 2-ethoxyethyl methoxycinnamate (1). The unpleasant sensations appear to be associated with the stimulation of cutaneous nerve endings specialized in pain transmission, called nociceptors.

Some authors (2) hypothesized a correlation between sensitive skin and constitutional anomalies and/or other triggering factors such as occupational skin diseases or chronic exposure to irritants; others (3) supported the fact that no constitutional factors play a role in the pathogenesis of sensitive skin, though the presence of dermatitis demostrates a general increase in skin reactivity to primary irritants, which lasts for months.

In different epidemiological surveys, the correlation between sensitive skin with sex, race, skin type, and age has been studied. No sex-related significant differences have been found in the reaction pattern.

Some authors (4–6) documented a higher reactivity to irritants mostly in females, some others noted that male subjects were significantly more reactive than female (7), but other experimental studies did not confirm these observations (8,9).

Conflicting data were also reported on skin sensitivity among races: although blacks seem to be less reactive and Asians more reactive than Caucasians, data rarely reach statistical significance (10); recently, Arakami found significant subjective sensory differences between Asian and Caucasian women but no differences after sodium lauryl sulfate (SLS) testing, concluding that stronger sensations in Asians can reflect a different cultural behavior rather than measurable differences in skin physiology (11).

Studying the correlation between skin reactivity and skin type, subjects with skin type I were found to be more prone to develop sensitive skin (12); most common "stingers" were reported to be light-complexioned persons of celtic ancestry who sunburned easily and tanned poorly (13).

Moreover, skin reactivity tends to decrease with age: by testing croton oil, cationic and anionic surfactants, and weak acids and solvents, less severe skin reactions were observed in older subjects (14). Robinson, by testing sodium dodecyl sulphate, decanol, octanoic acid, and acetic acid, confirmed this lower reactivity in the older age cluster of subjects (15).

Aged skin seems to have a reduced inflammatory response either to irritants or to irritation induced by UV light (16,17). However, skin reactivity of women at the beginning of the menopause is increased, suggesting a role of estrogen deficiency on the observed impairment of skin barrier function (18).

TESTS FOR SENSITIVE SKIN

Clinical Parameters

It is difficult to find accurate parameters for categorizing skin as sensitive or nonsensitive; this condition often lacks visible, physical or histological, measurable signs. Subjects with subjective irritation tend to have a less hydrated, less supple, more erythematous and more teleangiectatic skin, compared with the normal population. In particular, significant differences were found for erythema and hydration/dryness (19). Tests for sensitive skin are generally based on the report of sensation induced by topically applied chemicals. Consequently, the use of self-assessment questionnaires is a valuable method to identify "hyperreactors" (6) and a useful tool for irritancy assessment of cosmetics (20).

SENSORY TESTING METHODS

Psychophysical tests based on the report of sensation induced by topically applied chemical probes have been increasingly used to provide definite information on sensitive skin. These methods of sensory testing can be validated by the use of functional magnetic resonance imaging (fMRI), which represent one of the most developed forms of neuroimaging. This technique measures changes in blood flow and blood oxygenation in the brain, closely related to neural activity manifested as sensory reaction.

When nerve cells are active, they consume oxygen carried by hemoglobin in red blood cells from capillaries. The local response to this oxygen use is an increase in blood flow to regions of increased neural activity, occurring after a delay of approximately one to five seconds. This hemodynamic response rises to a peak over four to five seconds, before falling back to baseline (and typically undershooting slightly). This leads to local changes in the relative concentration of oxyhemoglobin and deoxyhemoglobin and changes in local cerebral blood volume in addition to changes in local cerebral blood flow (21).

Quantitation of Cutaneous Thermal Sensation

In dermatology, thermal sensation testing analysis is the most used quantitative sensory testing (QST) technique (22). It assesses function in free nerve endings and their associated small myelinated and nonmyelinated fibers. This method enables quantitative measurement of the threshold for warm and cold sensation as well as hot and cold pain.

A small device, called thermode, based on Peltier elements, is in contact with the subject's skin. It consists of semiconductor junctions, which produce a temperature gradient between the upper and lower stimulator surfaces produced by an electrical current. In the center of the thermode, a thermocouple records the temperature.

TSA 2001® (Medoc company, Ramat Yshai, Israel) is considered one of the most advanced portable thermal sensory testing devices.

Basically, it measures the hot or cold threshold and the suprathreshold pain magnitude (Table 1).

TSA operates between 0°C and 54°C. The thermode in contact with the skin produces a stimulus whose intensity increases or decreases until the subject feels the sensation.

As the sensation is felt, the subject is asked to press a button. The test is then repeated two more times to get a mean value. Using this method, artefacts can occur because of the lag time the stimulus needs to reach the brain. This inconvenience can be avoided by using relatively slow rates of increasing stimuli.

The stimulus can also be increased stepwise, and the subject is told to say whether or not the sensation is felt. When a positive answer is given, the stimulus is decreased by one-half the

Table 1 Thermal Sensory Test

Parameters monitored	Sensory fibers
Warm sensation	C fiber (1–2°C above adaptation temperature)
Cold sensation	A-δ fibers (1–2°C above adaptation temperature)
Heat-induced pain	Mostly C fiber (45°C)
Cold-induced pain	Combination of both C- and A-δ fibers (10°C)

initial step and so on, until no sensation is felt. The subject's response determines the intensity of the next stimulus. The limitation of this second method is that a longer performance time is required.

Stinging Test

Stinging test represents a method for the assessment of skin neurosensitivity. Stinging seems to be a variant of pain that develops rapidly and fades quickly anytime the appropriate sensory nerve is stimulated. The test relies on the intensity of stinging sensation induced by chemicals applied on the nasolabial fold (13). The procedure differs depending on the chemical used.

Lactic Acid

After a 5- to 10-minute facial sauna, an aqueous lactic acid solution (5% or 10% according to different methods) is rubbed with a cotton swab on the test site, while an inert control substance, such as a saline solution, is applied to the contralateral test site. After application, within a few minutes, a moderate-to-severe stinging sensation occurs for the "stingers group." Subjects are then asked to describe the intensity of the sensation using a point scale. Hyperreactors, particularly those with a positive dermatologic history, have higher scores. Using this screening procedure, 20% of the subjects exposed to 5% lactic acid in a hot, humid environment were found to develop a stinging response (13). Lammintausta et al. confirmed these observations (23) identifying in his study 18% of subjects as stingers. In addition, stingers were found to develop stronger reactions to materials causing nonimmunologic contact urticaria and to have increased transepidermal water loss (TEWL) and blood flow velocimetry values after application of an irritant under patch test.

Capsaicine

An alternative test involves the application of capsaicin. Recently, a new procedure assessed by l'Oreal Recherche (24) appears to be more accurate and reliable for the diagnosis of sensitive skin. After a facial cleansing, five increasing capsaicine concentrations in 10% ethanol aqueous solution (3.16×10^{-5}%; 1×10^{-4}%; 3.16×10^{-4}%; 1×10^{-3}%; and 3.16×10^{-3}%) are applied on the nasolabial folds. The application of the vehicle alone serves as control and to exclude subjects who feel any sensation of discomfort prior to capsaicine application. The formulation of capsaicine in hydroalcoholic solution accelerates the action of capsaicin on the face in comparison with the previously used 0.075% capsaicine emulsion, without being associated with painful sensation.

The capsaicine detection thresholds are more strongly linked to self-declared sensitive skin than the lactic acid stinging test.

Dimethylsulfoxide

The alternative application of 90% aqueous dimethylsulfoxide (DMSO) has not the same efficacy of lactic acid or capsaicine stinging test and, after application, intense burning, tender wheal, and persistent erythema often occur in stingers.

Nicotinate and Sodium Lauryl Sulfate Occlusion Test

A different approach to identify sensitive skin relies on vasodilation of the skin as opposed to cutaneous stinging. Methyl nicotinate, a strong vasodilator, is applied to the upper third of the ventral forearm in concentrations ranging from 1.4% to 13.7% for a 15-second period. The vasodilatory effect is assessed by observing the erythema and the use of laser Doppler velocimetry (LDV). Increased vascular reaction to methyl nicotinate was reported in subjects with sensitive skin (25). Similar analysis can be performed following application of various concentrations of SLS.

Evaluation of Itching Response

Itchy sensation seems to be mediated by a new class of C fibers with an exceptionally lower conduction velocity and insensivity to mechanical stimuli (26).

Indeed, no explanation of the individual susceptibility to the itching sensation without any sign of coexisting dermatitis has been found. Laboratory investigations have also been limited.

An itch response can be experimentally induced by topical or intradermal injections of various substances such as proteolytic enzymes, mast cell degranulators, and vasoactive agents.

Histamine injection is one of the more common procedure: histamine dihydrochloride (100 μg in 1 mL of normal saline) is injected intradermally in one forearm. Then, after different time intervals, the subject is asked to indicate the intensity of the sensation using a predetermined scale, and the duration of itch is recorded. Information is always gained by the subject's self-assessment.

A correlation between whealing and itching response produced by applying a topical 4% histamine base in a group of healthy young females has been investigated (14). The itching response was graded by the subjects from none to intense. The data showed that the dimensions of the wheals do not correlate with pruritus. Also, itch and sting perception seem to be poorly correlated.

The cumulative lactic acid sting scores were compared with the histamine itch scores in 32 young subjects; all the subjects who were stingers were also moderate-to-intense itchers, while 50% of the moderate itchers showed little or no stinging response (14).

Furthermore, the histamine-induced itch sensation decreases after topically applied aspirin (27). This result can be attributed to the role that prostaglandines play in pain and itch sensation (28).

Localized itching, burning, and stinging can also be features of nonimmunologic contact urticaria, a condition characterized by a local wheal and flare after exposure of the skin to certain agents. Non-antibody-mediated release of histamine, prostaglandins, leukotriens, substance P, and other inflammatory mediators may likely be involved in the pathogenesis of this disorder (29). Several substances such as benzoic acid, cinnamic acid, cinnamic aldehyde, and nicotinic acid esters are capable of producing contact nonimmunologic urticaria and eliciting local edema and erythematous reactions in half of the individuals. Provocative tests are based on an open application of such substances and well reproduce the typical symptoms of the condition.

Washing and Exaggerated Immersion Tests

The aim of these tests is to identify a subpopulation with an increased tendency to produce a skin response.

In the washing test (30), subjects are asked to wash their face with a specific soap or detergent. After washing, individual sensation for tightness, burning, itching, and stinging is evaluated using a point scale previously determined.

The exaggerated immersion test is based on soaking the hands and forearms of the subjects in a solution of anionic surfactants (such as 0.35% paraffine sulfonate, 0.05% sodium laureth sulfate-2EO) at 40°C for 20 minutes.

After soaking, hands and forearms are rinsed under tap water and patted dry with a paper towel. This procedure is repeated two more times, with a two-hour period between each soaking, for two consecutive days. Prior to the procedure, baseline skin parameters are evaluated. The other evaluations are taken 2 hours after the third and sixth soaking and 18 hours after the last soaking (recovery assessment). All of the skin parameters are performed after the subjects have rested at least 30 minutes at 21°C ± 1°C.

BIOENGINEERING TESTS

Physiologic changes indicative of sensitive skin can be detected at low levels prior to clinical disease presentation by using noninvasive bioengineering tests.

Transepidermal Water Loss

TEWL is used to evaluate water loss that is not attributed to active sweating from the body through the epidermis to the environment and represents a marker of stratum corneum barrier function. TEWL assessment can be performed using different techniques (closed chambers method, ventilate chambers method, and open chambers method). Measurements are based on the estimation of water pressure gradient above the skin surface. The open chambers instruments consist of a detachable measuring probe connected by a cable to a portable main signal-processing unit. The probe is provided with chambers open at both ends with relative

humidity sensors (hygrosensors) paired with temperature sensors (thermistors). TEWL values ($g\ m^{-2}\ hr^{-1}$) are calculated by the signal processing units in the probe handle and main unit and are digitally displayed. The closed chamber instrument consists of a closed cylindrical chamber containing the sensors. The humidity sensor based on a thin-film capacitative sensor is integrated to a handheld microprocessor-controlled electronic unit provided with a digital readout for the TEWL value (31,32).

Corneometry

The corneometry is a method to measure stratum corneum water content (electrical measurements).

The instrument consists of a probe that should be placed to a hair-free skin surface with slight pressure. It is described as being a "capacitance"-measuring device, operating at low frequency (0.95–1.05 MHz), which is sensitive to the relative dielectric constant of material in contact with the electrode surface. In about 20 milliseconds, it estimates water content of the stratum corneum to an approximate depth ranging between 60 and 100 mm, using arbitrary units.

The presence of salts or ions on the skin surface can affect the reading.

Laser Doppler Velocimetry

A monochromatic light from a helium-neon laser is transmitted through optical fibers to the skin. The light is reflected with Doppler-shifted frequencies from the moving blood cells in the upper dermis at the depth of $\sim$1 mm. The LDV extracts the frequency-shifted signal and derives an output proportional to the blood flow. LDV is useful to evaluate the degree of skin irritation (33).

Colorimetry

Surface color may be quantified using the Commission Internationale de L'Eclairage (CIE) system of tristimulus values. The device uses silicon photocells. The measuring head of these units contains a high-power-pulsed xenon arc lamp, which provides two CIE illuminant standards. The color is expressed in a three-dimensional space. The coordinates are expressed as L* (brightness) a* value (color range from green to red) and b* value (color range from blue and yellow). The a* value, related to skin erythema, increases in relation to irritation and skin damage.

Corneosurfametry

This method (34) investigates the interaction of surfactants with the human stratum corneum. It is performed as follows: cyanoacrilate skin surface stripping (CSSS) is taken from the volar aspect of the forearm and sprayed with the surfactant to be tested. After two hours, the sample is rinsed with tap water and stained with basic fuchsin and toluidine blue dyes for three minutes. After rinsing and drying, the sample is placed on a white reference plate and measured by reflectance colorimetry (Chroma Meter® CR200, Minolta, Osaka, Japan).

The index of redness (CIM = Luminacy L* – Chroma C*) is taken as a parameter of the irritation caused by the surfactant. This index has a value of 68 $\pm$ 4 when water alone is sprayed on the sample and decreases when surfactant is tested, with stronger surfactants lowering the values.

Piérard et al. (35), testing different shampoo formulations in volunteers with sensitive skin, demonstrated that corneosurfametry correlates well with in vivo testing. A significant negative correlation ($p < 0.001$) was found between values of colorimetric index of mildness (CIM) and the skin compatibility parameters (SCPs) that include a global evaluation of the colorimetric erythemal index (CEI) and the TEWL differential, both expressed in the same order of magnitude.

In the same study, corneosurfametry showed less interindividual variability than in vivo testing, allowing a better discrimination among mild products.

An interesting finding showed that sensitive skin is not a single condition. Goffin (36) hypothesized that the response of the stratum corneum to an environmental threat might be impaired in different groups of subjects experiencing sensitive skin. Data of the corneosurfametry performed after testing eight different house cleaning products showed that the overall stratum corneum reactivity, as calculated by the average values of the corneosurfametry index

(CSMI) and the CIM, is significantly different ($p < 0.01$) between detergent-sensitive skin and both nonsensitive and climate/fabric-sensitive skin, as well.

Irregularity Skin Index

Irregularity skin index (ISI) can contribute to the identification of subjects with sensitive skin.

In a recent study (37) conducted on 243 subjects positive to the lactic acid stinging test, slides of cyanoacrylate skin surface stripping (CSSS), obtained from the volar aspect of the forearm, were examined by means of a computer-assisted fast Fourier transform (FFT) to determine the skin surface micro-relief. Acquisition of the images was performed by a stereomicroscope connected to an analogic video camera. The results confirmed a significant correlation ($p < 0.001$) between intensity of symptoms in "stingers" and ISI. This procedure represents a valuable and promising tool for the study and diagnosis of sensitive skin.

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9 Mechanisms of Skin Hydration

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INTRODUCTION

One of the main functions of the skin is to maintain a competent barrier to water loss (Table 1). Water is continuously lost from the outermost skin layers to the atmosphere (evaporative water loss); and to control the rate of water loss, the barrier integrity must be preserved. Maintaining the barrier to water loss is important since hydration affects the skin's appearance, mechanical properties, and cell signaling processes (1–9). The barrier integrity can be compromised by chemical insult (e.g., the use of surfactant-containing cleansing products or harsh chemicals), mechanical insult, dry relative humidity conditions, and sun exposure (10–16).

There are several excellent review articles discussing stratum corneum structure, biochemical processes, and the importance of maintaining well-hydrated skin (17–25). In this chapter, we will build on these reviews with data pertaining to the importance of cleansing with mild products, adaptability of the skin to changing environments, effect of excess water exposure, and influence of diet on skin hydration. This chapter begins by examining the skin's intrinsic mechanisms for maintaining adequate hydration and concludes by discussing the external influences that affect the skin's water content (i.e., the environment, cleansing products, moisturizing systems, and dietary practices).

STRATUM CORNEUM

The skin is divided into three main components: the epidermis, dermis, and subcutaneous fat tissue. The stratum corneum is the uppermost layer of the epidermis. It is, in most body sites, 10 to 20 μm in depth and is composed of intercellular lipids and dead cells known as corneocytes (26). Corneocytes are flat, hexagonal-shaped keratin-containing structures surrounded by a protein-strengthened envelope. The protein envelope is made up of a variety of proteins including involucrin, loricrin, filaggrin, proline-rich proteins, and keratolinin (27,28). Corneocytes originate from proliferative epidermal cells known as keratinocytes. As the keratinocytes divide and migrate up toward the outermost skin layers, by a process known as differentiation, they change their morphology and cell content. By the time they reach the stratum corneum, they become flattened, protein-rich sacs. The corneocytes have no nucleus or any other cell organelles. Although the stratum corneum is sometimes referred to as the nonviable epidermis, perturbation of this tissue initiates a cascade of events occurring in the stratum corneum as well as in the viable epidermis [e.g., changes in protease activity, lipid biosynthesis, aquaporin (AQP), and filaggrin expression, etc.] (29).

The epidermis is divided into four main continuous layers: the stratum corneum, stratum granulosum, stratum spinosum, and stratum basale. As Figure 1 illustrates, the character of the cells within each of these layers is quite distinct. The basal, keratinocyte cells are columnar in shape, are found in the deepest layer of the viable epidermis, and divide and migrate upward to eventually replace the corneocyte cells. The entire process from cell birth to the "desquamation" of the corneocyte cells takes three to four weeks. The cells in the stratum spinosum are more polygonal shaped and have spinelike projections that cross intercellular spaces and form desmosomes and tight junctions. It is within these cells that keratin synthesis is initiated. In the stratum granulosum, the cells begin to flatten and the major organelles (including the mitochondria and nucleus) begin to degenerate. The stratum corneum represents the skin's uppermost "horny layer" that consists of dead, keratin-filled corneocytes.

Elias and Friend DS (31–35) have proposed a model for the stratum corneum, known as the "brick and mortar" model. The rigid, keratin-filled corneocytes are the bricks, and the intercellular lipids are the mortar. The intercellular lipids, along with lectins, desmosomes, and

Table 1 Main Functions of the Skin

Functions of the skin	Activity
Protective shield	Protects body from mechanical insult, chemical penetration, germ invasion, and UV radiation
Barrier to water loss and foreign body penetration	Prevents the evaporation of excess water and thwarts the penetration of chemicals and pathogens
Temperature regulator	Contains sweat ducts that modulate body temperature
Detoxification system	Because skin continuously desquamates, it provides an avenue for the body to eliminate toxins
Early defense system	Langerhan cells capture and transfer foreign material (e.g., viruses and bacteria) to the lymph nodes for their safe removal from the body
Sensory organ	The presence of nerve endings and Merkel cells enables the sense of touch
Appearance	The skin defines a person's physical appearance
Wound repair	Natural restorative response to repairing tissue damage

Abbreviation: UV, ultraviolet.

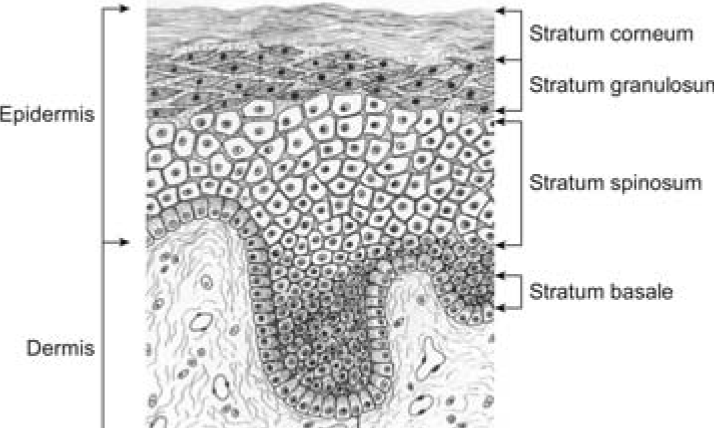

Figure 1 Schematic diagram of the skin's major epidermal layers. *Source*: From Refs. 10 and 30.

corneodesmosomes, bind to corneocytes that help to hold them in place (36). It is the physical arrangement of corneocytes and lipids, which enables the skin to resist high transepidermal water loss (TEWL) and prevent foreign microbial and chemical entities from gaining entry into the body.

Natural Moisturizing Factor

In addition to keratin, which can bind a substantial amount of water, the stratum corneum contains a number of other hydrophilic agents listed in Table 2. These materials are called natural moisturizing factors (NMF) (37–39). The NMF constitute about 20% to 30% dry weight of the stratum corneum (40) and are found intracellularly as well as extracellularly

Table 2 Composition of Natural Moisturizing Factor

Components	Mole percent (%)
Amino acids	40.0
Sodium pyrrolidone carboxylic acid	12.0
Lactate	12.0
Urea	7.0
Ions (e.g., Cl^-, Na^+, K^+, Ca^{2+}, Mg^{2+}, PO_4^{3-})	18.5
Sugars	8.5
Ammonia, uric acid, glucosamine, creatine	1.5
Citrate and formate	0.5

Source: From Refs. 18 and 25.

[e.g., sugars, hyaluronic acid (HA), urea, and lactate] (41). The major contributors to the intracellular NMF are basic amino acids and their derivatives, such as pyrrolidone carboxylic and urocanic acid, comprising up to 50% weight of the total NMF. The NMF concentration varies as a function of age and skin depth (20). Harding et al. report that for healthy skin, not exposed to surfactant damage, the NMF content is independent of depth until one approaches the filaggrin-containing levels of the skin (20). In the deeper stratum corneum layers of older individuals (50–65 years), the NMF concentration is low. This observation is a reflection of the skin's diminished ability to degrade filaggrin.

Because the NMF are effective humectants, they have a positive impact on the biochemical and mechanical properties of the stratum corneum. I.H. Blank communicated the importance of maintaining effective concentrations of water in the stratum corneum to prevent or reduce skin tightness, cracking, scaling, and flaking (12,14). In addition to enhancing the skin's water content, the NMF improve skin plasticity due to specific interactions with keratin. The NMF reduce the mobility of water as well as intermolecular forces between the keratin fibers (42). Neutral and basic amino acids appear to be the major contributors to the plasticization process. Removal of the soluble NMF can occur during water rinsing and cleansing (43). Mild cleansing systems should thus be used to minimize the NMF removal.

Most amino acid–based NMF (and their derivatives, pyrrolidone carboxylic and urocanic acid) are derived by the enzymatic hydrolysis (proteolysis) of the protein, filaggrin, and to a lesser extent by the hydrolysis of corneodesmosomes (17,44–46). Filaggrin is a protein found in the stratum granulosum layer. It is derived from the 500 kDa, highly basic profilaggrin protein found in the keratohyalin granules of the epidermis. Profilaggrin is degraded to filaggrin (via a dephosphorylation process) in the uppermost layers of the viable epidermis. Because profilaggrin is osmotically inactive, the skin has engineered a process to protect the water-rich epidermal cells from osmotic pressure–induced lysis (17). Conversely, the ability of filaggrin to degrade into the components of the NMF in the stratum corneum makes it possible for the outermost skin layers to maintain an adequate water supply when exposed to dry environments. The breakdown of filaggrin is strictly controlled by the water activity (1,18,47). On the basis of in vitro experiments, the degradation of filaggrin only occurs when the water activities are between 0.7 and 0.95. At higher activities, no breakdown occurs (48). At lower activities, the proteolytic enzymes are inactivated, and the desquamation process ceases. Consequently, when the skin is occluded (or when the relative humidity is high), there is minimal breakdown of filaggrin. Drier conditions lead to an increase in proteolytic activity, resulting in the production of more NMF. A mechanism is thus present that ensures adequate water content in the skin layer most influenced by changes in environmental conditions or chemical insult.

Using tape-stripping methods (49,50) and confocal Raman spectroscopy (43), investigators have shown that the concentration of NMF declines substantially as one approaches the stratum granulosum. This is consistent with the fact that filaggrin degradation begins in the stratum compactum, the lowest region of the stratum corneum. Given the higher water content, one expects that low amounts of NMF would be formed near the stratum granulosum/stratum corneum border. As the concentration of water decreases in the upper stratum corneum, an enhanced degradation of filaggrin occurs. Surprisingly, Egawa and Tagami reported no changes in the concentration of NMF (other than lactic acid and urea, which could have been produced via sweating) as a function of season (51). The only correlation was the panelist's subjective feeling of "not feeling dry" and higher amounts of NMF. In this same report, younger Japanese individuals (mean age: 32 years) had a lower amount of NMF versus older individuals (mean age: 67 years). This result was attributed to the faster stratum corneum turnover of the younger age group. Unlike what was reported previously (20), these authors showed a high amount of NMF at the skin surface that decreased as a function of depth. Typically, the uppermost layer of the stratum corneum has a lower NMF content than the mid–stratum corneum presumably because cleansers remove the surface material.

Some NMF behave as simple humectants and have other functions. Lactate and potassium, for example, affect the pH and stiffness of the stratum corneum (52). The L-isomer of lactic acid also stimulates ceramide biosynthesis and improves barrier function (53).

Two additional NMF, HA (54) and glycerol, have also been found in the stratum corneum. HA, a nonsulfated glycosaminoglycan, is a hygroscopic polymer of repeating disaccharide units of *N*-acetylglucosamine and glucuronic acid. It is a well-known component of the dermis, maintaining its hydrated state and providing structural integrity. In the stratum

corneum, it not only functions as a humectant but also interacts with the intercellular lipids and regulates the mechanical properties of the stratum corneum.

Glycerol may be derived from the breakdown of sebaceous triglycerides or originate from the conversion of phospholipids to free fatty acids. The importance of glycerol was revealed in a study completed by Fluhr et al. (55). These authors employed mice models where sebaceous glands (which produce triglycerides that degrade to glycerol) were largely absent and showed that although the permeability barrier responded to mechanical abrasion similar to the control, skin hydration was only enhanced by the addition of glycerol. Like HA, glycerol also influences the skin's pliability by interacting with skin lipids. Froebe et al. (56) and Mattai et al. (57) showed how glycerol could modulate the phase behavior of intercellular lipids favoring a more pliable, liquid crystalline structure at low relative humidities.

Stratum Corneum Lipids

Stratum corneum lipids play a major role in maintaining skin hydration. These intercellular lipids comprise approximately 40% to 50% ceramides, 20% to 25% cholesterol, 15% to 25% fatty acids (that have chain lengths between 16 and 30 carbons, C24:0–C28:0 being the most abundant), and 5% to 10% cholesterol sulfate; the approximate molar ratios of these lipids are 1:1:1 (ceramide: fatty acid: cholesterol) (58–60). They represent about 15% of the dry weight of the stratum corneum (61). These intercellular lipids are arranged in a highly organized lamellar arrangement (or bilayer) with only very small amounts of water present, presumably interacting with the lipid polar head groups (62). This compact lamellar structure is a very effective barrier to the TEWL. When the skin is exposed to solvents such as toluene, n-hexane, or carbon tetrachloride, which remove barrier lipids, the TEWL is increased (63). The ceramides are major components of the intercellular lipids, and this is reflected in their contribution to the structural organization of the lamellar bilayer. There are about nine major ceramides, which are synthesized from glucosylceramides, epidermosides (acylglucosylceramides), and sphingomyelin (64). These ceramides have complex structures varying in both their polar head groups and dual hydrophobic chains (Fig. 2) (65). Each ceramide contributes in specific ways to stratum corneum organization and cohesion and thus to the integrity of the barrier. In particular, the ω-hydroxyacyl portion of ceramide EOS (Fig. 2) completely spans a lamellar bilayer and the linoleate tail is believed to intercalate between a closely apposed bilayer, essentially linking two bilayers together (60,66). In fact, when any of the acylceramides is extracted, the periodicity of the lamellar bilayer structure is eliminated (67).

Figure 2 lists the structure and names of the nine identified ceramides. The ceramide (CER)-naming nomenclature was proposed by Motta et al. (68). Ceramides are designated: CER FB, where F is the type of fatty acid and B is the type of base. N represents normal fatty acids; A stands for α-hydroxy fatty acids; O represents ω-hydroxy fatty acids; and E represents ester linked linoleic acid. S, P, and H represent sphingosines, phytosphingosines, and 6-hydroxysphingosine, respectively.

Lipid Organization and Structural Models

Electron diffraction studies (69) have shown that as corneocytes migrate from the lower regions of the stratum corneum to the outer layers, there is a corresponding change in lipid packing from a more ordered, orthorhombic packing to a more fluid hexagonal phase. This observation is consistent with the known weakening of the barrier and complete loss of lamellar ordering in the topmost layers of the stratum corneum (70–72). Changes in the composition of the stratum corneum lipids in the upper stratum corneum (i.e., increased concentration of cholesterol sulfate, hydrolysis of CER EOS, increased concentration of short–chain length fatty acids, crystallization of cholesterol, and decreased levels of ceramides) presumably influence the loss of lamellar order (71). Indeed, factors that can affect lipid composition, such as washing with harsh cleansers, perturb the lamellar structure and adversely change the condition of the skin (72).

There are several models that have been proposed to describe the structural phases of the lipid bilayer (Table 3). The domain mosaic model suggests that lipids coexist as a mixture of liquid crystalline and gel phases (73). The more ordered gel phase allows for greater packing of the lipids and hence a more effective barrier.

X-ray diffraction studies of hydrated stratum corneum have shown two types of lamellar structures, having repeat distances of 13.2 to 13.4 nm (long periodicity phase) and 6.0 to 6.4 nm (short periodicity phase) (62,74). Bouwstra et al. (75) have proposed a molecular arrangement

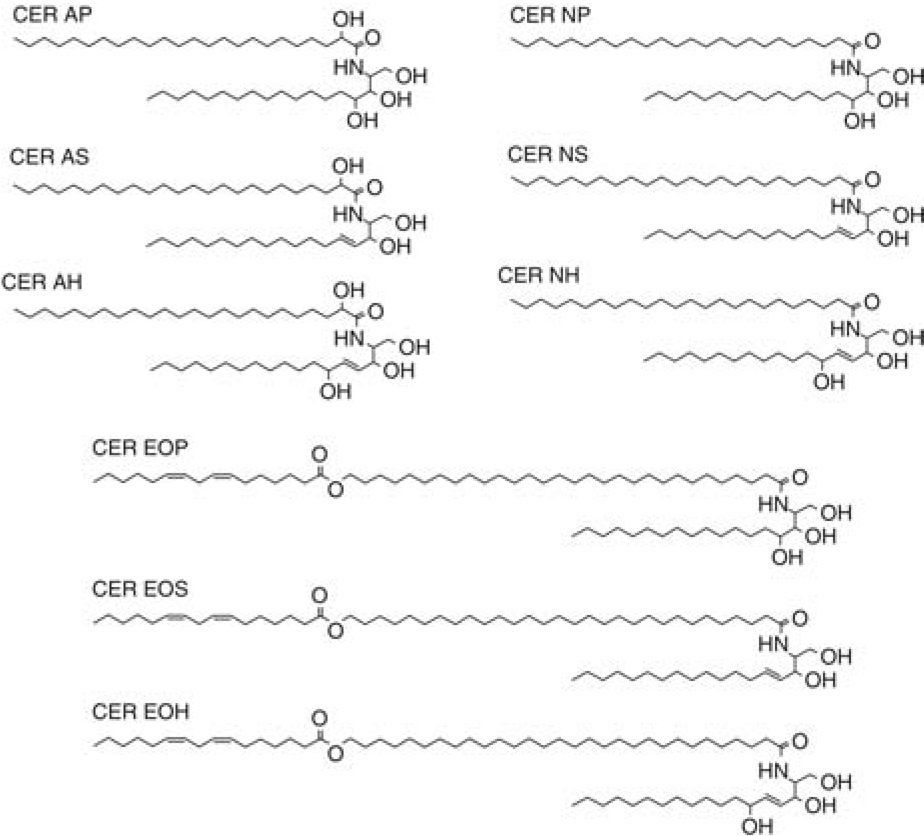

Figure 2 Chemical structure of stratum corneum ceramide lipids. The depicted ceramide naming, CER FB, was proposed by Motta et al. (68). F refers to the type of fatty acid and B to the type of base. N represents normal fatty acids; A stands for α-hydroxy fatty acids; O represents ω-hydroxy fatty acids; and E represents ester linked linoleic acid. S, P, and H represent sphingosines, phytosphingosines and 6-hydroxysphingosine, respectively.

Table 3 Proposed Models Describing How Barrier Lipids Structure Within Stratum Corneum

Skin barrier model	Description
Domain mosaic	Stratum corneum barrier lipids coexist in liquid crystalline (water permeable) and highly ordered gel phase (water impermeable) domains. Water is expected to be most permeable at the phase boundaries. The more fluid crystalline phase allows for the permeation of water.
Sandwich model	Proposes a more structured arrangement of liquid crystalline and gel domains. A narrow central lipid layer with fluid domains (3 nm wide) lies between two broad, crystalline lipid layers (6.4 nm wide).
Single–gel phase model	Skin barrier lipids exist as a single lamellar gel phase with no phase boundaries.

of the long periodicity phase, called the sandwich model, consisting of two broad lipid layers of about 5 nm each, with a crystalline structure separated by a narrow central lipid layer of about 3 nm with fluid domains. Cholesterol and ceramides are important for the formation of the lamellar phase, while fatty acids mostly impact the lateral packing of the lipids.

L. Norlen has proposed yet a third skin barrier model (76). This model suggests that the lipid matrix has a homogeneous lamellar gel phase with a low degree of lipid fluidity. Stratum corneum epidermal lipid heterogeneity, the long length of the fatty acids chains, and the presence of cholesterol are used to support this model since these factors have been shown to stabilize gel phases (77,78). This model does not require the presence of water or any bilayer conformation.

There are also different models describing the mechanism of skin barrier formation. The Landmann model suggests that "lamellar bodies" or stacked monobilayer vesicles separate from the trans-Golgi network, extrude into the intercellular space at the stratum granulosum/stratum corneum border, fuse with the cell plasma membrane of the stratum granulosum, and discharge the lipids into continuous multilamellar membrane sheets in the intercellular space (79). The membrane-folding model (80–82) argues against abrupt changes in lipid phase transitions that would result from the disruption, diffusion, and fusion of the lamellar bodies. On the basis of this model, the skin barrier formation takes place as a direct, continuous unfolding of a three-dimensional membrane into a flat, multilayered two-dimensional lipid structure (having only hexagonal hydrocarbon chain packing and no abrupt phase transitions) (80,81). By evaluating vitreous sections of non-pretreated, non-stained, full-thickness, hydrated, skin, using cryo-transmission electron microscopy, cubic-like membrane structures were observed. This organizational pathway was proposed to be more thermodynamically preferred to that previously described by Landmann (79). The cryo-transmission electron microscopy preparation was also reported to be improved over conventional electron microscopy methods because it does not require dehydration and chemical fixation of the sample. With additional innovations in instruments and instrumental techniques, active research will be sure to continue in this area.

Lamellar Lipid Arrangement and Water Permeability

The lamellar or bilayer arrangement, independent of the nature of the lipids from which it is derived, is a natural barrier to water permeability (83). In the skin, there is a relatively large gradient in water chemical potential between the viable epidermis, where the water content is about 70% by weight, and the stratum granulosum/stratum corneum junction, where the water content drops to 15% to 30% (84). Under this large water gradient, the stacked bilayer arrangement of lipids, which is a continuous region in the stratum corneum, provides an optimal way to reduce water loss through the skin. Water escaping from the stratum corneum would have to traverse the tortuous pathway of the bilayer (73,85). In addition, fully matured corneocytes would also increase the tortuosity and hence the diffusional path length of water (19). The combination of a lamellar arrangement of lipids and increased diffusional path length due to corneocytes reduce water diffusion to the atmosphere.

AQUAPORINS AND TIGHT JUNCTIONS

Another mechanism by which the skin maintains its hydrated state is the use of AQPs. These transmembrane proteins form water channels across cell membranes, facilitating the transport of small polar molecules across the cell membrane. Specific AQPs also have the ability to facilitate the transport of glycerol and urea. AQP3 is most relevant to skin hydration (19). AQP3 is localized in the basal and suprabasal layers of the epidermis, and is not expressed in the stratum corneum. In AQP3-deficient mouse skin, the skin is less hydrated, less elastic, the permeability of water and glycerol within the skin is reduced, and there is a delayed barrier recovery (86–88). Only by adding glycerol does the condition of the skin improve (89). Skin diseases associated with impaired barriers and low skin hydration also tend to have reduced expression of AQP3. Boury-Jamot et al. found that AQP3 expression was inversely correlated to the severity of patients with eczema and spongiosis (90).

Tight junctions consist of more than 40 transmembrane [i.e., claudins, occludin, and junctional adhesion molecules (JAMs)] and plaque proteins (zonula occludens) (91). This protein combination forms a semipermeable barrier between aligning cell membranes, making it very difficult for water to pass through the space between the epidermal cells. Ions or fluids must actually diffuse or be actively transported through the cell to pass through the tissue. Claudins, occludins, and JAMs are principally responsible for controlling water permeability. Claudin 1–deficient mice die within one day of birth because of excessive TEWL (92). The presence of organized tight junctions and an intact stratum corneum barrier ensures low values of TEWL. For those diseases due to which patients experience dry skin and a compromised barrier (e.g., psoriasis vulgaris and ichthyosis vulgaris), the location of tight-junction proteins may also be altered. Proteins that may be expressed homogeneously throughout the epidermis may be preferentially expressed in the upper or lower layers.

DESQUAMATION

So far, the above discussion has centered on natural ways in which the human skin has evolved to retain water. In addition to hydrating the skin, water also plays a crucial role in the exfoliation or desquamation of corneocytes. Corneocytes are linked in the lower stratum corneum by corneodesmosomes, which are macromolecular glycoprotein complexes. As the corneocytes move from the lower to the outer region of the stratum corneum, the corneodesmosomes are progressively degraded by hydrolytic enzymes. This leads to desquamation in the outer stratum corneum. These enzymes include serine proteases such as stratum corneum chymotryptic enzyme (SCCE) and stratum corneum tryptic-like enzyme (SCTE), which are more effective at neutral pHs and are most active on the outermost layers of the stratum corneum (19,47,93–95). The cathepsin family of proteases is more active under lower pH conditions and are present throughout the stratum corneum. Other proteases include cysteine proteases, sulfatases, and glycosidases. Many of these enzymes are localized in the intercellular space, and their activity is affected by both the lipid organization and water content (20,96). Clearly, low water content within the stratum corneum affects the activities of stratum corneum proteases, which leads to dry, flaky skin. Recently, these changes have been studied as a function of season, anatomical site, and skin depth (97). To maintain these processes, in vitro results suggest that optimally hydrated skin requires water content between 10% and 20% (13).

ENVIRONMENTAL IMPACT ON SKIN HYDRATION

Changes in lipid biosynthesis (71,98), epidermal DNA synthesis (9), barrier function (99), and skin thickness (100) are all influenced by the skin's water content. There are many studies showing that biochemical processes are also altered as a function of changes in the environmental relative humidity (101,102). Rawlings et al. demonstrated that dry conditions inhibit corneodesmosomal degradation, while increasing humidity increases corneodesmosomal degradation (103). Moreover, when the human skin was exposed to low humidity conditions (10%) even for short exposure periods (3 and 6 hours), a significant decrease in water content of the stratum corneum and increase in skin roughness was observed (3).

Even in humid conditions, the skin is still subject to a number of environmental insults that can negatively affect skin hydration. Excess UV radiation, for example, causes UV-induced erythema leading to a compromised barrier (104). Several animal studies have demonstrated that abrupt changes in the environment, such as going from humid (80% relative humidity) to dry (less than 10% relative humidity) conditions, increases the time required for barrier function to return to normal (99). In this situation, the skin does not have enough time to adapt to the new climatic conditions. Declercq et al. have further demonstrated that skin can adapt to dry climatic conditions (5). They found that the panelists living in a hot, dry climate such as Arizona had a better barrier function and less dry skin compared with the panelists living in New York, which had a more humid climate (5).

While prolonged exposure to conditions of low relative humidity (<20%) enhance barrier function, sustained exposure to high-humidity conditions leads to a gradual deterioration in the barrier (1). A relative humidity greater than 80% is associated with a decrease in NMF and corneocyte hydration in the epidermis of hairless mice (1). It has also been shown that when normal skin is exposed to a moist environment, the kinetics of barrier recovery is delayed because of a reduction in the number of epidermal lamellar bodies and lipid content, in direct contrast with what is observed at low humidities (102). Therefore, when the skin adapts to a high-humidity environment, its capacity to respond to external changes is decreased, partially because of a reduction in the reservoir of stratum corneum lipids.

It is remarkable that a human fetus has a mechanism to protect the outermost skin barrier to the damaging effects of amniotic fluid, an environment that would result in a loss of barrier function in adults (105). During the third trimester of gestation, a biofilm known as vernix caseosa forms and coats the prenatal skin. This film acts as a barrier and facilitates the formation of the acid mantle, which provides an optimal environment for inhibiting bacterial colonization (106,107). Vernix caseosa consists of ~80% water, 10% protein (corneocytes with no desmosomal attachments), and 10% lipids by weight (consisting of barrier and sebaceous

lipids not arranged in any lamellar structure). This material has been shown to have multiple functions, besides being an efficient moisturizer and osmoregulator (108). On the basis of transmission electron microscope images, the limited structure of vernix caseosa is very similar to that of the topmost layers of the stratum corneum. The body appears to have retained this structural feature of vernix caseosa during the course of stratum corneum maturation.

PERSONAL CARE PRODUCTS AND SKIN HYDRATION

The Effect of Cleansing Systems

Cleansers are designed to remove unwanted materials from the skin such as dirt, oils, and sebum. However, the use of harsh surfactants damages the skin barrier; increases the skin's susceptibility to environmental sources of irritation and sensitization; and reduces skin moisture and smoothness (109). Charged surfactants, such as anionic and cationic, are the most aggressive. Sodium lauryl sulfate (SLS) is a harsh surfactant that, given its small hydrodynamic radius, is the only surfactant that can extract the intercellular lipids and disrupt the lipid bilayer (110). It, along with most of the charged surfactants, adsorb skin proteins, causing them to denature and swell. Rhein et al. reported that the extent of protein denaturation is dependent on the surfactant monomer concentration and exposure time (111). As surfactants denature skin proteins, enzymatic reactions that control desquamation, inflammation, and oxidation processes are negatively impacted (112,113). The resulting enhanced barrier permeability leads to skin dryness, roughness, cracking, and inflammation (10,47,114).

Fortunately, there are a number of surfactants used commercially that are mild to the skin. These include mostly nonionic and amphoteric variants and the anionic variants: highly ethoxylated (at least 5-EO) alkyl sulfates, sulfosuccinates, isethionates, sarcosinates, taurates, alkyl phosphates, and alkyl glutamates. The aggressiveness of charged surfactants can be mitigated by reducing the concentration of the surfactant's monomer species, reducing the charge by incorporating various counterions and/or cosurfactants to form mixed micelles, and introducing ethoxylation (10). The improved mildness reduces the incidence of barrier damage, which aids in the maintenance of hydrated skin (i.e., nondrying cleansers).

Surfactants also negatively impact the skin hydration properties by removing NMF. Blank and Shappirio (14) showed that when isolated human stratum corneum was exposed to 1% solutions of soap, alkyl sulfate or alkyl benzylsulfonate, all surfactants reduced the ability of the tissue to absorb water from the atmosphere, relative to water. This water-holding capacity is correlated with the loss of NMF. A similar correlation has been found between natural saponified soaps and mild synthetic surfactants using confocal Raman spectroscopy (115) (Fig. 3).

There has been a great deal of research focused on delivering enhanced skin moisturization using cleansers (109). Emollient-containing cleansers have been found to alleviate the dry skin condition of people having rosecea, sensitive skin, and/or atopic dermatitis (116,117). Emulsion-based liquid body washes are commonly employed to mildly

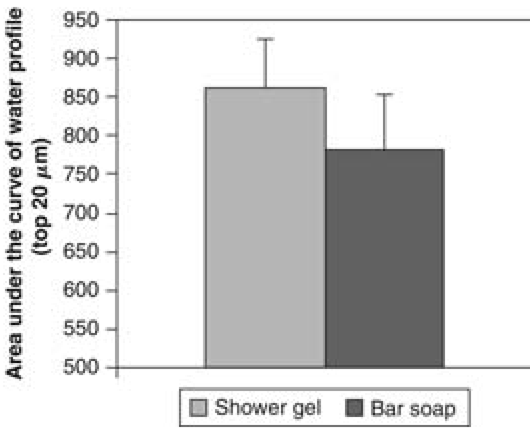

Figure 3 Water content as a function of cleanser type determined using confocal Raman spectroscopy. *Source*: From Ref. 115.

cleanse and moisturize the skin. Although delivering a moisturization benefit using lipophilic agents is difficult to achieve in a rinse-off system, clinical studies have confirmed that enhanced moisturization can be achieved in formulas containing a large quantity of oils and/or humectants (118). The patent literature is replete (but will not be further discussed in this chapter) with examples of approaches to improve the delivery of actives from cleansing systems. Invariably, it has been demonstrated that cleansing systems are able to remove dirt and bacteria while simultaneously depositing oils on the skin to improve skin feel, smooth desquamating corneocytes, and improve barrier function.

Research has demonstrated that oatmeal is a good choice for gentle cleansing and moisturizing dry, sensitive skin (119). Oatmeal has been used for centuries as a soothing agent to relieve itch and irritation associated with various xerotic dermatoses. Many clinical properties of colloidal oatmeal are derived from its chemical polymorphism. Its high-concentration of starches and β-glucan is responsible for the protective and water-holding functions of oat. The presence of different types of phenols confers antioxidant and anti-inflammatory activity. Some of the oat phenols are also strong UV absorbers. The cleansing activity of oat is mostly due to saponins. Many of its functional properties make colloidal oatmeal a good cleanser, moisturizer, buffer, as well as a soothing and protective anti-inflammatory agent (120).

Although cleansers have been formulated to successfully deliver oils to the skin, delivering humectants has been more challenging. Humectants are highly water soluble and, consequently, harder to deposit onto the skin during the washing process. Special delivery systems have yet to be developed to improve the competency in this area.

Moisturizing the stratum corneum using lotions and creams is typically the best way to hydrate the skin. This is typically accomplished by using emulsion formulas, which contain humectants, emollients, and/or occlusive agents (121). Humectants attract and hold on to water. Occlusive agents form a barrier across the skin, reducing the TEWL. "Emollient" comes from a Latin derivation meaning a material designed to soften and soothe the skin (122). Emollients can be occlusive or semiocclusive meaning they may not be very effective at preventing evaporative water loss, but are effective in smoothing skin.

Glycerol and urea are well-known humectants (123–125). Glycerol also prevents the crystallization of stratum corneum lipids at low relative humidity, which leads to less TEWL and higher water content of the skin. Previous studies evaluated the influence of glycerol on the recovery of damaged stratum corneum induced by repeated washings with SLS. The authors found that glycerol created a stimulus for barrier repair and improved stratum corneum hydration (126).

Petrolatum is a common occlusive agent. Application of hydrophobic materials such as petrolatum to prevent skin dryness may be as old as mankind itself. In recent times, however, manufacturers are incorporating lipids that can form lamellar bilayers in their formulations to enhance the barrier properties of the skin (127,128). They typically use ceramides or ceramide-like molecules to accomplish this goal and have found even greater benefit when they combine the lipid technology with glycerol (129). Niacinamide has also been shown to enhance lipid biosynthesis, which again improves barrier function (130). As in the above situation, the addition of glycerol further improves the clinical dry-skin condition.

Water in Excess

Skin exposure to extrinsic water is usually considered to be harmless. Often times it is used as the "control" site in experiments that investigate the way compounds interact with the skin. However, there is evidence that prolonged contact with water can negatively affect SC barrier function, similar to surfactants (131). In addition to eliciting erythema, inflammation, and intense dermatitis, excess water exposure can increase SC swelling and suppleness, weaken SC corneocyte cohesion, and increase the permeability of all substances, especially water. Warner et al. (114,131) showed that overexposure of skin to water causes a disruption of the SC intercellular lamellar bilayer ultrastructure in vitro as well as in vivo. Similar to surfactant exposure, the swelling response was time dependent, and wide intracellular clefts between corneocytes were observed. These studies as well as others show that prolonged hydration of the SC can directly disrupt the barrier lipids, leading to compromised skin (114,131,132).

DIETARY IMPACT ON SKIN CONDITION

It is generally stated that topically applied cosmetic products can be helpful in restoring normal hydration to dry skin. However, less recognized is the positive influence that drinking plenty of water can have on the skin's appearance. Approximately 45% to 70% of human body weight consists of water. One-third of the total body water is extracellular, and two-thirds are within the intracellular compartment (133). Water is free to move between the cell membranes with any net movement controlled by the effective osmotic and hydrostatic pressures. This balance of body fluid is dependent on the intake of water through drinking, food, and metabolism and the loss of water through natural processes. The three components of the skin, the epidermis, dermis, and subcutaneous fat tissue, play a major role in water regulation, with the SC water content helping to maintain many of the skin's biophysical properties (134). Soft, smooth skin has an optimally hydrated SC with a water content of approximately 20% to 30%, and a water content of less than 10% to 20%, resulting in abnormally dry skin (133,134). While the environment can play a role in TEWL, a good balance between water intake and loss is vastly important in helping to maintain healthy water content in the SC, which has a positive influence on skin hydration.

An increased intake of pure, healthy water helps to enhance nutrient absorption, skin hydration, detoxification, and virtually every aspect of better health. However, studies have also shown that drinking dietary natural mineral water or taking a food supplement containing pro-hydrating actives maintains adequate skin hydration as well. Mac-Mary et al. (135) showed that the magnitude of change in a Corneometer® measurement on the forearm of healthy subjects increased by 14% when 1 L of mineral water was consumed per day for 42 days, which was clinically significant and similar to the observed modifications with moisturizing cosmetic products (10–30%). Primavera and Berardesca (133) investigated how a capsule containing an active product based on vegetable ceramides, amino acids, sea fish cartilage, antioxidants, and essential fatty acids improved skin hydration after oral use. Significant improvement in Corneometer readings were seen in the active-treated groups (+30%), in addition to a decrease in skin roughness and improved skin smoothness after 40 days, as measured using a VisioScan®. Self- and clinical-assessment data confirmed the results of the biophysical measurements. These studies demonstrate that a proper diet with adequate water and mineral intake is just as important in the management of skin hydration as a complementary cosmetic approach. Puch et al. further showed that ingesting a probiotic-containing dairy product enriched in γ-linolenic acid (an ω-6-polyunsaturated fatty acid that has been shown to enhance the rate of barrier recovery when applied topically and when taken orally), vitamin E, and catechins improved barrier function after six weeks of taking twice a day dosage. The average improvement was 13% (136). The reduction in TEWL was observed throughout the six-month study, despite the changes in season.

SUMMARY

Maintaining hydration of the stratum corneum can be accomplished using a number of different mechanisms. From using mild surfactants that minimally compromise the skin barrier to delivering moisturizers (humectants, occlusive oils, and lipid modulating agents), these materials offer a means of adding moisture back to the skin or, alternatively, reducing water loss (137,138). The skin itself, in fact, has a natural process to minimize excess water loss. Through the water-dependent production of intercellular skin lipids and NMF, an intricate mechanism is in place to function optimally in an often arid, external environment. The skin is a remarkable organ, producing vernix caseosa to protect (as a barrier, anti-infective and antioxidant) the fetus while it is immersed in amniotic fluid, a potential damaging environment, and following birth enhancing the acid mantle development, which facilitates skin maturation during the postnatal period. The production of urocanic acid and free fatty acids in the stratum corneum further contributes to the regulation of stratum corneum pH (139,140). As for those living in dry climates, the skin is adaptable and can generate an improved barrier function and increased water content. The development of the confocal Raman spectrometer has allowed researchers to noninvasively monitor the skin's water content and composition changes as a function of the environment and product use

(43,141–143). The identification of AQPs and tight junctions provides increasing evidence for internal mechanisms that the skin is using to improve the opportunities for corneocyte hydration. More importantly, there is increasing data confirming the importance of maintaining an optimal skin's water content to insure the activity of processes that occur in the epidermis. Protecting and maintaining an adequate water content and barrier function of the skin are proving to be essential to achieving healthy, youthful-looking skin.

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10 Hydrating Substances

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INTRODUCTION

Hydrating substances are used in cosmetic products to retard moisture loss from the product during use and to increase the moisture content in material that is in contact with the product. This function is generally performed by hygroscopic substances, or humectants, which are able to absorb water from the surroundings. In the *International Cosmetic Ingredient Dictionary*, approximately 125 substances are listed as humectants and almost 200 hygroscopic materials are used to increase the water content of the skin (1).

Dry hair and dry skin are the target areas in the body for treatment with humectants. Sometimes mucous membranes also benefit from application of humectants. Dry hair is brittle and rough, has a tendency to tangle, and has hardly any luster. Humidity of the atmosphere is the only source of moisture to hair, except shampooing, and the addition of humectants to the hair will, therefore, facilitate its retention of water. The same is true for the skin, although it is constantly supplied with water from inside of the body. The skin forms a critical structural boundary for the organism and is frequently compromised as a result of under hydration. The water held by the hygroscopic substances in the stratum corneum (SC) is a controlling factor in maintaining skin flexibility and desquamation (2,3). Hydration plays an important role in maintaining the metabolism, enzyme activity, mechanical properties, appearance, and finally, barrier function of the skin.

The special blend of humectants found in the SC is called natural moisturizing factor (NMF) (4). NMF can make up about 10% of the dry weight of the SC cells (4). Substances belonging to this group are amino acids, pyrrolidone carboxylic acid (PCA), lactates, urea, and inorganic ions (Tables 1 and 2) (4). Furthermore, glycerol is found naturally in SC, and the mean amounts are found to be about 0.7 $\mu g\ cm^{-2}$ on the cheek and 0.2 $\mu g\ cm^{-2}$ on the forearm and sole (8). The proportion of the inorganic ions and lactate in the SC differs from that in sweat and also changes between winter and summer (9). The level of lactate and potassium in the SC appears to correlate with each other as well as with the physical properties of the SC (9). The levels of lactate have been found to be approximately 100 times higher than that of glycerol (8,9).

NMF is formed from the protein filaggrin, whose formation is regulated by the moisture content in the SC (2). In skin diseases such as ichthyosis vulgaris (10,11) and psoriasis (12), there is a virtual absence of NMF. In ichthyosis vulgaris, the stratum granulosum is thin or missing because of a defect in the processing of profilaggrin, which is also noticed as tiny and crumbly keratohyalin granules (13).

Glycerin is another humectant suggested to be important for the SC hydration (Tables 1 and 2). Skin dryness in sebaceous gland–deficient mice has been found to be linked to reduced levels of glycerin because of absence of triglycerides, which are the primary source for glycerin (14). This type of dryness may also be applicable to clinical situations where sebaceous glands are absent or involuted, such as in prepubertal children showing eczematous patches, which disappear with the onset of sebaceous gland activity. Moreover, xerosis in the distal extremities of aged skin and in patients receiving systemic isotretinoin for treatment of acne may be linked to glycerin depletion because of the lower sebaceous gland activity (14).

Physiologically occurring and synthetic substances are used as humectants in cosmetic products (Tables 1 and 2). The water-binding capacity of the sodium salts of lactic acid and PCA appears to be higher than that of glycerin and sorbitol (Table 3) (15,16). Treatment of solvent-damaged guinea pig footpad corneum with humectant solutions shows that the water held by the corneum decreases in the following order: sodium PCA > sodium lactate > glycerin > sorbitol (20). Urea also has strong osmotic activity (21,22). However, which of these substances most efficiently reduces xerosis or other dry skin conditions is not known. Besides differences in water-binding capacity, their absorption into the skin is important for the effect.

Table 1 Chemistry of Hygroscopic Substances

Name	CAS-No	Mw	Other names	Natural source
Butylene glycol	107-88-0	90.1	1,3-butanediol, 1,3-butylene glycol	
Glycerin	56-81-5	92.1	Glycerol, 1,2,3-propanetriol	Hydrolysis of oils and fats
Lactic acid	50-21-5	90.1	2-hydroxypropanoic acid	Sour milk and tomato juice
Panthenol	81-13-0	205.3	Dexpanthenol, pantothenol, provitamin B5	Plants, animals, bacteria
PCA	98-79-3	129.11	L-pyroglutamic acid, DL-pyrrolidonecarboxylic acid, 2-pyrrolidone-5-carboxylic acid	Vegetables, molasses
Propylene glycol	57-55-6	76.1	1,2-propanediol	
Hyaluronic acid	9004-61-9	5×10^4–8×10^6	Hyaluronan	Cock's combs, biofermentation
Sorbitol	50-70-4	182.17	D-glucitol	Berries, fruits
Urea	57-13-6	60.08	Carbamide, carbonyl diamide	Urine

Abbreviations: MW, molecular weight; PCA, pyrrolidone carboxylic acid.
Source: From Refs. 1, 5–7.

Table 2 Chemical Formulas of Humectants

Humectant	Formula
Butylene glycol	
Glycerin	
Lactic acid	
Panthenol	
PCA	
Propylene glycol	
Sorbitol	
Urea	

Table 3 Moisture-Binding Ability of Humectants at Various Humidities

Humectant	31%	50%	52%	58–60%	76%	81%
Butylene glycol						38[e]
Glycerin	13[c]11[b]	25[a]	26[b]	35–38[c,f]	67[b]	
Na–PCA	20[c]17[b]	44[a]	45[b]	61–63[c,f]	210[b]	
Na–lactate	19[b]	56[a]	40[b]	66[f]	104[b]	
Panthenol	3[d]		11[d]		33[d]	
PCA	<1[c]				<1[c]	
Propylene glycol					32[f]	
Sorbitol			1[a]		10[f]	

Abbreviation: PCA, pyrrolidone carboxylic acid.
[a]From Ref. 15.
[b]From Ref. 16.
[c]From Ref. 17.
[d]From Ref. 18.
[e]From Ref. 5.
[f]From Ref. 19.

Table 4 Parameters to Consider During Product Development to Obtain the Desired Effect

Formulation related	Effect on the target area
Price and purity?	Product claim?
Chemical stability during production and shelf life?	Substantivity in rinse-off products?
Sensitive to heat? UV-light? pH?	Penetration characteristics?
Incompatibilities with other ingredients?	Hygroscopicity?
Adsorption to the packaging material?	Adverse effects?
Effects on the preservation system?	

Hence, the in vitro humectancy should be distinguished from the in vivo moisturizing effect (23). Some factors to consider during product development are highlighted in Table 4.

This chapter will provide basic information about some commonly used humectants, which are primarily used for treatment of the skin. Moreover, safety information will also be provided.

BUTYLENE GLYCOL

Description

Butylene glycol usually means 1,3-butanediol, but the term can also be used for 2,3-butanediol (Tables 1 and 2). The alcohol is a viscous, colorless liquid with sweet flavor and bitter aftertaste (5). It is soluble in water, acetone, and castor oil, but practically insoluble in aliphatic hydrocarbon (5).

General Use

Butylene glycol is used as humectant for cellophane and tobacco (5). It is also used in topical products and as solvents for injectable products. Butylene glycol is claimed to be most resistant to high humidity and is often used in hair sprays and setting lotions (24). The alcohol also retards loss of aromas and preserves cosmetics against spoilage by microorganisms (24).

Safety

Butylene glycol is considered safe by the Cosmetic Ingredient Review (CIR) Expert Panel (25). Human skin patch test on undiluted butylene glycol produced a very low order of primary skin irritation, and a repeated patch test produced no evidence of skin sensitization (25). The substance is reported to be less irritating than propylene glycol (26,27). Few reports of contact allergy exist, but the substance does not seem to cross-react with propylene glycol (26).

GLYCERIN

Description

In 1779, the Swedish scientist, C.W. Scheele, discovered that glycerin could be made from a hydrolyzate of olive oil. The alcohol is a clear, colorless, odorless, syrupy, and hygroscopic liquid (Tables 1 and 2) (5,12), approximately 0.6 times as sweet as cane sugar (5,12). It is miscible with water and alcohol, slightly soluble in acetone, and practically insoluble in chloroform and ether (12,13).

General Use

Glycerin is used as a solvent, plasticizer, sweetener, lubricant, and preservative (5). The substance has also been given intravenously or by mouth in a variety of clinical conditions in order to benefit from its osmotic dehydrating properties (6). This effect can also be used topically for the short-term reduction of vitreous volume and intraocular pressure of the eye (6). Moreover, concentrated solutions of glycerin are used to soften earwax (6) and suppositories with glycerin (dose 1–3 g) promote fecal evacuation (6).

Effects on Skin

The importance of glycerin in skin care products is well established. To explain its benefits, studies have focused on its humectant and protecting properties. Levels ranging between a few percent and 20% to 25% are used in moisturizers for treatment of dry skin conditions (28). Glycerin not only attracts water but has also been suggested to modulate the phase behavior of SC lipids and to prevent crystallization of their lamellar structures in vitro at low relative humidity (29). Incorporation of glycerin into an SC model lipid mixture enables the lipids to maintain the liquid crystal state at low humidity (29). The biochemical consequences of these properties may be due to the influence of the activity of hydrolytic enzymes crucial to the desquamatory process in vivo (30). Thereby, the rate of corneocyte loss from the superficial surface of human skin increases, probably because of an enhanced desmosome degradation (2,30).

The mode of action of glycerol both on SC hydration and epidermal barrier function seems to be related to the aquaporin 3 channel. The aquaporins are a family of small, integral membrane proteins that function as plasma membrane transporters of water and in some cases small polar solutes [reviewed in (31)]. Glycerol is transported very slowly into the epidermis, and thus, its transport rate is sensitive to the intrinsic glycerol permeability of the basal keratinocyte layer. Repeated tape stripping taken from skin treated with 15% glycerin cream indicates that glycerin diffuses into the SC to form a reservoir (32). During some hours after application, a decrease in transepidermal water loss (TEWL) has been noted (32–35) followed by increased values after some hours in animal skin (35). No evidence of deterioration of the skin barrier function has been noted after long-term treatment of normal and atopic skin with 20% glycerin (36,37). Instead, glycerin has been found to accelerate barrier recovery after acute external perturbations (38). Moreover, in human skin, its surface profile, electrical impedance, and increase in the coefficient of friction were found to accompany an improvement in the skin condition, as assessed by an expert (33). Glycerin is also suggested to induce a shrinking of superficial corneocytes, which was independent from osmotic effects (39). This contraction might give a more compact SC and reduce the risk for irritant contact dermatitis (39).

Safety

Very large oral or parenteral doses can exert systemic effects because of the increase in the plasma osmolality, resulting in the movement of water by osmosis from the extravascular spaces into the plasma (6). Glycerin dropped on the human eye causes a strong stinging and burning sensation, with tearing and dilatation of the conjunctival vessels (40). There is no obvious injury, but studies have indicated that glycerin can damage the endothelial cells of the cornea (6,40). Glycerin has been shown to have excellent skin tolerability, and treatment with 20% glycerin did not show any signs of adverse effects on atopic dry skin (28).

HYALURONAN (HYALURONIC ACID)

Description

The earliest work on skin was devoted predominantly to the cells that make up the layers of skin: epidermis, dermis, and underlying subcutis. Now it is beginning to be appreciated that the materials that lie between cells, the matrix components, have major instructive roles for cellular activities. This extracellular matrix endows skin with its hydration properties. The components of the extracellular matrix appear amorphous by light microscopy, but form a highly organized structure of glycosaminoglycans (GAGs), proteoglycans, glycoproteins, peptide growth factors, and structural proteins such as collagen and, to a lesser extent, elastin. The predominant component of the extracellular matrix, however, is hyaluronan; one of the first extracellular matrix component to be elaborated in the developing embryo [reviewed in (41)]. The term "hyaluronan" is used to cover both hyaluronic acid and sodium hyaluronate. Hyaluronan is a member of the class of amino sugars containing polysaccharides known as the GAGs widely distributed in body tissues. The polymer provides the turgor for the vitreous humor of the eye and the name "hyaluronic acid" derives from the Greek hyalos (glossy, vitreous) and uronic acid. Molecular weight is within the range of 50,000 to 8×10^6, depending on source, methods of preparation, and determination (5). Hyaluronic acid is a regulator of cell behavior and influences cellular metabolism. Moreover, the molecule binds water and functions as a lubricant between the collagen and the elastic fiber networks in dermis during skin movement. A 2% aqueous solution of pure hyaluronic acid holds the remaining 98% water so tightly that it can be picked up as though it was a gel (42).

The skin is the largest reservoir of hyaluronic acid, containing more than 50% of the total body. The papillary dermis has the most prominent levels of hyaluronic acid than the reticular dermis. Hyaluronic acid is extracted from cock's comb or obtained from streptococci (Lancefield Groups A and C) (6). During manufacturing, the large, unbranched, noncross-linked, water-containing molecule is easily broken by shear forces (42). The carbohydrate chain is also very sensitive to breakdown by free radicals, UV radiation, and oxidative agents (42).

General Use

A viscous solution of sodium hyaluronate is used during surgical procedures on the eye and is also given by intra-articular injection in the treatment of osteoarthritis of the knee (6). Hyaluronic acid is also applied topically to promote wound healing. Topical application of 0.1% solution in patients with dry eye has been suggested to alleviate symptoms of irritation and grittiness (6).

Effects on Skin

High molecular weight hyaluronic acid solutions form hydrated viscoelastic films on the skin (42). The larger the molecular size, the greater the aggregation and entanglement of the molecules, and hence, the more substantial and functional the viscoelastic film associated with the skin surface (42). Owing to the high molecular weight, hyaluronic acid will not penetrate deeper than the crevices between the desquamating cells. The polymer may also be injected to obtain a smoother surface and reduce the depth of wrinkles.

Safety

Sodium hyaluronate is essentially nontoxic. When the substance is used as an ophthalmic surgical aid, transient inflammatory ocular response has been described (6).

LACTIC ACID

Description

Lactic acid is colorless to yellowish crystal or syrupy liquid, miscible with water, alcohol, and glycerol, but insoluble in chloroform (6). Lactic acid is an α-hydroxy acid (AHA), i.e., an organic carboxylic acid in which there is a hydroxy group at the two, or α, position of the carbon chain (Table 2). Lactic acid can exist in a DL, D, or L form. The L and the D forms are enantiomorphic isomers (mirror images). Lactate is also a component of the natural hygroscopic material of the SC and constitutes about 12% of this material (Table 1) (4).

Formulations containing lactic acid have an acidic pH in the absence of any inorganic alkali or organic base. The pH is increased in several formulations by partial neutralization.

General Use

Lactic acid has been used in topical preparations for several decades because of its buffering properties and water-binding capacity (6,20). Lactic acid and its salts have been used for douching and to help maintain the normal, acidic atmosphere of the vagina. Lactic acid has also been used for correction of disorders associated with hyperplasia and/or retention of the SC, such as dandruff, callus, keratosis, and verrucae (viral warts) (6). Moreover, lactic acid has been suggested to be effective for adjuvant therapy of mild acne (43). Also, ethyl lactate has been proposed to be effective in the treatment of acne, due to its penetration into the sebaceous follicle ducts with subsequent lowering of pH and decrease in the formation of fatty acids (44).

Investigators have also reported increases in the thickness of viable epidermis (45,46) as well as improvement in photoaging changes (45,47). Lactic acid in combination with other peeling agents is used to produce a controlled partial-thickness injury to the skin, which is believed to improve the clinical appearance of the skin (48).

Effects on Skin

In guinea pig footpad corneum, it has been shown that both lactic acid and sodium lactate increase the water-holding capacity and skin extensibility (20). Potassium lactate has been suggested to restore SC hydration more effectively than sodium lactate, suggesting that potassium ion itself may play certain roles in maintaining the physical properties of the SC (9). With increasing pH, the adsorption of lactic acid decreases, because of the ionization of the acid (20). In another study on strips of SC from human abdominal skin, the uptake of water by sodium lactate was greater than that by lactic acid, but the SC was plasticized by lactic acid and not by sodium lactate (15). Lactic acid also reduces the cohesion between the corneocytes and interferes with the bonding between the cells, which causes an increased cell turnover, especially at pH around 3 (49–51).

The concentrations used for treatment of ichthyosis and dry skin have ranged up to 12% (52). After treatment with 5% lactic acid combined with 20% propylene glycol, increased TEWL has been noted in patients with lamellar ichthyosis (53). However, lactic acid has been suggested to stimulate the ceramide synthesis and improve skin barrier function (54,55).

Safety

Lactic acid is caustic to the skin, eyes, and mucous membranes in a concentrated form (40). Compared to other acids, lactic acid has no unusual capacity to penetrate the cornea, so its injurious effect is presumably attributable to its acidity (40).

Immediately after the application of an AHA, stinging and smarting may be noticed; this is closely related to the pH of the preparations and the substances themselves (50,51,56). The emulsion type has been reported to influence the degree of stinging, where water-in-oil emulsions induced less stinging than ordinary oil-in-water ones (57). In normal skin, irritation and scaling may be induced when the acids are applied in high concentrations and at low pH (58). At a fixed lactic acid concentration, the desquamative effect is highly pH dependent, while at fixed pH, the turnover rate of skin is concentration dependent (51). Increased sensitivity to UV-light has also been detected, which raises concerns over long-term use (59). Due to insufficient safety data, the FDA recommends that lactic acid should be used up to a maximum level of 2.5% and a pH ≥ 5 (59).

PANTHENOL

Description

D-Panthenol is a clear, almost colorless, odorless, and viscous hygroscopic liquid, which may crystallize on prolonged storage (Tables 1 and 2) (6). Panthenol is an alcohol, which is converted in tissues to D-pantothenic acid (vitamin B_5), a component of coenzyme A in the body. The substance can be isolated from various living creatures, which gave the reason for its

name (Table 1) (Panthoten is Greek for everywhere) (60). Panthenol is very soluble in water, freely soluble in alcohol and glycerol, but insoluble in fats and oils (18). The substance is fairly stable to air and light if protected from humidity, but it is sensitive to acids and bases and also to heat (18). The rate of hydrolysis is lowest at pH 4 to 6 (18).

General Use

Panthenol is widely used in the pharmaceutical and cosmetic industry for its moisturizing, soothing, and sedative properties (60,61). It is also found in topical treatments for rhinitis, conjunctivitis, sunburn, and wound healing (ulcers, burns, bed sores, and excoriations); usually 2% is used (6,60). The mechanisms of action are only partly known. The hygroscopic alcohol can further be used to prevent crystallization at the spray nozzles of aerosols (18).

Effects on Skin and Hair

Topically applied panthenol is reported to penetrate the skin and hairs and to be transformed into pantothenic acid (60,62). Treatment of sodium lauryl sulfate (SLS)-induced irritated skin with panthenol accelerates skin barrier repair and SC hydration (61). Moreover, skin redness decreased more rapidly by panthenol treatment (61). Pantothenic acid can be found in normal hair (18). Soaking of hair in 2% aqueous solution of panthenol has been reported to increase the hair diameter up to 10% (63).

Safety

Panthenol has very low toxicity and is considered safe to be used in cosmetics (62). Panthenol and products containing panthenol (0.5–2%) administered to rabbits caused reactions ranging from no skin irritation to moderate-to-severe erythema and well-defined edema (62). Low concentrations have also been tested on humans, and those formulations did not induce sensitization or significant skin irritation (62). Contact sensitization to panthenol present in cosmetics, sunscreens, and hair lotion has been reported, although allergy to panthenol among patients attending for patch testing is uncommon (60,64).

PCA AND SALTS OF PCA

Description

"PCA" is the cosmetic ingredient term used for the cyclic organic compound known as 2-pyrrolidone-5-carboxylic acid (Tables 1 and 2). The "L" form of the sodium salt is a naturally occurring humectant in the SC at levels about 12% of the NMF (4) corresponding to about 2% by weight in the SC (17). The sodium salts of PCA are among the most powerful humectants (Table 3). PCA is also combined with a variety of other substances, such as, arginine, lysine, chitosan, and triethanolamine (1).

Effects on Skin

A significant relationship has been found between the moisture-binding ability and the PCA content of samples of SC (17). Treatment with a cream containing 5% sodium-PCA also increased the water-holding capacity of isolated corneum compared with the cream base (65). The same cream was also more effective than a control product containing no humectant, and equally effective as a similar established product with urea as humectant, in reducing the skin dryness and flakiness (65).

Safety

In animal studies, no irritation in the eye and the skin was noted at concentrations up to 50%, and no evidence of phototoxicity, sensitization, or comedogenicity was found (66). Minimal, transient ocular irritation has been produced by 50% PCA (66). Immediate visible contact reactions in back skin have also been noted after application of 6.25% to 50% aqueous solutions of sodium PCA (67). The response appeared within five minutes and disappeared 30 minutes after application. PCA should not be used in cosmetic products in which *N*-nitroso compounds could be formed (66).

PROPYLENE GLYCOL

Description

Propylene glycol is a clear, colorless, viscous, and practically odorless liquid having a sweet, slightly acrid taste resembling glycerol (Tables 1 and 2) (7). Under ordinary conditions it is stable in well-closed containers, and it is also chemically stable when mixed with glycerin, water, or alcohol (7).

General Use

Propylene glycol is widely used in cosmetic and pharmaceutical manufacturing as a solvent and vehicle, especially, for substances unstable or insoluble in water (7) (5,60). It is also often used in foods as antifreeze and emulsifier (5,7). Propylene glycol is also used as an inhibitor of fermentation and mold growth (5).

Effects on Skin

Propylene glycol has been tried in the treatment of a number of skin disorders, including ichthyosis (53,68,69), tinea versicolor (70), and seborrheic dermatitis (71), because of its humectant, keratolytic, antibacterial, and antifungal properties (7,72).

Safety

Propylene glycol has been given an acceptable daily intake (ADI) value of 25 mg/kg by the Joint FAO/WHO Expert Committee of Food (7,73). Poisoning has been found after oral doses of around 100 to 200 mg/kg to children (74–76) and after topical treatment with high concentrations in burn patients (77), but the alcohol is considered safe for use in cosmetic products (78).

Clinical data have shown skin irritation and sensitization reactions to propylene glycol in normal subjects at concentrations as low as 10% under occlusive conditions and in dermatitis patients as low as 2% (27,78). The nature of the cutaneous response remains obscure and, therefore, the skin reactions have been classified into four mechanisms: (*i*) irritant contact dermatitis, (ii) allergic contact dermatitis, (*iii*) nonimmunologic contact urticaria, and (*iv*) subjective or sensory irritation (79). This concept allows a partial explanation of effects observed by different authors (79).

PROTEINS

Description

Proteins and amino acids for cosmetics are based on a variety of natural sources. Collagen is the traditional protein used in cosmetics. Collagen has a complex triple helical structure, which is responsible for its high moisture retention properties. Vegetable-based proteins have grown in importance during recent years as an alternative to using animal by-products. Suitable sources include wheat, rice, soybean, and oat.

In cosmetics, native proteins can be used, but perhaps the most widely used protein types are hydrolyzed proteins of intermediate molecular weight with higher solubility. An increased substantivity is obtained by binding fatty alkyl quaternary groups to the protein. Improved film-forming properties can be obtained by combining the protein and polyvinylpyrrolidone into a copolymer. Such modifications may increase the moisture absorption compared with the parent compound. Potential problems with proteins are their odor and change in color with time. Furthermore, as they are nutrients, their inclusion in cosmetics may require stronger preservatives.

Efficacy and Safety

Amino acids belong to the NMF and account for 40% of its dry weight (4). Because of their relatively low molecular weight, they are capable of penetrating the skin and cuticle of the hair more effectively than the higher molecular weight protein hydrolyzates.

Salts of the condensation product of coconut acid and hydrolyzed animal protein (80) and wheat flour and wheat starch (81) are considered safe as cosmetic ingredients by CIR. The most frequent clinical presentation of protein contact dermatitis is a chronic or recurrent dermatitis (82). Sometimes an urticarial or vesicular exacerbation has been noted a few minutes

after contact with the causative substance (82,83). Hair conditioners containing quaternary hydrolyzed protein or hydrolyzed bovine collagen have induced contact urticaria and respiratory symptoms (83). Atopic constitution seems to be a predisposing factor in the development of protein contact dermatitis (83).

SORBITOL

Description

Sorbitol is a hexahydric alcohol appearing as a white crystalline powder, odorless, and having a fresh and sweet taste (Tables 1 and 2) (6). It occurs naturally in fruit and vegetables and is prepared commercially by the reduction of glucose. Sorbitol is most commonly available as 70% aqueous solution, which is clear, colorless, and viscous. It is easily dissolved in water, but not so well in alcohol. It is practically insoluble in organic solvents.

Sorbitol is relatively chemically inert and compatible with most excipients, but it may react with iron oxide and become discolored (7).

General Use

Sorbitol is used in pharmaceutical tablets and in candies when noncariogenic properties are desired. It is also used as sweetener in diabetic foods and in toothpastes. Sorbitol is also used as laxative intrarectally and believed to produce less troublesome side effects than glycerin (6). Its hygroscopic properties are reported to be inferior to that of glycerin (Table 3) (15,84).

Safety

When ingested in large amounts (>20 g/day), it often produces a laxative effect (6,7).

UREA

Description

Urea is another physiological substance occurring in human tissues, blood, and urine (Tables 1 and 2). The amount is of the order of 2% in urine. The extraction of pure urea from urine was first accomplished by Proust in 1821, and pure urea was first synthesized by Wöhler in 1828 (85).

Urea is a colorless, transparent, slightly hygroscopic, odorless or almost odorless, prismatic crystal, or white crystalline powder or pellet. Urea is freely soluble in water, slightly soluble in alcohol, and practically insoluble in ether (6). Urea in solution hydrolyzes slowly to ammonia and carbon dioxide, which may cause swelling of the packaging (6).

General Use

Urea is used as a 10% cream for the treatment of ichthyosis and hyperkeratotic skin disorders (85,86) and in lower concentrations for the treatment of dry skin. In the treatment of onychomycosis, urea is added to a medicinal formulation at 40% as a keratoplastic agent to increase the bioavailability of the drug (87).

Effects on Skin

An increased water-holding capacity of scales from psoriatic and ichthyotic patients has been observed after treatment with urea-containing creams (86,88).

Concern has been expressed about the use of urea in moisturizers, with reference to the risk of reducing the chemical barrier function of the skin to toxic substances (21). The increase in skin permeability by urea has been shown in several studies, where it has been found to be an efficient accelerant for the penetration of different substances (89–91). Not all studies, however, support the belief that urea is an effective penetration promoter (92,93), and treatment of normal skin with moisturizers containing 5% to 10% urea has been found to reduce TEWL and also to diminish the irritative response to the surfactant SLS (94,95). One moisturizer with urea also reduced TEWL in atopic patients (36,96) and made skin less susceptible against irritation to SLS (97). Improvement in skin barrier function has also been shown in dry skin (98) and in ichthyotic patients (86).

Safety

Urea is a naturally occurring substance in the body, as the main nitrogen containing degradation product of protein metabolism. Urea is an osmotic diuretic and has been used in the past for treatment of acute increase in intracranial pressure due to cerebral edema (6). No evidence of acute or cumulative irritation has been noted in previous studies on urea-containing moisturizers, but skin stinging and burning are reported after treatment with 4% to 10% urea creams in dry and lesioned skin (98–100).

CONCLUSIONS

A number of interesting humectants are available as cosmetic ingredients. Most of them have a long and safe history of use, and several are also naturally occurring in the body or accepted as food additives. The low–molecular weight substances are easily absorbed into the skin, providing a potential drawback of stinging sensations from some of them. The high–molecular weight substances usually do not penetrate the skin, but instead, they are suggested to reduce the irritation potential of surfactants. However, case reports of urticarial reactions have been reported after exposure to modified proteins (83).

The advantage with the larger and chemically modified materials are that they have an increased substantivity to target areas, whereas it is apparent that small amounts of several low–molecular weight hygroscopic substances have a questionable contribution to the water content of hair and SC in rinse-off products (Table 4).

Another issue worth considering is whether the obtained humectancy is the only mode of action. Some humectants may modify the surface properties and increase the extensibility of SC without influencing the water content. Furthermore, humectants may also modify skin barrier function and influence specific metabolic processes in the skin. One should also keep in mind that humectants can improve the cosmetic properties of the formulation, and some of them also facilitate marketing of the product just because of their names.

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11 Skin Care Products

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AN OVERVIEW OF EMULSION-BASED SKIN CARE PRODUCTS

A variety of skin care products exist in today's marketplace. They fulfill a variety of functions by either acting directly on the skin (e.g., moisturizers) or being a cosmetically elegant vehicle for the delivery of specific active ingredients (e.g., sunscreens or antipuretic or antiacne medicaments). In general, these products are categorized in the United States into three functional groups:

- Drugs. To prevent or ameliorate diseases by altering the structure and/or function of the body.
- Cosmetics. To beautify and improve the feeling or sensory aspects of normal and/or nondiseased skin. Dry skin would be included in this category.
- Cosmeceuticals. An intermediate classification for cosmetic products that may enhance the function of the skin. Currently, the Food and Drug Administration (FDA) does not recognize this category (1).

The three product groups can also be classified by their physical properties. Most common forms of skin care products are emulsions. Emulsions are mixtures of two insoluble materials that are stabilized against separation. An example is mixture of oil and water, which will not mix unless an intermediate emulsifier is incorporated into the mixture.

Different Types of Emulsions

Emulsifiers can act as solubilizers and spreading or dispersing agents. Correct use of emulsifiers permits one to formulate homogeneous mixtures, dispersions, or emulsions of oily, waxy substances with water. Solids may be dispersed in liquids or insoluble liquids within other liquids. Greasy anhydrous ointments can be designed to be more washable. These types of properties may be achieved by appropriate selection of emulsifiers, active ingredients, and other compatible ingredients in the vehicle.

Emulsions may be formulated of water in oil (w/o), oil in water (o/w), aqueous gel, and silicone in water. Other products may be formulated as semisolids containing oleaginous ingredients, absorption bases, and water-soluble types containing polyethylene glycol (PEG). Recently, there has been a growing interest in water-in-oil-in-water (w/o/w) emulsions, also referred to as multiple emulsions.

O/W emulsions are the most commonly formulated. These types of emulsions tend to feel less greasy and have a lower cost formulation because of a higher water content. W/O emulsions have historically been less popular because of a characteristic greasy, oily feel on application to skin. However, the development of newer emulsifiers has enabled a skilled formulator to develop w/o emulsions of a lighter texture. Silicone formulation aids may also be used to form stable water-in-silicone (w/Si) or w/o emulsions. These are polymeric surface-active agents with long bond lengths and wide bond angles. This provides for free rotation of functional groups permitting formulations of w/o and w/Si emulsions with exceptional elegance and good coverage when applied to skin (2). This enables formulation of stable emulsions with medium-to-low viscosity. These different chemical-type emulsions are commonly referred to as vehicles when "cosmetic"-active or drug-active ingredients are incorporated into them (Table 1).

Not all emulsifiers behave in the same way. Properties of the emulsifier will determine the emulsion type. Their compatibility with oils having different polarities is also of a critical concern. Emulsifiers will impact the desired sensory properties of the product such as color, odor, and desired viscosity (e.g., lotion or cream consistency).

Table 1 Examples of Vehicle Types

Type of emulsion	Examples
W/O	Cold creams, cleansing or evening creams (overnight creams)
O/W	Common moisturizers, hand and body lotions
Oleaginous	Petrolatum
Water soluble	Polyethylene glycol-based ointments
Aqueous gels	Lubricating jelly. Gelling agents such as Carbomers®, hydroxyethylcellulose, and magnesium aluminum silicate may be used in the formulation.
Absorption bases	Hydrophilic petrolatum; these vehicles may contain raw materials able to function as w/o emulsifiers permitting large quantities of water to be incorporated as emulsified droplets.

Abbreviations: W/O, water in oil; O/W, oil in water.
Source: From Ref. 3.

Different Types of Emulsifiers

Emulsifying agents, which are surface-active agents (surfactants), are available in a wide range of chemical types. These include nonionic, hydrophobic, lipophilic, ethoxylated, and nonethoxylated. A recent trend is to lower or even eliminate surfactants in an effort to minimize the already low irritation potential of the formulation. It is possible to formulate emulsifier-free emulsions with cross-linked acrylic polymer derivatives. These materials are hydrophilic polymers that are hydrophobically modified by adding an alkylic chain. These molecules, known as polymeric emulsifiers, provide additional formulation options for new product development (4).

FORMULATING HYDRATING CREAMS AND LOTIONS

The continuing development of biophysical instrumentation and test techniques has enabled formulation of highly effective skin care formulations. Formulators now have several options with respect to formulating new products. When initiating formulation development, it is important to understand project/product requirements, type of product(s), performance and aesthetics needs, formulation cost constraints, packaging needs, product claims, and formulation safety. To what part of the body will the formulation be applied, and at what time of the day, morning or overnight? Will makeup be applied over the product; will clothing come into contact with the product? Will the targeted consumer apply a fragrance to the body after application of the product, and if so, will the fragrances conflict? Once these requirements are defined, the formulator can consider active ingredients, emulsion systems, preservative systems, color, and fragrance.

Emulsions allow the formulating chemist to combine otherwise incompatible ingredients into an effective commercially desirable cosmetic product. The key to product development is the technique employed to select appropriate raw materials. Commonly used emulsifying agents are ionic (anionic or cationic) or nonionic. The function of the emulsifying agent is dependent upon the unique chemical structure of the emulsifier. Each emulsifier has a hydrophilic (water-loving) and lipophilic (oil-loving) part. Examples of hydrophilic moieties are polyhydric alcohols and polyethylene chains. Lipophilic parts may be a long hydrocarbon chain such as fatty acids, cyclic hydrocarbons, or combination of both. Nonionic agents may have hydrophilic action generated by hydroxyl groups and ether linkages, such as polyoxyethylene chains. Nonionic emulsifying agents can be neutral or acidic, giving formulators greater flexibility regarding pH requirements for cosmetic actives. Nonionics can be used in formulating w/o or o/w type emulsions and will help to mitigate the characteristic oily feel of w/o emulsions.

Thousands of emulsifying agents are available on the world market today. Choosing the best agent is the key responsibility of the formulator. Many agents used in the cosmetic and drug industry are classified by a system known as HLB number or hydrophilic-lipophilic balance number. This system, developed in the mid-1950s, is a useful starting point in emulsifier selection. In this system, each surfactant having a specific HLB number is used to emulsify an oil phase having an HLB required for a stable emulsion. Using an emulsifier or combination of emulsifiers matching the required HLB of the oil phase will form a stable emulsion. Limitations to this method include incomplete data for required HLBs of many cosmetic ingredients. Combinations of or single emulsifying agents giving the appropriate theoretical HLB may not be the optimal combination for emulsion stability or product

performance. Other emulsifying agents may work better and provide a more elegant formulation with greater efficacy. In addition, theoretical HLB numbers of complex mixtures may not follow a linear additive rule specified in the calculation (2).

In this classification system, emulsifying agents with an HLB of 10 would indicate a more water-soluble agent compared with one having an HLB of 4.

For nonionic detergents of the ester type:

$$\text{HLB} = 20\left(1 - \frac{s}{a}\right)$$

s = saponification number of the material
a = acid number of the fatty acid moiety of the product

For ethoxylated esters and ethers, when the saponification value is not known:

$$\text{HLB} = E + \frac{P}{5}$$

E = percentage of ethylene oxide
P = percentage of polyalcohol in the molecule

When the hydrophobic portion contains phenols and mono-alcohols without poly-alcohols, the equation can be simplified to:

$$\text{HLB} = \frac{E}{5}$$

Most nonionics fall into this category; manufacturers who provide HLB values in their product specifications most frequently use the latter formula (Table 2).

Mixtures of anionic and nonionic agents obtain the best emulsion; mixtures of cationic and nonionic emulsifiers may not be as elegant. Examples of nonionic emulsifiers are alcohol ethoxylates, alkylphenol ethoxylates, block polymers, ethoxylated fatty acids, sorbitan esters, ethoxylated sorbitan esters, and ethoxylated castor oil. The solubility of nonionic surfactants in water can often be used as a guide in approximating the HLB and usefulness.

Oil-in-Water Emulsions

O/W emulsions typically contain 10% to 35% oil phase; a lower-viscosity emulsion may have an oil phase reduced to 5% to 15%. Water in the external phase of the emulsion helps hydrate the stratum corneum of the skin. This is desirable when one desires to incorporate water-soluble active ingredients in the vehicle. Oil droplets in emulsions have a lower density than the phase they are suspended in. To have a stable emulsion, it is important to adjust the specific gravity of the oil and water phases as closely as possible. Viscosity of the water phase (external phase) may be increased to impede the upward migration of the oil particles.

Table 2 Relationship Between HLB Range and Water Solubility

Water solubility	HLB range
No dispersibility in water	1–4
Poor dispersion	3–6
Milky dispersion after agitation	6–8
Stable milky dispersion	8–10
Translucent to clear dispersion	10–13
Clear solution	13+
HLB	Application
4–6	W/O emulsifier
7–9	Wetting agent
8–18	O/W emulsifier
13–15	Detergent
15–18	Solubilizer

Abbreviations: HLB, hydrophilic-lipophilic balance; W/O, water in oil; O/W, oil in water.
Source: From Ref. 5.

Addition of waxes to the oil phase will increase specific gravity, but may have a profound effect on the appearance, texture, and feel on application to skin of the product. Increasing water phase viscosity is one of the most common approaches. Natural thickeners (alginates, caragenates, xanthan) and cellulosic (carboxymethyl cellulose) gums are used for this purpose.

Carbopol® resin is perhaps the most popular gum thickener for contributing toward emulsion stability, especially at higher temperatures. The addition of a fatty amine to a Carbopol resin will further enhance stability by strengthening the interface of the water and oil phases through partial solubilization into the oil droplets. Electrolytes and cationic materials will have a destabilizing effect on anionic sodium carboxymethyl cellulose and should not be used together. Veegum, an inorganic aluminum silicate material is also commonly used to thicken emulsions. Carbopol and Veegum may be used together to modify the characteristic draggy feel of Carbopol when used at the higher levels.

Emulsifier blends with HLBs ranging from 7 to 16 are used for forming o/w emulsions. In the blend, the hydrophilic emulsifier should be formulated as the predominate emulsifier to obtain the best emulsion. A popular emulsifier, glycerol monostearate and polyoxyethylene stearate blend is a self-emulsifying, acid-stable blend. Emulsifiers are called self-emulsifying when an auxiliary anionic or nonionic emulsifier is added for easier emulsification of the formulation. Formulating with self-emulsifying materials containing nonionic emulsifiers permits a wide range of ingredient choice for the formulator, especially with acid systems. In alkaline formulations, polyoxyethylene ether type emulsifiers are preferred with respect to emulsion stability.

An alternative to glycerol monostearate self-emulsifying emulsifier is emulsifying wax, National Formulary (NF). This emulsifier, when used with a fatty alcohol, will form viscous liquids to creams depending on the other oil-phase ingredients. Use levels may vary from 2% to 15%; at lower levels, a secondary emulsifier such as the oleths or PEG glycerides will give good stability. This system is good for stabilizing electrolyte emulsions or when other ionic materials are formulated into the vehicle. Polysorbates are o/w emulsifiers, wetting agents, and solubilizers that are often used with cetyl or stearyl alcohol at 0.5% to 5.0% to produce o/w emulsions (6).

Water-in-Oil Emulsions

Although less popular than o/w emulsions, these systems may be desirable when greater release of a medicating agent or the perception of greater emolliency is desired. Emulsifiers having an HLB range of 2.5 to 6 are frequently selected. When multiple emulsifiers are used, the predominant one is generally lipophilic with a smaller quantity of a hydrophilic emulsifier. These emulsions typically have a total of 45% to 80% oil phase.

During the last few years, formulators have become interested in more elegant w/o emulsions by formulating with new emulsifying agents, e.g., emollient such as esters, Guerbet alcohols, and silicones. Selection of a suitable emollient depends on ability of the material to spread on skin with low tack, dermal compatibility, and perceived elegance by the user. In achieving this elegance, some researchers suggest a correlation of emollient and molecular weight of the emollients. In these studies, viscosity of w/o creams has correlated with molecular weight of the emollients used in test formulations. High molecular weight coemulsifiers formulated with high molecular weight emollients gave more stable w/o emulsions. The polarity of the emollients used was found to be important as well. Emollients or mixtures of emollients with medium polarity gave test lotions the most desirable stability results (7). Anionic emulsifiers are generally inefficient w/o emulsion stabilizers, because more surface-active agents are often needed to stabilize these emulsions. Sorbitan stearates and oleates are effective emulsifiers when used at 0.5% to 5.0%; sorbitan isostearates, being branched chain materials, give a very uniform particle size for w/o emulsions.

Multiple Emulsions

Multiple emulsions are of interest to the skin care formulator because of the elegant appearance and less greasy feel of these formulation types. Two types of multiple emulsions are encountered in skin care, w/o/w, where the internal and external water phases are separated by oil, and oil-in-water-in-oil (o/w/o), where the water phase separates the two oil phases. The method of preparation for each multiple emulsion type is similar.

Benefits of these types of formulations are the claimed sustained release of entrapped materials in the internal phase and separation of various incompatible ingredients in the same formulation.

A suggested technique for forming a w/o/w emulsion is to first create a w/o primary emulsion by combining water as one phase with oil and a lipophilic emulsifier as the second phase in the traditional method. Next, water and a hydrophilic emulsifier is combined with the w/o primary emulsion at room or warm (i.e., 40°C) temperature with mixing forming a w/o/w multiple emulsion. These emulsions typically contain about 18% to 23% oil and 3% to 8% lipophilic emulsifier. The continuous oily phase is stabilized with about 0.5% to 0.8% magnesium sulfate. W/O emulsifiers have an HLB less than 6 and are frequently nonionic or polymeric. O/W emulsifiers have an HLB greater than 15 and are ionic with high interfacial activity. For o/w/o multiple emulsions, w/o emulsifiers have an HLB less than 6 with similar properties as a w/o/w w/o emulsifier. O/W emulsifiers have an HLB greater than 15 and are nonionic with lower interfacial activity.

Water-in-Silicone Emulsions

Silicone compounds have evolved into a class of specialty materials used for replacement, substitutes, or enhancers for a variety of organic surface-active agents, resulting in the ability to formulate products with unique properties. Previously, silicone compounds were available as water-insoluble oily materials almost exclusively. Newer silicone compounds such as polyethylene-oxide bases grafted to polydimethylsiloxane hydrophobic polymers, known as dimethicone copolyol emulsifiers, have been developed. These types of emulsifiers permit formation of water-in-cyclomethicone emulsions. Further work in this field led to adding hydrocarbon chains to silicone polyether polymers. This resulted in improved aesthetics to oil in silicone emulsions as well. Silicone copolyols exhibit high surface activity and function similarly to traditional emulsifiers. Unlike hydrocarbon emulsifiers with higher molecular weights, high molecular weight silicone emulsifiers can remain fluid. This gives very stable viscoelastic films at the water/oil interface. The ability to make silicones more formulator-friendly has led to development of several new silicone-based surfactants. Both a water-soluble and an oil-soluble portion are needed to make a surface-active molecule. Silicone surfactants substitute or add on silicone-based hydrophobicity creating a distinctive skin feel and other attributes of typical silicones as well as attributes of fatty surfactants. These emulsions may be prepared in a traditional two-phase method, e.g., 2% to 3% weight/weight (w/w) of laurylmethicone copolyol in 23% w/w oil phase can be mixed in a separate water phase with electrolyte to form a hydrating cream (8).

Water-Soluble Ointment Bases

PEG polymers are available in a variety of molecular weights. These materials are water-soluble and do not hydrolyze or support mold growth. For these reasons, PEGs make good bases for washable ointments and can be formulated to have a soft-to-hard consistency. PEGs dissolve in water to form clear solutions; they are also soluble in organic solvents. PEG ointment Unites States Pharmacopeia (USP) is a mixture of PEG 3350 and PEG 400 heated to 65°C, cooled, and mixed until congealed. To formulate a water-soluble ointment base, water and stearyl alcohol may be incorporated into this base.

Absorption Bases and Petrolatum

Absorption bases can serve as concentrates for w/o emollients; water may be added to anhydrous absorption bases to form a cream-like consistency. Petrolatum, a component of some absorption bases, has been shown to be absorbed into delipidized skin and to accelerate barrier recovery. Bases can be made washable by addition of a hydrophilic emulsifier. For example, formulation with polysorbate-type emulsifiers with polyoxypropylene fatty ethers will improve washability. These surfactants will form o/w emulsions with rubbing on skin. W/O petrolatum creams can be formulated by mixing 50% to 55% petrolatum with a sorbitan sesquioleate at 5% to 10% having an HLB of about 3 to 7 in one phase and water in a second phase. Both phases are blended at 67°C to 70°C with mixing.

OTHER INGREDIENTS

Consumer-perceived benefits of a cream or lotion are often a result of ingredients remaining on the skin after water and other volatile materials have evaporated. Emollients and other skin conditioners are commonly used for this reason. Following are frequently used ingredients to modify the feel of the emulsion on skin (Table 3).

Table 3 Examples of Moisturizer Ingredients and Their Functions

Ingredient	Use level (%)	Comments
Emollient esters	5–25	Modify the oily, greasy feel of mineral oil and petrolatum, light-to-moderate feel on skin.
Triglyceride oils	5–0	Light-to-heavy feel, often used as spreading agents.
Mineral oil/petrolatum	5–70	Heavy, oily feel, provides occlusion for appropriate vehicles.
Silicone oils	0.1–15.0	Helps to prevent soaping of formulations, improves spread on skin, is water repellent, and has skin-protective properties.
Humectants (Glycerin, Propylene Glycol, Sorbitol, Polyethylene glycol)	0.5–15.0	Moisture-binding properties help retard evaporation of water from formulation, control viscosity, and impact body and feel of emulsion.
Thickeners (Carbopol®, Veegum)	0.1–2.0	Help obtain viscosity, enhance stability, bodying agents.

Preservative Systems

Most formulations require preservative systems to control microbial growth. Microbial contamination with pathogenic microorganisms can pose a health risk to the consumer, especially from *Pseudomonas* infection in the eyes or from an existing illness. Microbial contamination may cause an emulsion to separate and/or form off-odors. Contaminated products are also subject to recall, which is undesirable from a commercial viewpoint.

Preservatives can be divided into two groups: formaldehyde donors and those that cannot produce formaldehyde. The former group includes DMDM hydantoin, diazolidinyl urea, imidazolidinyl urea, quaternium 15, and the parabens (esters of *p*-hydroxybenzoic acid), whereas preservatives such as Kathon GC, phenoxyethanol, and iodopropunyl butylcarbamate work by alternative mechanisms. The formulator is advised to consult appropriate preservative manufacturers to select the optimal preservative system for the emulsion (Table 4).

Table 4 Examples of Emulsifiers

Nonionic	
Polyoxyethylene fatty alcohol ethers	Very hydrophobic to slightly hydrophobic
Polyglycol fatty acid esters	Very hydrophobic to slightly hydrophobic
Polyoxyethylene modified fatty acid esters	Very hydrophilic to slightly hydrophilic
Cholesterol and fatty acid esters	Slightly lipophilic to strong lipophilic
Glyceryl dilaurate	Secondary emulsifier
Glycol stearate	Secondary emulsifier
Anionic	
Disodium laureth sulfosuccinate	
Sodium dioctyl sulfosuccinate	
Alcohol ether sulfate	
Sodium alkylaryl sulfonate	
Cationic	
PEG-alkyl amines	
Quaternary ammonium salts	
Self-emulsifying bases (form o/w emulsions)	
PEG-20 stearate and cetearyl alcohol	
Cetearyl alcohol and polysorbate 20	
Glyceryl stearate SE	
Absorption bases	
Lanolin alcohol and mineral oil and octyldodecanol	
Petrolatum and ozokerite and mineral oil	

Abbreviation: PEG, polyethylene glycol.

SKIN CARE EMULSIONS FOR THE AGING POPULATION

Consumers frequently refer to young skin as having a healthy glow, radiance, or vitality that tends to diminish over time. These changes in appearance in part are related to the diminished ability of older skin to retain moisture. Cosmetic and cosmeceutical products that address the needs of the aging population by enhancing appearance are predicted to grow in product sales at twice the rate of the overall cosmetic market in the near future (9).

Early moisturizers were formulated primarily with lipids on the basis of the assumption that fats and oils make the skin soft and supple. In reality, it is difficult to specify exactly how much water content of skin is required for adequate moisturization. The water content of keratinocytes in the basal layer is about 70%. This decreases to about 15% to 20% as mature stratum corneum reaches the desquamating layers (10). Current moisturizing strategy is to:

- Increase water-holding capacity of the stratum corneum by external application of hydroscopic ingredients, known as humectants. These ingredients act in the same way as natural moisturizing factor (NMF) in skin; some materials used in moisturizers such as lactic acid and urea are components of NMF.
- Hold water in the stratum corneum by deposition of a water-insoluble oily material on the skin surface; these materials are known as occlusive agents. Oily materials mimic the effect of the natural lipid bilayers of the skin to restrict evaporation from the surface, i.e., petrolatum.

In general, required levels of occlusive agents are relatively high and will cause a formulation to become tacky when applied to skin. Emulsification of occlusive agents in combination with hydroscopic agents can reduce the ability of the agent to be effectively occlusive in the finished product. Humectants are used to improve moisturization of the skin, but there are conditions when humectants may actually deprive the skin of water. Once a humectant has absorbed water, the activity coefficient of water is lowered. "If the water in skin tissue does not have a lower water activity compared to the surrounding humectant-water blend, water molecules will not be transferred to skin." Consideration should be given in the selection of humectant to ensure that the formulation does not hamper the enzyme-controlled normal desquamatory process. Glycerin is frequently the humectant of choice for this reason. More recent formulations contain hydrophilic polymers (Table 5) that may function as humectants and help smooth skin as well (10) (Table 6).

Table 5 Hydrophilic Polymers Used in Skin Care Moisturizers

Alginic Acid
Chitosan (and salts)
Collagen
Hyaluronic Acid

Source: From Ref. 10.

Table 6 Examples of Common Skin Care Moisturizing and Conditioning Agents

Emollients	Humectants	Occlusives
Acetylated lanolin	Acetamide MEA	Acetylated lanolin alcohol
C14-15 alcohols	Ammonium lactate	Caprylic/capric triglyceride
Dimethicone copolyol	Copper PCA	Cetyl ricinoleate
Hexyl laurate	Glucuronic acid	Dimethicone
Isopropyl myristate	Glycerin	Hydrogenated lanolin
Lanolin	PCA	Mineral oil
PPG-20 cetyl ether	Propylene glycol	Myristol myristate
Squalene	Sodium PCA	Petrolatum
Sucrose oleate	Sorbitol	Soybean lipid
Wheat germ glycerides	Urea	Squalane

Source: From Ref. 10.

Emulsion formulators are aware that the health of the epidermis may be affected by

- the intracorneal lipid layer, its formation, hydrolysis, and oxidation;
- enzymatic dependency of synthesis of NMF; and
- climatic changes.

A disadvantage of formulating with glycerin-based moisturizers is that they are poor solvents for cosmetic lipids (10). When it is desirable to have a lipophiloic "cosmetic active" in the formulation, the formulator must use skill and experience to optimize the formulation.

FORMULATING FOR IMMEDIATE IMPROVEMENT IN APPEARANCE AND TEXTURE OF SKIN

Various strategies are available to formulate emulsions that provide immediate cosmetic benefits to skin. Epidermis of young skin is translucent; it allows light to partially pass through it. Skin that appears translucent will exhibit a shine or glow. The layer between the epidermis and dermis has ridges known as rete pegs. In aging skin, this region becomes smaller and flatter, tending to reduce the translucent effect of skin. Further, keratinocytes at the surface of the skin do not slough off as quickly. This results in skin that has a dull and uneven appearance. Other contributing factors to loss of "skin glow or radiance" are the irregular pattern of melanocytes that tends to develop in aging skin.

In normal daylight, one observes light that is partially reflected from the surface of stratum corneum and light that is partly reflected back from the dermis. Younger-looking skin will reflect light from lower epidermis and blood vessels in the dermis with color contributed from melanin and hemoglobin. Incident light reflecting off dry skin will not penetrate as deeply and reflect back with a dull appearance.

Interference Pigments

One approach to altering the way light is reflected back from skin is to formulate with interference pigments. This approach initially used in facial products has recently found popularity in body moisturizers. Effect-enhancing pigments are used to "add natural, transparent luster to skin"; they can improve the tactile qualities of the skin by giving the emulsion a silky feel. The same effect-enhancing pigments may be used to impart an elegant luster to the appearance of the product (11).

Effect pigments are composed of thin, translucent platelets that produce luster by partially reflecting and partially transmitting light. Pigments are available as natural pearl, mica, and bismuth oxychloride-based materials. Bismuth oxychloride crystals have a "brilliant" white pearlescense; some grades create metallic effects while other grades provide a "subtle luster and smooth feel." Natural pearls can provide a "satiny luster" to emulsions. Metal oxide-coated mica pigments with thin films of iron oxide or titanium dioxide are most commonly used. The colors in these materials will shift with the viewing angle to create complex iridescence on curved body surfaces. Smaller platelets provide a "satiny-smooth, silky luster, while larger ones provide sparkle, glitter, and a lively appearance (11)." Use of appropriate particle size and color combinations can give the skin a "radiant glow."

Interference pigments are formulated in skin care products at levels of 0.1% to 2.0% by weight, depending upon the qualities the formulator wishes to achieve. The selection of particle size can help diminish the appearance of age spots, fine lines, and uneven skin color. Interference effects are maximized when a variety of particle sizes are formulated.

Soft Focus Effects

Fine particles, such as microspheres, are used in emulsions and anhydrous formulations to enhance the feel and appearance of skin. The chemical compositions of microspheres are diverse. Examples are polymethyl methacrylate, polyethylene, ethylene/acrylayes copolymer, nylon, polyurethane, silicone resins, and silica. Selection of the appropriate material can provide "optical blurring" effects to the formulation, minimizing the appearance of fine lines and uneven skin tone. Some skin care products can deposit a transparent layer on the skin, making fine lines more visible to the eye. Formulation with appropriate microspheres can help

Table 7 Examples of Refractive Indexes (Various Sources)

Material	Refractive index
Air	1.00
Perspiration	1.33
Polyethylene	1.45
Titanium dioxide	2.51
PMMA	1.49
Silica	1.45
Skin	1.62
Microspheres (general)	1.41–1.53
Propylene glycol dibenzoate (ester)	1.54
Phenyl trimethicone (silicone)	1.46
PPG-3 benzyl ether myristate (ester)	1.465
Dimethicones, cyclomethicones (silicone)	1.375–1.403, 1.394–1.398

to minimize this effect and give the skin an enhanced appearance (12). Formulating with varied particle size will further help minimize the appearance of uneven skin (13).

When formulating with interference pigments and soft focus materials, a critical consideration is the refractive index (RI) of the primary vehicle and the material(s) to be incorporated into the vehicle. When the vehicle is applied to skin, the portion of the vehicle remaining on the skin after evaporation is considered the "primary vehicle." For example, an emulsion of oils and polymers applied to skin, the oil/polymer portion will be the primary vehicle after the water has evaporated from the skin's surface. In general, the RI of the light-diffusing particle must be greater than that of the skin and the vehicle to be effective (Table 7).

Emollient Esters

Chemically, esters are the covalent compounds formed between acids and alcohols. Esters can be formed from inorganic and carboxylic acids and any alcohol. Esters, when formulated in cosmetic emulsions, have diverse functions. They serve as emollients, skin conditioners, solvents, fragrance compounds, and preservatives (14).

More recently, emollient esters have been used in place of more expensive silicones to provide aesthetic benefits to cosmetic emulsions. Esters can be formulated with silicones to enhance stability and feel of the emulsion when applied to skin (15). Esters that function as co-emulsifiers provide improved skin adhesion of the reduced formulation tackiness and can improve hydration properties of humectants.

Esters display properties that reflect their chain length and structural arrangement of their two starting materials. For this reason, different esters will have differing emollience. A simple monoester of a short-chain fatty alcohol or acid will possess a light feel. Branched esters will feel nongreasy; chemically more "complex" pentaerythrityl esters will have a "cushiony feel" (14). The structural composition of the ester will also affect its spreading behavior on skin. Branched esters typically have a higher spreading factor. Spreading will begin to decrease as the molecular weight increases. Emollient esters affect the viscosity of the emulsion, either improving texture and formulation aesthetics or detracting if incorrectly formulated. When formulating with coated pigments, one must ensure that the selected ester is compatible with the coating. Another consideration is the pH of the finished product. Below a pH of 3.4, esters tend to hydrolyze, resulting in a product that may develop an undesirable odor (16).

Polymers

Polymers are small molecules that are chemically connected in long repeating units. Polymers are ubiquitous in nature. The DNA of all living cells and the protein and starches in our foods as well as the tires of our automobiles are all composed of polymers. The use and function of polymers in cosmetic emulsions are equally diverse. Polymeric emulsifiers, such as those based on silicone or polyacrylic acids, are used as emulsifiers. These polymers have cationic charges that are substantive to skin and impart a smooth, conditioning effect. Others polymers are formulated in emulsions to create the sensation of firming skin, minimize interference pigments and other solid particles from rub off to clothing, and provide water resistance to sunscreen containing emulsions. These polymers form a film on the skin's surface (Table 8).

Table 8 Examples of Polymers (Various Sources)

Polymer	Type	Potential application
Acrylates/C10-30 alkyl acrylate cross-polymer	High molecular weight polyacrylate	Primary emulsification (O/W)
Carbomer	Acrylic acid	Synthetic thickener
Acrylates/steareth-20 methracylate copolymers	Acrylic polymer emulsion/anionic	Thickener
PEG-150/decyl alcohol/SMDI copolymer	Hydrophobically modified nonionic polyol	Low pH formulations, cationic conditioners, O/W sunscreens, cationic silicone emulsions
Caprrylic/capric triglyceride sodium acrylates copolymer	Polyacrylic acid	W/O emulsions
PVP/eicosine copolymer	Copolymers of vinylpyrrolidone	Oil soluble, rub resistance in sunscreen
Tricontanyl PVP	Copolymer of vinylpyrolidone	Oil soluble, rub resistance for pigments and sunscreens

Abbreviations: W/O, water in oil; O/W, oil in water.
Source: From Ref. 14.

FORMULATING EFFECTIVE COSMECEUTICALS FOR AN AGING POPULATION

An aging consumer population seeks products to address fine lines and wrinkles, improve the appearance of an uneven skin tone, smoothen rough-textured skin, and reduce skin dispigmentation referred to as "age spots." Advances in molecular biology have enabled research investigators to develop numerous in vitro screening protocols demonstrating the potential of various cosmeceutical ingredients to help improve the appearance of aging skin.

Peptides, Vitamin Derivatives, Botanicals

In vitro data may produce very dramatic results supporting efficacy of cosmeceutical agents. Many of these agents must be properly formulated and should be properly tested in vivo to confirm they will function as desired to meet consumer expectations. Cosmeceutical agents must be compatible and stable in the vehicle they are formulated in to be effective. For example, peptides are available with variations in the number of amino acids and sequence. The peptide must be designed to have the ability to penetrate skin in order to be effective. One approach is to add a lipohilic chain, such as a palmitate (Table 9), to the peptide. A copper peptide is commercially available; to be effective, it must be formulated at significantly higher levels compared with the palmitoyl pentapeptide (17–19).

Published literature supports claims that retinoic acid improves the appearance of wrinkles, promotes collagen formation, and evens skin tone. Retinoic acid has limited stability, and consumers frequently experience dry, irritated skin during product use. To promote stability, formulation exposure to oxygen and light should be minimal. Formulating with an antioxidant and encapsulation of the retinoic acid are other options. The primary package should be designed to be oxygen and light impermeable. Irritation potential may be reduced by formulating with an appropriate retinoic acid derivative. Retinol is better tolerated by skin than *trans*-retinoic acid (20). Incorporation of anti-inflammatory agents may further mitigate irritation. Sugar amines such as glucosamine and *N*-acetyl-glucosamine can help hydrate skin and reduce fine lines/wrinkles and facial hyperpigmentation. Glucosamine tends to be unstable in formulations formulating with antioxidants, and at an acidic pH it may help overcome this problem (21). Formulations with glycolic acid are associated with an increase in sensitivity to solar exposure and sunburn cell formation in skin. For this reason, products with glycolic acids should also contain sunscreens.

Medicinal and cosmetic use of botanicals has a long history spanning many centuries. Selection of a botanical is influenced by experience passed on from generation to generation. Despite this long history of use, traditional medicine has not been officially recognized by many countries (22). In recent years, interest has increased regarding use of botanicals in skin care. Data addressing safety, quality, efficacy, and guidelines for formulating with botanicals to achieve optimal benefits are lacking. The formulator of botanical-based products

Table 9 Cosmeceutical Ingredients

Ingredient	Claimed benefit	Mechanism	Formulation consideration
Botanicals: soy, green tea, pomegranate, red clover, curcumin, resveratrol (in skin and seeds of grapes)	Soy: skin tone evening, improvement in dyspigmentation	Inhibition of PAR-2 activation by protease inhibitors	Source of soy important.
	Green tea: UVB-induced formation of thymine dimers (a marker for DNA damage) inhibited a 5% pretreatment prior to UVB exposure inhibited keratinocyte damage	Quenching of reactive oxygenating species (ROS); also, modulation of NF-$\kappa\beta$ pathway, a signal transduction pathway responsive to UV radiation	Green tea extract tested in vivo, applied topically to skin in a solution of ethanol or water. A 5% GTP solution was effective, 10% was optimal (1–10% solutions demonstrated a dose-dependent response) (23).
Milk thistle (silibinin)	Protect skin from UVR	Antioxidant, free radical scavenger, downregulates chemically induced lipoxygenase, TNFα, and IL-1α in mouse skin.	
Hydroxyacids, e.g., Alpha, beta, poly, and bionic acids	Photoaging and hyperkeratosis (age spots and hyperkeratotic lesions)	Antioxidant	pH of final product is low, $\leq$ 4.0 hydrolysis of esters in formulation will occur causing an off-odor.
	Increased dermal thickness	Bionic acid inhibits matrix metalloproteinase enzyme activity responsible for degradation of skin's matrix and structural integrity (wrinkle formation, skin laxity, and telangiectasia) Increased production of collagen and fibroblast proliferation	Sun sensitivity with α and β Acids.
Peptides, e.g., palmitoyl pentapeptide	Improve appearance of fine lines and wrinkles of the eye area	Stimulation of type I and type III collagens and fibronectin production	Peptide lipidated to penetrate skin.

(*Continued*)

Table 9 Cosmeceutical Ingredients (*Continued*)

Ingredient	Claimed benefit	Mechanism	Formulation consideration
Other peptides, e.g., acetyl hexapeptide-3	Wrinkle reduction (limited data available)	Inhibits calcium-dependent catecholamine release from and assembly of SNARE protein complex	Short amino acid sequence to facilitate cell membrane permeability.
Miscellaneous vitamins:			
Vitamin C (ascorbic acid, ascorbyl phosphate)	Wrinkle reduction, improvement in skin tone evening and texture	Improvement in skin collagen, reduced pigment transfer from melanocyte to keratinocyte	Stability, proper formulation pH, penetration into skin.
Vitamin B3 (niacinamide and its esters)	Improved skin tone, reduced dyspigmentation	Antioxidant	Formulate at appropriate pH to avoid hydrolysis.
Vitamin E (tocopherol and tocopherol acetate)	Protection against UV-induced effects to skin, reduced inflammation of skin	Antioxidant	Stability against oxidation, oil-soluble forms are less elegant, acetate form is subject to hydrolysis in formulation.
Retinoic acid (functional form of vitamin A)	Wrinkle reduction via thickened skin.	Increased epidermal thickness and ground substance inhibit collagenase production.	Oxygen and light render material unstable. Antioxidants may improve stability.
	Reduce appearance of dyspigmentation	Reduce expression of tyrosinase	
Sugar amine, e.g., *N*-acetyl-glucosamine	Moisturization, reduce fine lines/wrinkles	Precursor of hyaluronic acid, a water-binding component of skin	Tends to be unstable, creating a brown colored product.
	Improve skin tone	Inhibits tyrosinase, thus inhibiting melanin production	

in addition to information supplied by the manufacturer is advised to consult other resources such as the World Health Organization (22), Journal of Nutrition (24), and other reliable published data.

Two promising groups of botanically derived agents appear to hold promise, as chemotherapeutic treatments for aging skin are polyphenolic antioxidants (catechins and flavonols) and isoflavones. Green tea contains epigallocatein-3-gallate (EGCG), and grape seed contains polyphenolic antioxidants. Silymarin found in milk thistle and genistein found in soybean extract are other examples of useful ingredients for photoaging (25).

Many botanical extracts are available to the formulator. Plant constituents of extracts vary with respect to chemical compounds. Variations in solubility and stability have potential to cause shelf life and stability challenges of the finished product over time. Many extracts have a dark color or an odor that may create aesthetic concerns. Extraction methods intended to lighten color or mitigate odor may remove a compound with the desired activity. To minimize aesthetic and stability concerns, formulators should consult with the extract manufacturer regarding availability of technical information addressing polarity of plant-derived oils and optimal formulation pH range for extracts containing alkaloids. Botanically derived lipids are often not hydrogenated and are subject to oxidation promoting product rancidity (26). Pharmaceutical grade extracts are typically 5 to 10 times stronger than cosmetic grade extracts. Cosmetic extracts may be aesthetically acceptable in emulsions. They may lack key desirable chemical constituents. Alternatively, pharmaceutical grade extracts are very resinous, dark in color, and not soluble in many cosmetic formulations (27).

Notes from a Herbalist: Formulating with Botanical Extracts

A tincture is a solution of soluble plant constituents in a solvent known as the menstrum. Poor filtration, exposure to light, temperature changes from warm to cold, or chemical degradation of extractives can cause precipitation to occur. The precipitate may contain active constituents or inert proteins. Precipitation can be minimized by storage at constant temperature and avoidance of exposure to light. Massive precipitation, development of a marked color change, and "off" odor indicate that the tincture should be discarded. Alkaloids in extracts have diverse medicinal benefits. Acidification of the extraction solvent may increase potency, but efficacy may be neutralized by mixing with tannins. Glycerine is commonly used as an extraction solvent when it is undesirable to use alcohol. This type of extract is referred to as a glycerite. Glycerites tend to be less potent than alcoholic extracts and have a shorter shelf life.

Vegetable oils are good extraction solvents for many plant constituents. Herbalists are concerned that they are also good solvents for pesticides and herbicides. For this reason it is advisable to formulate with organic certified organic vegetable oils, ideally cold-pressed oils (28).

FUTURE FORMULATION CHALLENGES

Cosmeceutical ingredients have been popular for many years, and new cosmetic active agents are continuously being identified. Many of these active ingredients have excellent in vitro data to support claims, but are lacking in vivo data. Further, formulators often formulate the active in an existing prototype rather than employing a strategy of formulation optimization. Consumers have come to expect functional cosmetic products. Products that fail to deliver on consumer expectations are unlikely to succeed long term in the marketplace (29).

Future formulation challenges will be to:

- Determine the optimal emulsion system to effectively deliver the desired ingredient to the viable epidermis via the stratum corneum (partition coefficients, penetrant polarity).
- Understand the influence of formulation characteristics on skin delivery (influence of the emulsifier, solubility characteristics of the primary emollient, or solvent and influence of emollients in general).
- Continuously advance regarding knowledge of skin molecular biology, specifically the intended region of product use on the body.

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12 Tests for Skin Hydration

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INTRODUCTION

Writing about skin hydration means writing simultaneously about dry skin and its treatment by moisturizers (1). Dry skin has never really been defined in a repeatable way. In fact, this expression prejudices into believing that the skin does have reduced water content, although this was never confirmed or denied. Hopefully, the recent availability of near-InfraRed (IR)-based water measurement will now allow to resolve this issue (2).

Experimental models used for measuring skin hydration are basically clinical models using or not using noninvasive bioengineering measurements. To ensure meaningful results, the outlines of the intended studies should be of modern design, incorporating blinding, randomization, and a suitable statistical control (particularly if different products are to be compared). This last point means including a predetermined adequate number of subjects in the study. The general ethical and legal frames of such clinical studies required for claim support are well defined in corresponding monographs or publications covering extensively the general procedures to be followed and the prerequisite information needed about the products to be tested (3,4).

Regardless of the method used, a further important point concerns standardization of the experimental conditions. To obtain acceptable and reproducible results, measurements should be performed with relaxed patients and/or volunteers already acclimatized for at least 20 minutes to controlled ambient temperature and relative humidity conditions. Both factors mainly affect activity of the sweat gland, but other parameters should equally be considered with attention to, e.g., anatomical skin site, test products remaining or not on the skin, and correct handling of the measuring equipment, if any. All these possible influences on measurement outcome have been discussed in detail in recent guidelines and in pertinent reviews (5–8).

A CLINICAL EVALUATION: THE REGRESSION METHOD

The dermatologist can perfectly clinically grade a given state of skin dryness (e.g., surface roughness, squames, and fissures). Clinical evaluation and grading of skin hydration are based on visual and tactile evaluation of clinical signs. There are numerous possibilities of testing, but basically these rely on the regression method, published in 1978 by Kligman (9), which is still used as an industry standard. Briefly, female subjects with moderate to severe xerosis of the legs are selected following strict criteria. The test products are applied under controlled conditions by trained employees twice daily five days a week for three weeks. Three days after the treatment ends, the follow-up period begins. Scoring is also completed three and seven days later. Treatment period may be shortened to two weeks, if necessary. Following a published guideline ensures that clinical scoring of the hydration state of the skin surface will be conducted on the basis of the same definitions (5). Caution is given upon scoring by the subjects themselves, as their perception of their skin condition may not be the same as that of the dermatologist's perception (5,10).

USING BIOENGINEERING MEASUREMENT METHODS

A large number of bioengineering methods are now available to evaluate hydration (or dryness) of the skin directly or indirectly. Inclusion of these methods in the study protocol opens many possibilities for getting meaningful results such as design variations, optimization

of the claim support, and also, most importantly, improvement of cost effectiveness by shortening the duration of experiment, using a lower number of subjects, and strengthening the statistical evaluation.

Concerning the numerous techniques available for the evaluation of skin hydration, the reader is referred to recent monographs describing these methods in a detailed fashion (10–15). They mainly include measurements of electrical properties, spectroscopic methods such as IR absorption spectroscopy and emission, evaluation of the barrier function of the stratum corneum (SC), measurement of mechanical properties, transient thermal transfer, nuclear magnetic resonance imaging, skin surface topography, and scaling evaluation. Most frequently, bioengineering techniques based only on the electrical properties of the SC together with measurement of transepidermal water loss (TEWL) are used. Other methods remain confined to research laboratories. However, as stated in the introduction, recent availability of near-IR-based water measurement will now allow to improve hydration measurements and to better define product efficacy (2,16,17).

Static Measurements

Short-Term Tests/Single Application

The tests are conducted most of the time on the inner side of the forearm of healthy subjects and allow a randomized side-to-side comparison of test products with a placebo or vehicle, a known active product, and an untreated control skin. Four to six products may be simultaneously tested. The products are applied at the rate of 2 mg/cm^2. Two different experimental designs may be used:

1. The test products are left in place for one hour (or another suitable duration, e.g., 3 hours) (18). Measurements are conducted at different times thereafter. Removal of excess or non-penetrated product is preferable before measuring, especially if the preparation contains a high proportion of lipids. Most moisturizers show a rapid increase of measured hydration values (Fig. 1).
2. The test products may be applied on similar areas at the same rate but under occlusion, with a standard occluding patch overnight for 16 hours. The next morning, measurements are conducted in the same way as in part 1 beginning one hour after removal of the occlusion patch (Fig. 2). This last procedure better picks up the activity of a humectant contained in the test preparation, whereas the vehicle effect is strongly attenuated by the uniform conditions encountered under the occlusion patch.

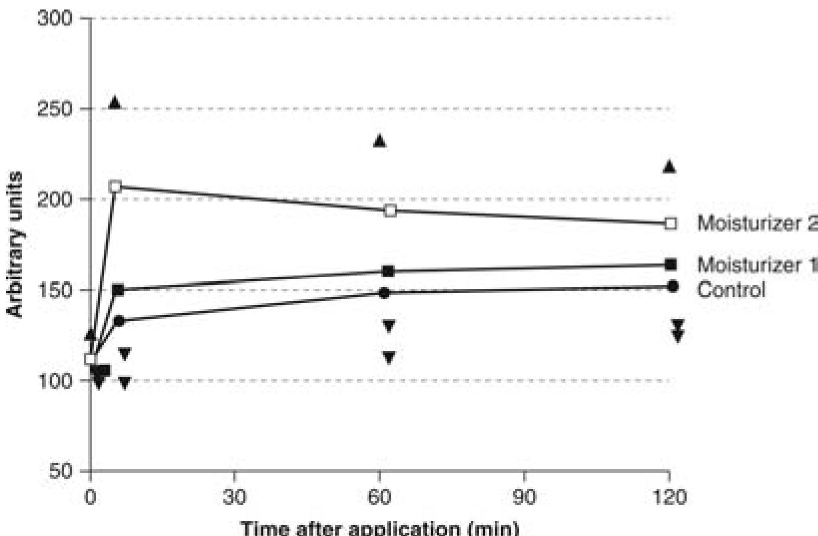

Figure 1 Example of hydration changes over time after one-hour application of two different O/W moisturizers containing both 2% urea as humectant (measurements conducted with the NOVA DPM 2003; means ± ½SD, ▲▼). ■, moisturizer 1; □, moisturizer 2; ●, control (untreated skin). Start values (time = 0) measured before application of the products.

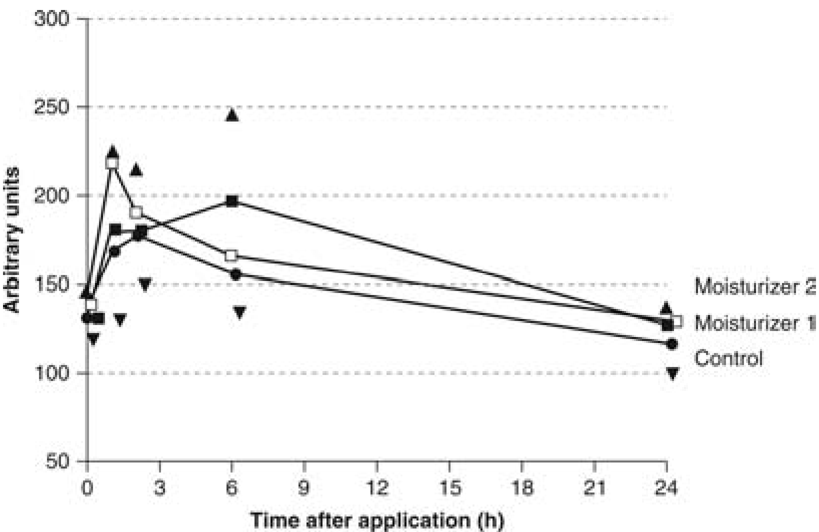

Figure 2 Example of hydration changes over time after 16-hour application of two different O/W moisturizers containing both 2% urea as humectant (same products as in Fig. 1; measurements conducted with the NOVA DPM 2003; means ± ½SD, ▲ ▼). ■, moisturizer 1; □, moisturizer 2; ●, control (untreated skin). Start values (time = 0) measured before application of the products.

Long-Term Tests/Multiple Applications

The design of these tests and selection of subjects is similar to the regression method previously described but with a modified and shortened regression protocol (19). The treatment period extends over one week only, and the regression phase takes place over the following week. Bioengineering measurements are conducted 12 to 16 hours after the treatment or moisturizer application, and for the last time on the Monday following the regression week. Inclusion of these noninvasive measurements allowed rapid and reliable product performance evaluation.

Dynamic Measurements

These tests, in addition to the classic evaluation of skin hydration, provide information on dynamic properties of the SC (20,21). These properties are likely to be modified by the humectants (e.g., glycerol, urea, α-hydroxy acids) incorporated in the moisturizers used for treatment. Generally speaking, dynamic function tests are characterized by the assessment of the skin's response to a given external stimulus that can be physical (e.g., water, occlusion, stretch, and heat) or chemical (e.g., drugs and irritants) in nature. These dynamic tests may be used either during short-term or long-term product testing, and will usually be performed before and at different time points after treatment.

The Sorption-Desorption Test

This test gives information about the water-binding capacity of the uppermost layers of the SC (20,21). It is best conducted using measurement devices that are able to measure hydration on a wet surface and that give instantaneous readings on contact with the skin.

The first value represents the hydration state of the SC. Then 50 μL of distilled water is pipetted onto the skin, left in place for exactly 10 seconds, and wiped with a soft paper towel. Then hydration is immediately measured. Further measurements are taken at 0.5, 1, 1.5, and 2 minutes. Parameters such as hygroscopicity, water sorption capacity, water-holding capacity, and accumulated water decay may be calculated from the measurement curve and used to characterize the state of the SC and/or different properties of the tested products (Refs. 20 and 21, Fig. 3).

The Moisture Accumulation Test

This test gives information about the quantity of moisture the SC may accumulate during a given time (20,21). It is conducted with a device that can measure continuously after bringing the probe in contact with the skin surface. The probe then remains on the skin for three

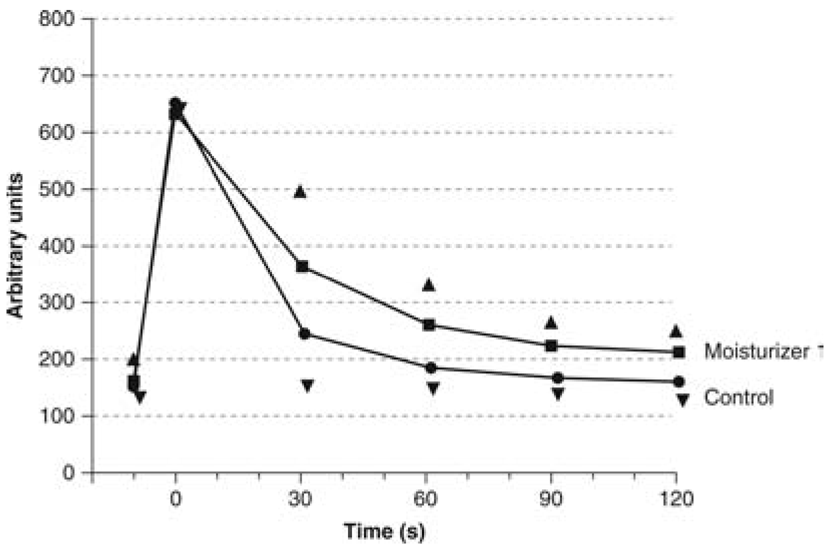

Figure 3 Time course of hydration changes during a SDT performed 60 minutes after a single one-hour application of a moisturizer (moisturizer 1 from Figs. 1 and 2; measurements conducted with the NOVA DPM 2003; means ± ½SD, ▲). ■, moisturizer 1; ●, control (untreated skin). *Abbreviation*: SDT, sorption-desorption test.

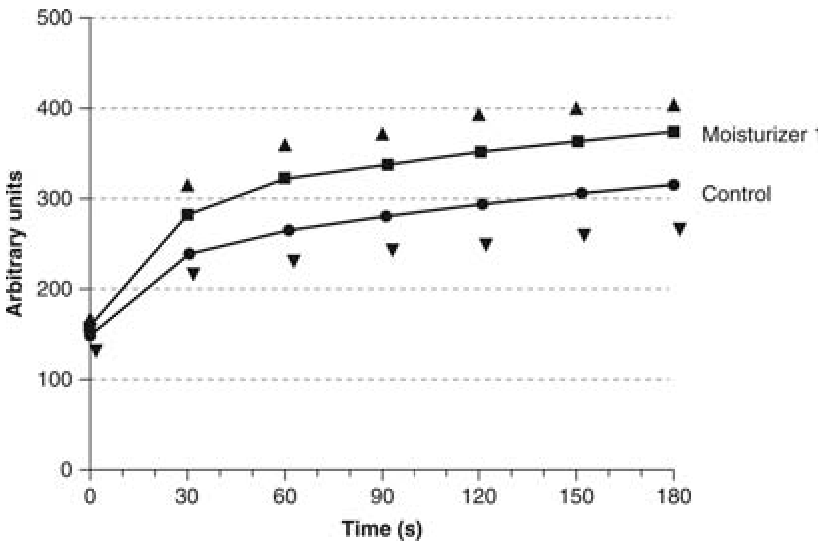

Figure 4 Time course of hydration changes during a MAT performed 60 minutes after a single one-hour application of a moisturizer (moisturizer 1 from Figs. 1 and 2; measurements conducted with the NOVA DPM 2003; means ± ½SD, ▲ ▼). ■, moisturizer 1; ●, control (untreated skin). *Abbreviation*: MAT, moisture accumulation test.

minutes, thereby creating occlusive conditions. The moisture accumulation test (MAT) measures the accumulation of water under the probe every 0.5 minutes. Water accumulation is evaluated by calculating the area under the time curve until three minutes (Fig. 4).

The Plastic Occlusion Stress Test

The plastic occlusion stress test (POST) may also be considered a dynamic test. It gives information about SC hydration, integrity of the barrier function, and SC water-holding capacity (20,21). It consists of occluding the skin with a plastic chamber (e.g., Hilltop chamber or a similar occlusive device) for 24 hours. Then the occlusion is removed, and the evaporation of the accumulated water is measured each minute for 30 minutes as TEWL. The TEWL-technique has been thoroughly described in recent reviews and guidelines (8,13–15,22,23). The measurement is called skin surface water loss (SSWL) and not TEWL, because it does not

Table 1 Moisture-Related Skin Types and Corresponding Corneometer CM 825 Units

Clinical grade	Arbitrary units (Corneometer CM 825)
Very dry	< 30
Dry	30–40
Normal	> 40

represent the true TEWL but the sum of the TEWL and the evaporation of water trapped within and over the SC under the occlusive equipment, at least at the beginning of the measurement period. The SSWL decay curve appears biexponential. During the first minutes of evaporation, the SSWL is proportional to SC hydration. At the end of the dehydration time, SSWL is greatly reduced and mainly TEWL is measured.

Near-IR-Based Spectroscopic Measurements

Methods using near-IR spectroscopy for evaluating the water content of the SC have been used for several years. They are very sensitive to changes in the water content of the tissue, and they allow fast determination, thus avoiding occlusive conditions that would change the water content (2,16,17,24). However, a major inconvenience has been the uncertainty related to the variations in skin penetration of the different wavelengths in the skin.

This has now been eliminated through the recent introduction of Raman spectroscopy. For the first time, in vivo measurements of the water content of the SC at different levels of depth are possible. The applications of this technique are numerous, and the development potential for skin hydration testing appears huge (2,16,17).

CLINICAL RELEVANCE OF BIOENGINEERING MEASUREMENTS

A recent study, including several research centers but featuring the same experimental conditions, has investigated the relation between measurements of very dry, dry, and normal skin using one of the most popular device, the Corneometer CM 825, and clinical grading of dry skin following stringent criteria (25). Categories that could be defined are shown in Table 1.

This allows for the first time relating in a reasonable manner a clinical score of skin dryness to a bioengineering measurement.

CONCLUSION

During the evaluation of SC hydration in vivo, it must be kept in mind that no absolute determination of a water content or concentration is possible if measurement methods other than the near-IR-based spectrophotometric determination are used. This holds for clinical evaluation and for bioengineering measurements as well. For this reason, several measurement techniques should be used simultaneously during a study. Not only is the information gained from these different experimental approaches complementary and of great benefit if they are integrated in a clinical evaluation, but one should also remember that moisturizers may influence skin hydration in different ways. Thus, different aspects of hydration changes need to be investigated, such as water binding, water retention, or emolliency, which is also a further part of a moisturizer's action. Lastly, it should be remembered that, to obtain meaningful results, proper design of the study, inclusion of a suitable number of subjects, strict standardization of measurement conditions, and all other relevant factors need to be tightly controlled. Only by assuring the best quality level will results be obtained that will help to design and use optimal moisturizers.

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13 Skin Capacitance Imaging

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INTRODUCTION

Recently, a special type of non-optical skin surface imaging was designed under the heading of skin capacitance imaging (SCI). This method is an application of the silicon image sensor (SIS) technology, which was primarily developed for recording fingerprints for security reasons (1)]. The sensor is composed of 92160 microcapacitors dispersed on a 18×12.8-mm sensor plate measuring skin capacitance every 50 μm. These microcapacitors are protected by a thin silicon oxide layer. SkinChip® (ST Microelectronics, Geneva and L'Oréal, Paris, France) is not yet commercially available. It represents a dedicated device for computer recordings of the skin's surface hydration and microrelief (1–3). The device must be closely applied to the skin surface for five seconds at the most not to interfere with the water flux and content inside the stratum corneum (SC). SCI images are acquired and displayed in real time on a computer screen where the capacitance values are transformed into a range of 256 gray levels to form a non-optical image. On a flat surface, the darker pixels represent high capacitance spots, and the clear ones, the lower capacitance values. Besides the regular software providing images, other softwares were developed for routinely characterizing some other specific skin parameters (3). The mean gray level (MGL) of the image represents the average skin surface hydration. The so-called corner density (CD) parameter corresponds to the number of crossings between the primary lines per centimeter square (4). The main orientations of the primary lines of the skin microrelief can also be assessed.

SKIN SURFACE PATTERNS

SCI scrutinizes the skin surface texture. Indeed, most of the features defining the skin microrelief (lines, pores, furrows, and wrinkles) appear as whitish objects because their deeper portions are not in close contact with the measuring probe (1,3,5–7). The gray levels of the skin surface in close contact with the measuring sensor correspond to the capacitance, i.e., the water content of the outer SC (Fig. 1). The primary and secondary lines of the microrelief network are well identified using SCI. In young subjects, the method shows two main, almost perpendicular, orientations of the skin microrelief and their rotation when the skin is stressed. According to age, CD varies from about $250/cm^2$ to $400/cm^2$ on the forearms. The lower lip exhibits a distinctive SCI map. Fine transversal furrows are present, and a whitish and drier area is located at the most internal part of the lip (6)] (Fig. 2).

Skin aging is in part characterized by changes in the main orientations of the microrelief lines (5,6) (Fig. 3). Indeed, the skin of elderly subjects shows microrelief lines mostly oriented along one single direction. In addition, wrinkles are visible as larger whitish lines.

DERMATOGLYPHICS

"Dermatoglyphics" is a term applied to the configurations of ridged skin. Dermatoglyphics not only have characteristic patterns, but the ridges are interrupted and branched in a way, which is unique for any individual. In the human hand, the distal segment of each digit has one of three configurations, namely a whorl, a loop, or an arch (Fig. 4). The systematic classification of ridge patterns, as a means of personal identification or for use in studies of inheritance, requires numerical procedures, such as counting the ridges between specified points or measuring angles. These aspects are conveniently highlighted using SCI.

Of particular interest, however, is the fact that distortions of the dermatoglyphic patterns occur in relation to chromosomal aberrations. For example, various alterations have been

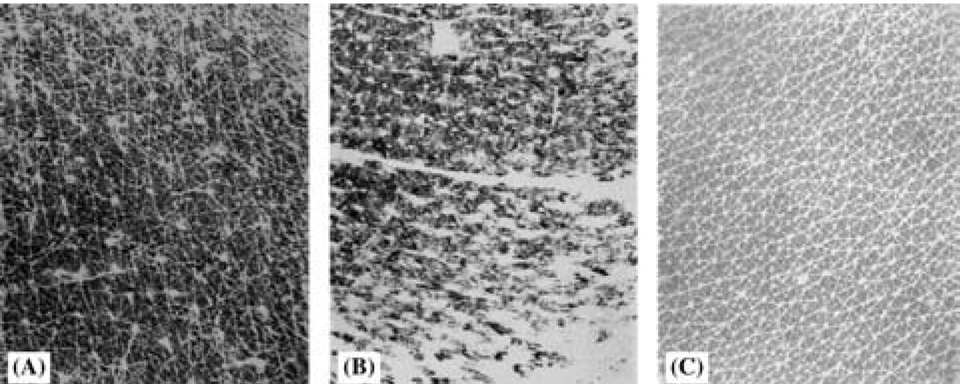


Figure 1 SCI at different anatomical sites. (**A**) Lateral side of the neck with numerous pore openings, (**B**) Wrinkles of the face (crowfeet), (**C**) Inner aspect of the arm with a dense criss-cross network of the microrelief lines. *Abbreviation*: SCI, skin capacitance imaging.

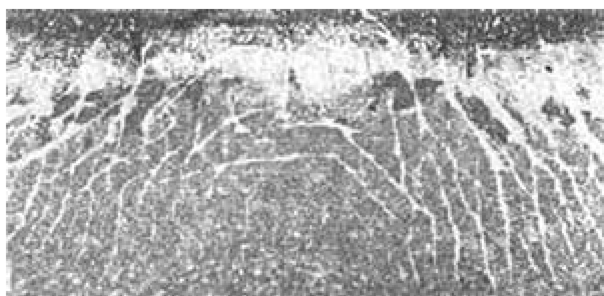


Figure 2 SCI of the lower lip: the inner portion appears drier than the outer part. *Abbreviation*: SCI, skin capacitance imaging.

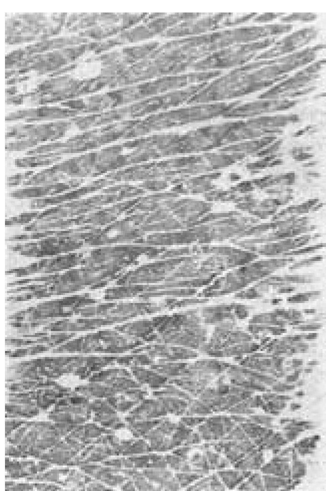


Figure 3 Heterogeneous aspects of the skin of the inner aspect of the forearm of an elderly men. The microrelief lines are mainly oriented along one direction, and CD is decreased. *Abbreviation*: CD, corner density.

described in Down's syndrome, Klinefelter's syndrome, and Turner's syndrome. Deficient ridge formation has also been reported in some dermatoses including Darier disease, alopecia areata, and psoriasis (8).

SCI is a rapid inkless procedure useful for recording dermatoglyphics. The observed features may bring an aid to diagnosis in some medical conditions.

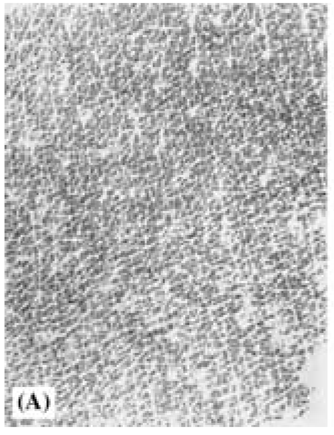
Figure 4 SCI of dermatoglyphics. *Abbreviation*: SCI, skin capacitance imaging.

SKIN SURFACE HYDRATION

The SCI-derived MGL correlates with the average capacitance values given by the Corneometer® (C+K electronic, Cologne, Germany) (1,3). Both methods establish a partial contact with the skin surface because of its microrelief. The Corneometer® gives the average capacitance of the whole contact area with the probe, while SkinChip® displays a more detailed distribution histogram of the capacitance values.

Any prolongation over five seconds of the contact time with the SkinChip® probe may increase the density in darker pixels owing to accumulation of sweat, transepidermal water loss (TEWL), and water saturation of the superficial SC. Similarly, SCI aspects are modified by the application of moisturizers. Images become darker with increased hydration, and sometimes the texture of the skin can be improved after treatment (Fig. 5).

On chronically photo-exposed skin, SCI presentation usually appears heterogeneous. Some areas look quite dry in close vicinity with other areas looking unremarkable. Such a patchy heterogeneity in hydration of the skin surface could be related to focal variations in the



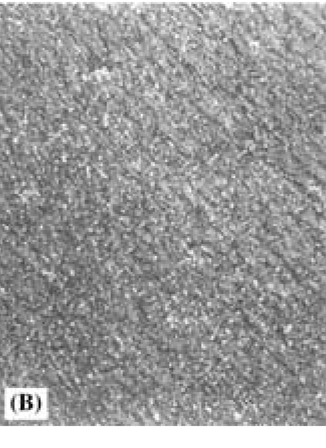


Figure 5 Skin of the neck of a young adult before (**A**) and immediately after (**B**) application of a moisturizer. The moisturizer dramatically darkens the SCI aspect. *Abbreviation*: SCI, skin capacitance imaging.

epidermal differentiation of photo-exposed skin (Fig. 4). Whether or not these aspects are in part related to field cancerogenesis is yet unsettled (9,10).

SKIN PORES

"Skin pore" is a dermocosmetic term, which does not encompass one single defined structure. It is replaced to the best advantage by the acroinfundibulum and the acrosyringium for distinguishing the openings of the folliculo-sebaceous ducts and the sweat gland apparatus, respectively (7).

Measurement of the TEWL is often used as a convenient assessment of the SC barrier function. A number of variables affect TEWL measurements, including person-linked factors as well as environmental and instrumental variables. Among them, it is acknowledged that physical, thermal, and emotional sweating need to be controlled. Therefore, a premeasurement of 15- to 30-minute rest without any physical activity in a temperature-controlled room of 20°C to 22°C is taken into consideration in most studies. The same considerations apply to the electrometric measurements performed under occlusion (11–13), including SCI. In these different technical approaches, it is impossible to control the so-called imperceptible perspiration. The contribution of this physiological parameter in the TEWL values has never been thoroughly evaluated and is neglected in the interpretation of TEWL data.

The clinically imperceptible perspiration is easily observed using SCI. Tiny black dots mark the active sweat gland openings (Fig. 6). In our experience, this aspect has no effect on the casual TEWL determinations. When sweating is more active, SCI black dots become larger, and some merge to form irregular black areas. Because sweat appears as black dots, it is possible to measure its contribution to the SCI-derived MGL by thresholding the histogram values. The activity of antiperspirants can be conveniently assessed by this method.

Pilosebaceous openings at the skin surface appear as whitish dots (Fig. 6). The open comedones and the keratin-filled funnel-like acroinfundibular structures are highlighted (7,14). These structures are revealed as whitish low capacitance spots. This aspect is in part due to the absence of contact between the probe and the epithelial lining of each empty infundibulum, or to the low hydration of the constitutive cornified cells of the microcomedo.

SCI of acne highlights a peculiar heterogeneous patchwork of electrical properties of the skin. Among the typical whitish pinpoint pattern of normal-looking acroinfundibula, microcomedones and open comedones appear as larger low-capacitance objects (Fig. 7). Inflammatory papules appear as targetoid structures centered by a whitish comedo surrounded by a darker rim. The latter structure reveals a weakened skin barrier function and the presence of a discrete serosity exsudate (15) (Fig. 7). These electrometric features are not perceived clinically, but may be important for antiacne drug delivery according to their hydrophilic or hydrophobic characteristics.

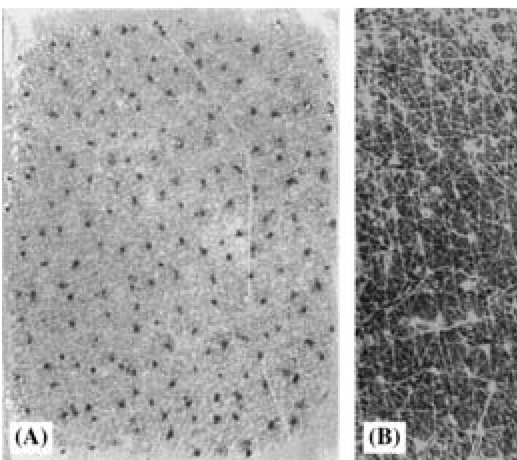


Figure 6 Skin pores. (**A**) Imperceptible perspiration. Tiny black dots mark the active sweat gland opening. (**B**) Pilosebaceous openings at the skin surface appear as whitish dots.

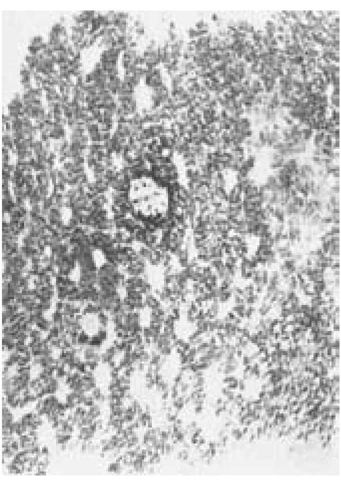


Figure 7 SCI of acne. The larger white spots (low capacitance) correspond to comedones, and inflammatory papules are identified as targoid structures centered by a whitish comedo surrounded by a well-circumscribed darker rim. *Abbreviation*: SCI, skin capacitance imaging.

SURFACTANT-INDUCED REACTION

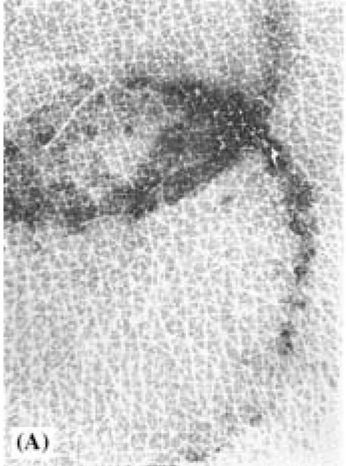
The dynamics of SC reactivity to surfactants is quite complex. Surfactants present in hygiene and skin care products are in part adsorbed at the skin surface, and they also permeate the SC where they interact with proteins and lipids. A number of physicochemical interactions exist between corneocytes and surfactants (16). One of the earliest events following surfactant-induced protein denaturation is perceived as corneocyte swelling (17). This condition leads to a paradoxical and transient SC hydration, following surfactant challenge in vivo (18). The structure and physical properties of the SC are further altered following prolonged contact with anionic surfactants (17,19,20). As a result, minimal to severe irritation usually develop. Full-blown lesions show erythema, increased TEWL, altered cutaneous microrelief, increased skin surface roughness, and impaired desquamation. The SC water content can be assessed in vivo using devices measuring changes in electrical properties of skin at different frequencies and at different depths inside the SC (12,21,22). SCI has an added value to the conventional assessment methods because its sensitivity discloses focal and minute changes that are blurred by the methods averaging data on a relatively large area corresponding to the size of the sensor probe.

Two discrete effects of mild surfactants on human SC were assessed using SCI. The short-term patch-testing procedure (23) and the open method close to the in-use conditions were used (24). Both experimental procedures disclosed the early step of corneocyte swelling induced by surfactants. Delayed assessments after a couple of hours as well as repeated surfactant insults were responsible for a second event corresponding to a skin surface–drying effect. The earliest change in the irritation zone was revealed by darker pixels, corresponding to water-enriched corneocytes in contact with the probe. This aspect probably resulted from the transient intracellular accumulation of unbound water. In a second step, this hydration state was replaced by the opposite dehydrated condition pictured as white pixels (Fig. 8). As a result, SCI reveals the surfactant-induced irritation kinetic with high sensitivity. A correlation was also found between SCI and data gained by the corneosurfametry bioassay (24).

HYPERKERATOTIC DERMATOSES

Hyperkeratosis is a typical feature of pityriasis (tinea) versicolor corresponding to a *Malassezia* spp infection. The condition is conveniently highlighted using SCI because the skin surface is dryer than the surrounding skin. The method allows to detect small lesions of pityriasis (tinea) versicolor almost invisible to the naked eye (Fig. 9). Interestingly enough, lesional skin appears anhidrotic, perhaps due to the occlusion of each acrosyringium (25).

Psoriasis is the paradigm of inflammatory hyperkeratotic dermatoses. SCI reveals a patchwork of different electrical properties on lesional skin (26). Whitish low capacitance is typical for stable hyperkeratotic plaques. More inflammatory and evolving plaques show



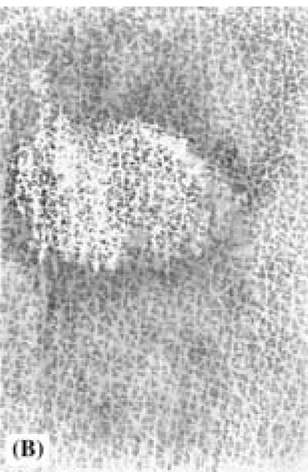


Figure 8 Corneocyte reactivity to anionic surfactant. (**A**) Skin surface imaging of the partial overlap between two successive patch tests performed with a diluted anionic surfactant. (**B**) Skin area examined two days after the condition depicted in (**A**). The overlap region shows a white appearance, indicating a decreased water content in corneocytes. *Source*: From Ref. 23.

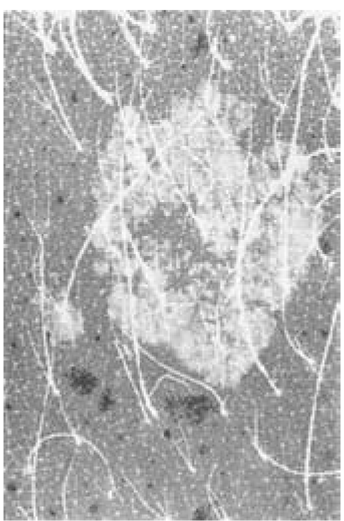


Figure 9 SCI of small lesions of pityriasis (tinea) versicolor. The aspect in bunch characteristic of this kind of lesion is well highlighted. The lesion appears anhidrotic. *Abbreviation*: SCI, skin capacitance imaging.

darker high-capacitance spots (Fig. 10). This aspect is likely related to sites exhibiting increased TEWL (27). SCI can thus provide clues of disease activity in the plaque stage of psoriasis and can be used to monitor therapy.

KERATOTIC OR PIGMENTED TUMORS

Viral warts are easily identified using SCI. They exhibit a dry hyperkeratotic aspect of their surface (25,28,29). No difference in capacitance reduction was found between different types of warts (Fig. 11).

Melanocytic nevi and pigmented seborrheic keratoses may be difficult to distinguish during the clinical inspection. SCI shows variable aspects irrespective of the nature of these lesions. Low capacitance is commonly yielded, but increased capacitance is also possible, particularly on minimally inflammed lesions (30). Inflammation in the superficial dermis produces edema and discrete transudate through the epidermis. Such a water flux ultimately steeps the SC. Inflamed lesions of seborrheic keratoses and melanocytic nevi exhibit a capacitance map, which is not uniform. Spotty areas of decreased capacitance are dispersed in a buckshot pattern over the background. The lesions are commonly rimmed by a thin border of lower capacitance (Fig. 12). This situation was also observed in inflammatory lesions of acne and acute psoriasis (Figs. 7 and 10).

Figure 10 SCI of a psoriatic lesion, combining white hyperkeratotic areas and darker inflammatory sites. *Abbreviation*: SCI, skin capacitance imaging.

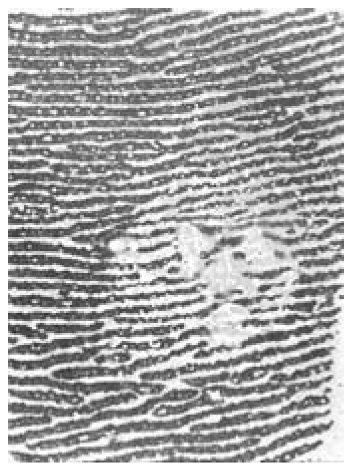
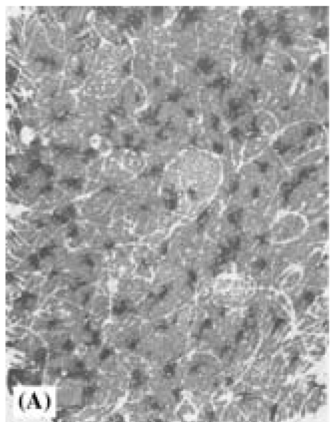


Figure 11 SCI of plantar warts. *Abbreviation*: SCI, skin capacitance imaging.



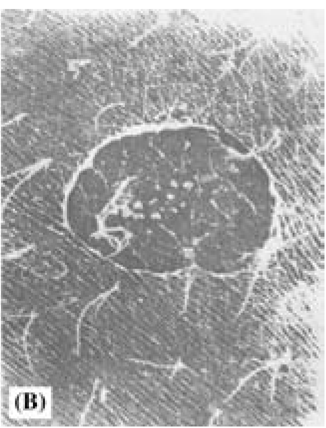


Figure 12 SCI of keratotic pigmented tumors. (**A**) Small lesions of seborrheic keratosis with white low-capacitance dots corresponding to horny plugs. (**B**) Moderately inflamed melanocytic naevus. *Abbreviation*: SCI, skin capacitance imaging. *Source*: From Ref. 29.

HAIR SHAFT MOISTURIZATION

Similar to the SC, the cuticle of hair shafts can show variations in hydration. The kinetics of water sorption and desorption is possibly altered following some hair weathering and damage. It is also influenced by the application of some hair care products. SCI determinations can be used for assessing these modifications in hair shaft moisture.

CONCLUSION

In conclusion, SCI provides non-optical pictures showing aspects invisible to the naked eye. It represents a procedure allowing both visualization and quantification of the skin microrelief, SC and hair shaft hydration, acneiform follicular cornification, imperceptible perspiration, and active sweating. The method brings unique and sound information in dermocosmetology, also giving insights in the physiopathology of skin disorders.

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14 Confocal Raman Spectroscopy for In Vivo Skin Hydration Measurement

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INTRODUCTION

Confocal Raman microspectroscopy of skin in vivo is nowadays routinely being applied in human panelist studies. Ever since the first Raman spectra of skin were presented, it is known that these spectra contain unique information on the chemical composition of the skin. The ability to measure the chemical composition of living biological tissues nondestructively is a valuable tool in the skin sciences. Raman spectroscopy has qualities that make it unusually attractive for such measurements. Especially the ability to measure the chemical composition of tissues noninvasively at defined depths, using confocal optics (optical sectioning), is unique. Because of the complexity of biological tissues, Raman spectroscopy has only in the last decade begun to make significant contributions in skin science. A very recent review discusses the in vivo applications of Raman spectroscopy in the measurement of the composition of skin, including topically applied compounds and their effects on skin composition, in the context of pharmaceutical applications (such as transdermal drug delivery) (1).

Skin research increasingly depends on more detailed knowledge of the molecular composition of skin and the spatial distribution of skin constituents. On a microscopic scale (the scale of the confocal Raman measurement), the skin is highly heterogeneous. Its molecular composition and structure vary tremendously over different body sites and at different depths below the surface of the skin. In the stratum corneum (SC) especially, concentration gradients (e.g., water gradients, pH gradients, diffusion kinetics) play a role in biochemical or skin physiological processes. The composition of the skin is also affected by skin disorders, environmental factors such as sun exposure, seasonal variation, diets, and bathing habits, and cosmetic or medical treatments. Skin treatments may also bring about changes in dimension, such as an increase in SC thickness due to swelling. The spatially complex skin tissue can be excellently studied using Raman spectroscopy with a confocal approach, where spatial resolution can be achieved that is consistent with the size of many features of interest (~5 μm in depth and ~1 μm horizontally).

Noninvasive methods are particularly welcome. This is partly because they cause less discomfort for the patient or volunteer subject, but also because noninvasive methods enable investigation of the skin in its natural state without affecting its integrity, morphology, or molecular composition. Noninvasive measurements can be performed repeatedly on the same skin area in vivo and can thus be used to monitor time-dependent changes in the skin brought about by skin treatments.

Caspers et al. presented the first in vivo confocal Raman spectra of human skin (2) and were able to clearly show compositional differences at different depths below the skin surface. For example, large changes in composition were observed near the SC–stratum granulosum interface, from which the SC thickness could be derived in vivo. This was confirmed shortly after by combined confocal microscopy and Raman spectroscopy (3), (see discussion below).

Whereas the aforementioned, more general review, discusses the measurement of the main chemical composition of the SC (1), this chapter focuses on how measurements of water concentration gradients can be used to study the moisturization process of the skin in its outer layer.

ALTERNATIVE METHODS TO MEASURE SKIN HYDRATION

It has been known for centuries that humans continuously lose water through the skin, for instance, from experiments in which human subjects and their ingested and excreted liquids are carefully weighted. The detailed hydration process of the skin and the dynamic transport

of water through the skin have been subject to investigations for decades. Modern methods for studying the moisturization of the skin can be coarsely classified, using the following criteria:

1. the relationship of the measured parameters to the hydration state of the skin (direct or indirect, straightforward, or complex);
2. spatial resolution, parallel and perpendicular to the skin surface;
3. the extent to which the measurement influences the skin state (invasiveness and the ability to resample the probed site); and
4. the ease of use (both the measurement procedure and the data processing and interpretation).

It is beyond the purpose of this chapter to discuss the features of the various methods in use. However, a short and simplified classification, using the aforementioned criteria, along with the newest method of confocal Raman spectroscopy, will help clarifying the quite remarkable position of the latter. The methods included in the comparison are Karl Fischer titration, light microscopy, electron microscopy, electrical methods (capacitance and conductance), transepidermal water loss (TEWL), magnetic resonance imaging (MRI), near infrared spectroscopy (NIRS), Fourier transform infrared spectroscopy using attenuated total reflection sampling (ATR-FTIR), opto-thermal transient emission radiometry (OTTER), and of course Raman spectroscopy. In Table 1, these methods are ranked according to their favorability toward the four criteria (from −− to ++). The table also contains a short comment. In the right column, some key reference papers are suggested for further reading. The selection of key references is a personal selection by the authors. It will provide the reader with more details, helpful in gaining a better understanding of the different ways in which skin hydration may be measured. The authors by no means claim the selection to be complete or to effectively represent the whole field of science.

Since the hydration of the skin is so closely related to water gradients that reside within the very thin SC, confocal Raman seems unusually suitable, especially when one takes its in vivo applicability into account. Despite its apparent complexity, of which we believe may be mainly due to relative unawareness of Raman spectroscopic technologies, the measurement routine is not difficult to master. It is the experience of the authors that an instrument operator (with no prior knowledge of spectroscopy) can be trained in one to two days, enabling him to carry out the in vivo measurements of water depth concentration profiles on human volunteers.

A method that appears to potentially possess comparable features as confocal Raman microspectroscopy is MRI. An impressive spatial resolution of 4 μm (in all directions) has already been demonstrated on very small (single biological cell) isolated samples (22). However, the extremely difficult challenges to overcome for large samples, such as human volunteers, will be the size and stability of the magnet and foremost the definition and stability of the magnetic field gradient. Whereas the Raman methodology already allows for measurement of the overall chemical composition (1), this is not yet possible for in vivo MRI of large samples. Finally, the cost of ownership of MRI equipment can become very high.

REQUIREMENTS FOR IN VIVO RAMAN METHODOLOGY

Qualitative Description of the Raman Effect

In Raman spectroscopy, a sample of interest (this can be gaseous, liquid, or solid) is illuminated by a laser beam. The light in the laser beam is of single (or very narrow) wavelength nature. The electric component of the electromagnetic fields within the laser beam drives the electronic cloud of the molecules present in the sample. The driven (and oscillating) electronic clouds reemit most of the collided laser light without energy loss (by the physical laws of induction, an oscillating electronic charge emits electromagnetic radiation at the oscillating frequency); the only difference may be the direction in which the photons are ejected out of the molecules. This process is referred to as elastic scattering (or Rayleigh scattering). A very small amount of the laser light, however, scatters inelastically; the ejected photons have a different energy than the injected laser photons. The difference in energy is taken up or released by the molecules and is used to promote or demote, respectively, the

Table 1 Brief Comparison of Some Features of Current Methods to Assess Skin Hydration

Method	Water direct/indirect	Spatial resolution	Invasiveness	Ease of use	Key references
Karl-Fisher	++ Direct and absolute	−−	−− Destructive	−− Long preparation	(4)
Light microscopy	+ Swelling can be observed	+ 0.5 μm	−− Sections required	− Long preparation	(5,6)
Electron microscopy (SEM and STEM)	+ Indirect	++ <0.01 μm	−− Cryo-sections	−− Long prep, complex instrument	(7–9)
Capacitance and impedance	− Influenced by products	− Probes top 30 μm	+ Contact probe	++ Push button	(10–12)
Conductance	− Influenced by products	− Probes top 1–10 μm	+ Contact probe	++ Push button	(13–15)
TEWL	+ Measures flux	− cm	+ Contact probe	++ Push button	(16,17)
MRI	++ Direct	− 70 μm in vivo	++	−− Complex instrument	(18)
NIR	+ Difficult to quantify	− Probes top 1–2 mm	++ Noncontact	+ Moderate complex	(19)
ATR-FTIR	+ Difficult to quantify	+/− Probes top 1–2 μm	+ Contact probe	+ Moderate complex	(20)
OTTER	+ Direct, but theoretical modeling required	+ > 10 μm	++ Noncontact	+/− Complex instrument	(21)
Confocal Raman	++ Direct, quantitative relative to keratin	+ 1 μm lateral, 5 μm depth	+ Contact probe	+ Moderate complex	This paper

Abbreviations: SEM, scanning electron microscopy; STEM, scanning transmission electron microscopy; TEWL, transepidermal water loss; NIR, near infrared; ATR-FTIR, attenuated total reflection sampling–Fourier transform infrared spectroscopy; OTTER, opto-thermal transient emission radiometry.

vibrational energy levels of the molecules. The mechanism of interaction, leading to the energy difference, involves a modulation of the electromagnetic field, because of oscillating electronic cloud, by the much smaller electromagnetic field generated by the ever-vibrating nuclei present in the molecules. By measurement of the intensity and energy of the reemitted light of different frequencies than the laser frequency, a Raman spectrum is obtained. The differences in energy correspond to transitions in vibrational energy levels. In this respect, a Raman spectrum contains the same kind of information as an infrared spectrum, but the way by which this is obtained (photon scattering) is different from IR spectroscopy (photon absorption).

In Vivo Raman Methodology

In vivo, Raman spectra are obtained by focusing a laser beam through a microscope and allowing the microscope objective to project the focused laser beam on and below the surface of the skin. Subsequently, the Raman light is measured in the backscattered direction through the same microscope objective. Numerous technical challenges have to be overcome before Raman measurements on biological tissues can be fast enough for practical use in in vivo clinical studies. General purpose Raman instruments, available in most well-equipped analytical laboratories, are not capable of practically useful measurements on skin. Recently, however, Raman instrumentation has been developed, employing advanced technologies, and made commercially available, which is capable of practical use in these demanding applications. Figure 1 shows a photo of this first commercially available Raman skin analyzer.

Most critical factors in an optimized Raman skin analyzer are selection of lasers, choice of optical materials, detector quality, opto-mechanical stability, and for practical utility, software that is easy to use and can effectively handle the large volumes of data that are generated in in vivo panel studies. Laser safety considerations also create limiting technical requirements that must be met, thereby strongly influencing the overall engineering of a Raman skin analyzer. Indeed, a capable Raman skin analyzer may be thought of as being composed of four components, each of which must meet critical requirements: (*i*) a laser light source and associated light conditioning optics, (*ii*) an NIR (the optimal wavelength applied in the measurement of skin) optimized microscopic measurement stage, (*iii*) the Raman spectrometer, and (*iv*) specialized operating and data analysis software. Each component, as well as the implications of laser safety, will now be briefly discussed below.

Laser Excitation Source and Optics

Firstly, the laser(s) used must emit light at wavelengths at which no photo(bio)chemical reactions are brought about and at which minimal fluorescence is stimulated in the skin. This places a lower limit on the usable laser wavelength at approximately 660 nm. Secondly, the Raman-scattered photons must be detected with the highest possible efficiency and the lowest possible noise. State-of-the-art technology for this purpose is a charge coupled device (CCD) detector for which the detection is limited to wavelengths shorter than about 1100 nm. Detection of a Raman spectrum in the so-called fingerprint spectral region (400–2000 cm^{-1}) therefore sets an upper limit to the laser excitation wavelength of about 900 nm.

Figure 1 The river diagnostics model 3510 skin composition analyzer is the first Raman instrument optimized for in vivo analysis of skin.

Thus, the choice of laser wavelengths is restricted to a "biological and technical window" in the NIR, approximately in the range of 660 to 850 nm. Typically, solid-state diode lasers are applied. For diffraction limited laser focusing (required for the best spatial resolution), a single mode laser is required. The laser must be stable in power output and wavelength, and its emission line must be narrow to allow for achievement of high spectral resolution. Unwanted laser diode background radiation or satellite emissions must be removed (filtered) before the laser light is injected into the microscope. The laser power out of the measurement device (the microscope) must meet the requirements derived from the laser safety limitations (see below the subsection "Laser Safety Considerations"). Finally, a strict requirement is set on all optical materials in the laser light path; only minimal fluorescence or other background contributions are allowed.

Microscope Measurement Stage

The Raman signal is collected back through the microscope objective, and the microscope must have uncompromised confocal optics. The entire optical train must very efficiently transmit the signal to the spectrometer and finally to the detector. The spatial resolution of the microscope must be better than the thickness of the SC, otherwise no information about the distribution of materials (such as water) within this skin layer is to be obtained. The best microscope objective to this end must be custom designed and optimized for the NIR wavelengths (660–950 nm). Also critical is the absence of any difference in refractive index in the optical path from the objective to the skin. In the skin analyzer (Fig. 1), this is managed by positioning the microscope objective below a measurement window of identical refractive index as the skin and the objective. The space between the objective and the measurement window is filled with a refractive index matching immersion oil. The sampled skin rests and is locally conveniently fixed on the measurement window. If, on the other hand, a large difference in refractive index is present between the microscope objective and the skin (e.g., by focusing through air), a severe degradation of depth resolution results. The microscope objective must be movable in the axial direction (z axis) under precise control. This allows spectra to be recorded at successive depths in the skin, from which composition-depth profiles are obtained. The microscope stage must allow for convenient orientation of human subjects. Usually an inverted configuration is used. The most common measurement site at present is the volar aspect of the forearm, (Fig. 1).

Raman Spectrometer

A very high laser wavelength rejection and again a high transmission at optical interfaces (low reflection and scattering losses) are required to preserve as many of the information-bearing photons as possible. Of course, the detector must also be of high performance. Any general purpose Raman spectrometer would benefit from these requirements, but in the measurement of biological samples, the information sought is often in small spectral differences. Therefore, the spectra must be of very high quality. Furthermore, these high-quality spectra must be routinely obtainable in a time scale compatible with panel studies and the patience of volunteer panelists. Hence, maximizing the signal-to-noise ratio (S/N) by employment of an optimized spectrometer design is of great importance. In a clinical research environment, data recorded today must compare meaningfully to data recorded before or after. Therefore, mechanical and optical stability and measurement repeatability are further important considerations. In clinical environments, where more than one spectrometer is in use, it is further required that results obtained on one skin analyzer will be directly and reliably comparable with results obtained on another skin analyzer. This places very high demands on the accuracy and reproducibility of instrument calibration and correction for instrument response effects.

Software

Data acquisition software for in vivo Raman measurements must have specialized features to handle the often large number of measurements in typical panel studies and to satisfy requirements that are not normally encountered in other types of Raman analysis. For example, the software must enable the operator to quickly select locations of interest on the skin surface. Also, since depth information (usually changes in composition as a function of

depth) is important, the software must incorporate a reliable and accurate means of locating the skin surface for reference. Further, the software must have minimal data acquisition "dead time" between sequential spectrum acquisitions, to maximize throughput, when thousands of spectra are typically acquired in a day. Because of the many experimental variables in a typical skin study design, the number of spectra to handle can become very large. Therefore, the data-processing software must incorporate special features. In conventional spectroscopic processing software, spectra can be manipulated and analyzed typically one by one or batch by batch. For the larger numbers of spectra, typical for in vivo studies, the time, simply to read in each single spectrum and export the result after analysis, can become prohibitive. Even in a batch-processing mode, the time to sort, select, and read in the spectra to define the batches for analysis can become a bottleneck. Therefore, the software must feature ways to enter the experimental design and use this to select and process the spectra accordingly.

Laser Safety Considerations

The International Laser Safety Standard, IEC 60825-1 (2001), prescribes maximum permissible exposure (MPE) limits for the skin, which are dependent on the wavelength of the laser light and the duration of the exposure. The MPEs for skin are formal limits based on extrapolations of exposure to sunlight and do not represent actual damage thresholds, which may be considerably higher. To provide a "flavor" for MPE magnitude, the configuration of the instrument (Fig. 1) results in an MPE limit of 30 mW for 785-nm laser excitation, and 20 mW for 671-nm excitation. These values are not to be taken in general for the wavelengths cited, but must be determined for any instrument design intended for in vivo skin analysis.

There must not be any significant risk of eye damage from exposure to the laser beam, while the measurement window is not covered by the skin to be measured. Practically speaking, laser exposure of the eye is not a difficult risk to manage in a properly designed instrument since the laser beam diverges at a high angle when emerging from the microscope objective, but the risk must nevertheless be properly managed. The instrument (Fig. 1) operates well within the limits of a class 2M laser device, which means that the instrument is eye safe. Incidental direct observation of the beam is not an eye hazard, provided that no optical instruments are used to observe the beam. Each instrument is tested for compliance with the class 2M laser device classification.

When these five elements, an appropriate laser light source, microscopic measurement stage, NIR-optimized Raman spectrometer, specialized software, and in vivo laser safety provisions, are combined in a Raman instrument, valuable information hitherto unavailable to researchers becomes accessible.

RAMAN METHODS FOR THE STUDY OF HYDRATION OF THE SKIN

Relationship Between the Raman Spectrum of Skin and the Local Water Concentration

In the Raman method for measurement of hydration of the skin, a signal is isolated from the Raman spectrum, which is mainly because of the water present in the skin. Note that the signal itself also depends on the depth from which the signal originates; signals that are recorded at greater depths will be weaker. This effect is easily understood, since the skin is not infinitely transparent for the laser and Raman light, it exhibits rather a bit turbid character. In Raman spectroscopy in general, this effect is usually compensated by dividing the measured intensity of the signal by the intensity of a reference signal that may be selected. Requirements for a good reference signal are that firstly it must be due to a substance that is present more or less homogeneously in the sample measured, and secondly, its Raman signal must be sufficiently strong. Since the reference signal is attenuated by exactly the same factor as the analyte signal (e.g., water), the division will cancel out the depth-dependent attenuation. In biological samples, often a signal due to a protein is used. The Mendelsohn group, for example, uses a signal due to phenylalanine to this end (23); others use the overall signal of keratin, which represents the major dry mass fraction in the SC (24).

In 2000, Caspers et al. published the first in vivo water concentration measurements in skin as a function of depth below the skin surface (24). In this paper, the method to calculate a water concentration in mass percentages of wet tissue is discussed in detail. It also involves internal normalization of the water signal, in this case by a signal due to keratin. In Figure 2, a

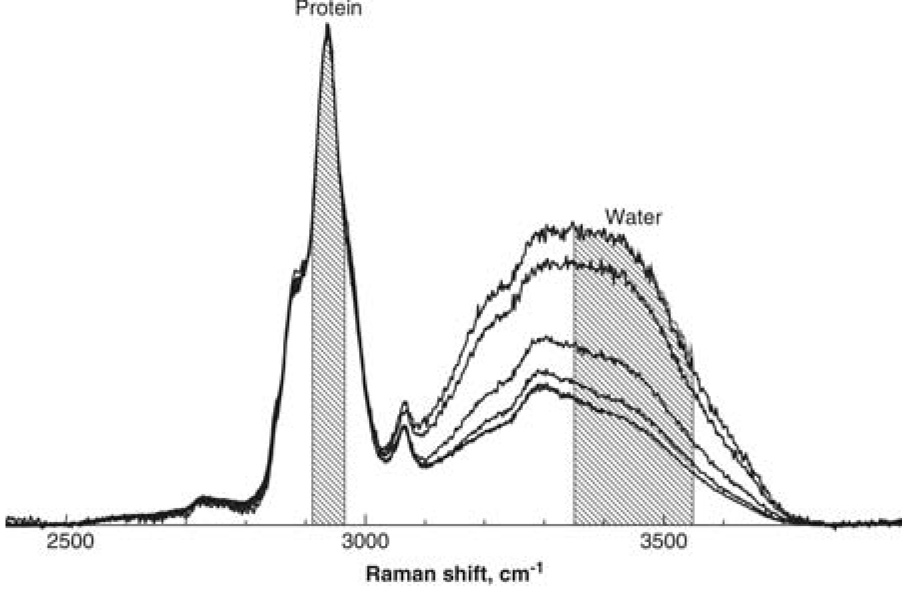

Figure 2 High wave number part of the spectral baseline corrected in vivo Raman spectrum of SC on the thenar. Indicated are integration boundaries for signals due to keratin and water.

part of a typical Raman spectrum of untreated SC skin is shown. In the Figure 2, the signals due to keratin and water are indicated. Furthermore, integration boundaries for the signals due to water and keratin are drawn after spectral baseline subtraction. From Raman spectral measurement of solutions of protein of known concentration, Caspers was able to set up a calibration, equating the ratio of Raman signal intensities due to water (W) and keratin (K) to the mass percentage of water present in the skin (for wet tissue) (24):

$$\text{water(mass\%)} = \frac{W/K}{(W/K) + R} \times 100\% \qquad (1)$$

Where R is a calibration constant derived from the measurements of the protein solutions.

In Figure 3, typical water depth concentration profiles recorded within a $2 \times 2\ \text{cm}^2$ area on the ventral forearm are shown.

As can be verified from Figure 3, the four repeat measurements do not coincide. This is caused by the biological inhomogeneity of the skin. This implies that for accurate water contents, repeat measurements and averaging must be carried out. In the Figure 3, the approximate SC–epidermis boundary is indicated. At this boundary, the water concentration gradient changes its slope. This feature is further discussed below.

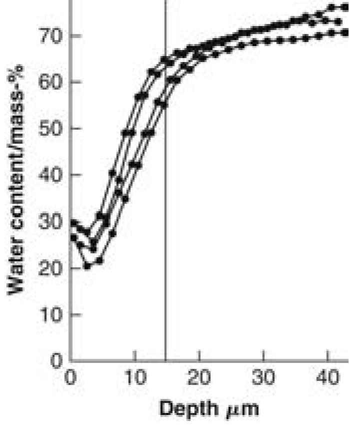

Figure 3 Measured water concentration versus depth, from confocal Raman measurements, at four locations within a small area ($2 \times 2\ \text{cm}^2$) on the volar aspect of the forearm. The line indicates the approximate SC–epidermis interface.

The time to record a single water depth concentration profile over about 30 μm of skin on the volar forearm (covering the SC and the upper part of the epidermis) is about 15 seconds.

In 2005, L'Oréal researchers presented in vivo results on human volar forearm skin, using an in-house built confocal Raman setup (25). The work discusses depth concentration profiles from water and other components, but for the present purpose, only the water results are highlighted. In their analysis of the Raman spectra, only a ratio between the intensities of signals due to water and a reference signal was used. The ratio chosen was taken from an older paper, on confocal Raman spectroscopy of cornea (26). No quantification of the water contents was carried out.

Validation of the Quantitative In Vivo Water Concentration Measurement

To the best of our knowledge, no independent method to quantify in vivo depth-dependent water concentration in skin exists. Therefore, validation against a "golden standard" is not possible. However, there are possibilities for comparison with in vitro methods. If we compare the water depth concentration profiles as proposed by Warner et al. in 1988 (7) to the results (Fig. 3), the agreement is striking. The two methods are completely independent. Warner et al. obtained their quantitative estimate from an area analysis of scanning transmission electron microscopy (STEM) images of thin cryosections of skin, taking into account the densities of keratin and ice. Caspers' method, on the other hand, is based on the Raman spectra of prepared solutions of proteins (24). Both methods result in a concentration in the 20% to 30% range for the outer surface (the upper layer of the SC), increasing to about 70% at the interface with the epidermis (note: the 70% concentration in Warner's method was an assumption and not a result of his method).

Very recently, Wu and Polefka presented direct validation results for extracted pigskin SC (27). Samples were equilibrated at different relative humidities and subsequently cut in half. For one half set, the absolute water concentration in the SC was determined with Karl Fischer's titration method. The other half set was analyzed according to the Raman method of Caspers (24). This approach allowed for a direct correlation of water concentrations from independent analysis methods. The correlation proved remarkably good, an R^2 of 0.989 was found. It was further noted that the precision of the Raman method for water concentrations above 30% was better than for the Karl Fischer method. In their paper, Wu and Polefka also reported correlations of conductance measurements with the Raman measurements. The same paper also covered moisturization efficacy results on pigskin SC (see the "Applications" section).

Water Concentration Gradients and Measurement of the Stratum Corneum Thickness

Knowledge of the thickness of the SC is essential in understanding the efficacies of products. Obviously, moisturization of the SC means adding water, and adding water implies adding volume. It is therefore expected that the SC will swell. The changing dimension of the SC under the action of any treatment has consequences for the calculation of efficacies; this applies to not only the degree of moisturization but also, for example, to the content of a constituent of interest before and after application.

Before confocal Raman spectroscopy became available, the shape of the water concentration gradient and its change upon treatment was already known from in vitro experiments or theoretical calculations.

STEM of biopsied and rapidly frozen human epidermis has already been applied for more then 20 years to study the water concentration gradient; see, for example, the work by Warner et al. from 1988 (7). These results showed that a water concentration gradient must reside in the SC.

In 1984, experimental dynamic water flux measurements of in vitro SC as a function of its water content enabled the calculation of water concentration gradients that must exist in the in vitro SC samples (28). These calculations were based on Fick's law of diffusion. All profiles were found to be steep and linear in the SC, and the model accurately described the swelling of the SC as a function of the water content and also as a function of the surface water content.

In 1997, Norlén et al. (6) applied light microscopy and confocal laser-scanning microscopy (CLSM) to study the swelling of extracted pieces of human SC. They found that after incubation of dried SC in distilled water for 90 minutes, the observed swellings were 26.3 ± 16% in the thickness dimension and only 4.1 ± 1.4% in the lateral dimension. Thus, swelling after addition of water to the SC mainly takes place in the thickness direction.

In Caspers' original paper (24), he pointed out that the shape of the water profiles could be linked to the SC thicknesses. At different body sites, the water concentration profiles changed slopes at different depths. Caspers confirmed the results in 2001 and pointed out that the observed steep increase in water concentration at a particular depth below the surface of the skin indicates the SC–epidermis interface (29). The general appearance of the in vivo water concentration profiles are in agreement with in vitro water concentration profiles as determined by X-ray microanalysis (7). The SC thicknesses on the thenar and forearm are approximately 110 and 15 μm, respectively. Further and conclusive confirmation of the fact that the steep increase in water concentration occurs at the boundary between SC and living epidermis was presented in 2003 by Caspers et al. (3). Boundaries, as determined by confocal video microscopy, corresponded precisely to the boundaries as derived from the water concentration profiles.

Recently, a number of groups published estimations of the SC thickness, on the basis of confocal Raman measurements and their corresponding water depth concentration profiles. Sieg et al. studied water depth concentration profiles for forearm skin (30). They propose to model the profiles with a sigmoid-like function (Weibull function). One of the fit parameters is the location of the steepest gradient; this is indicative of the SC thickness (it is not the thickness itself). Their work is further discussed in the "Applications" section.

In their study of the delivery of retinol to the viable epidermis, by confocal Raman microspectroscopy, Pudney et al. calculated the approximate location of the SC–epidermis boundary from water concentration profiles and from concentration profiles of the components of the natural moisturizing factor (NMF) recorded at the same location (31). By selecting a depth at which 30% of the maximum content of NMF is found and a second depth at which the water concentration is 55% by mass, two closely spaced locations of the interface are obtained. Subsequently, these two estimators are averaged. Although the criteria for depth selection may be arbitrary, their approach allowed for a systematic estimation of the location of the boundary for every individual measurement spot. This information was then used to verify whether the retinol was delivered to the viable cells or not.

Egawa et al. proposes yet another method to arrive at the location of the SC–epidermis boundary (32). Their estimation is taken from the depth at which the derivative of the water concentration profile is almost zero and coined this as the SC apparent thickness (SCAT; also see the "Applications" section).

The criteria discussed above to arrive at the SC thickness are not fully objective and probably do not represent the real thickness. Also, the models lack a physical rationale. Van der Pol et al. first proposed the more objective method for fitting of the water profiles, on the basis of diffusion of water through the SC–epidermis bilayer (33). The bilayer is thought of as two homogeneous media with two different (but constant) water diffusion coefficients. The water flux is considered constant. Under these conditions (Fick's law), the water concentration gradients must be linear in both media. The experimental water depth concentration profile is modeled simply with two linear functions (one in the SC and the other in the epidermis). This model function is further convoluted by an optical point spread function (a Gaussian function with a full width at half maximum of 5 μm), to account for the spatial resolution of the confocal Raman technique. The only variables for the model function are the location of the discontinuity at the interface of the SC and the epidermis and the two slopes of the water concentration gradients in both media. The method is now automated and implemented in routine moisturization efficacy studies on human panelists (34).

APPLICATIONS

Confocal Raman microspectroscopy is now a tool in the study of epidermal and dermal skin in various skin research groups. For a general review of the role of confocal Raman microspectroscopy in skin science, including the study of penetration of topically applied materials, see reference (1). In this section, the published Raman work related to the in vivo study of the moisturization of the skin is highlighted.

Moisturizing the Skin

A simple way of moisturizing is to wet it with water. In Caspers' 2000 paper, this was demonstrated using a wet towel (24). The resulting water depth concentration profiles changed

dramatically after application of the wet towel. In the SC, the water concentration increased to 50% to 60%, and swelling of the SC was noted.

Chrit et al. studied the in vivo short-term efficacy of a moisturizing cream (35). A number of 26 volunteers (Caucasian, female, dry skin) received a treatment of the volar forearm site with an emollient without hydrating agent and a treatment with a 3% glycerol-containing cream. A control measurement was included. The normalized water signal was measured at different depths after one hour of treatment. Signal intensities were then averaged over the depth range 0 to 20 μm. The glycerol-based cream induced a significant increase in average water content as compared with baseline, and at every depth between 0 and 20 μm, the water concentration was higher after the treatment. It is further noted that the shapes of the water depth concentration profiles did not exhibit a clear change of slope, at the expected depth of about 15 μm, where the SC–epidermis interface is located. This is most likely caused by a degraded optical resolution. In this study, a so-called dry microscope objective was applied; in other words, there was an air gap in between the objective and the skin. This caused a deterioration of the spatial resolution.

Sieg et al. presented an in vivo 14-volunteer study of forearm skin, but now for a cumulative treatment (3 weeks) with cosmetic moisturizers (30). The authors calculated the area under the water concentration profiles, for the entire thickness of the SC. During the treatment, the thickness of the SC changed, and this was taken into account. A formulation containing niacinamide was shown to increase the total water content of the SC much more (up to 2 or 3 times) than the other tested formulations.

Very recently, Stamatas et al. presented in vivo confocal Raman spectroscopy data of skin penetration and occlusive potential of two vegetable oils and a paraffin oil (36). Petrolatum was used as a positive control. The products were applied topically on the forearms of nine volunteers and seven infants, and Raman depth concentration profiles of both the oils and water were acquired before and at 30 and 90 minutes following application. It was shown that paraffin and vegetable oils penetrate the top layers of the SC with similar concentration profiles, a result that was confirmed both for adult and infant skin. The three oils tested demonstrated modest SC swelling (10–20%) compared to moderate swelling (40–60%) for petrolatum. The swelling was assessed using the method of van der Pol et al. (33). No statistical difference between the paraffin oil and vegetable oils in terms of skin penetration and skin occlusion was observed.

The already mentioned work by Wu and Polefka (27) included moisturization experiments using products whose effect was already known. On isolated pigskin SC samples, the following products were tested: lotion, commercial soap bar, syndet bar, non-emollient shower gel and emollient-containing shower gel. The results were consistent with what was expected. The water content on the skin treated with lotion was significantly higher than the nontreated control. Syndet bar-treated skin had significantly higher water content than soap-based bar-treated sites. Non-emollient shower gel washed sites were more moisturized than soap-based bar-treated samples. Finally, emollient shower gel-treated skin was significantly more hydrated than non-emollient shower gel–washed skin.

Water Distribution in the Skin for Different Skin Types

Understanding the hydration processes of the skin also requires knowledge of the state of the skin prior to treatment. It is likely that different types of skin will respond differently to equal treatments. Therefore, it is of interest to study differences in water distribution in the skin of human volunteers of different skin type. Such knowledge will no doubt contribute to the development of products targeted to these different skin types. The first papers using confocal Raman microspectroscopy to study different skin types are being published now.

In 2006, Matsumoto et al. presented the results of a systematic study of the water distribution in the skins of an "old" male Japanese group of volunteers ($N = 20$, average age 64.0 ± 2.5 years) and a "young" male Japanese group ($N = 20$, average age 27.8 ± 1.6 years) (37). Water concentration profiles were recorded on untreated areas on the volar aspect of the forearm, down to a depth of 200 μm; note that this is well in the dermis. Surprisingly, no differences in water concentration profile could be detected in the SC and the epidermis. However, the water content in the upper dermis was found significantly lower for the young group. Possibly, the mechanically more worn dermis of the old group contains more damages

such as voids. These voids may be filled with water. It was concluded that the water content in the dermis may be a useful parameter for evaluations of aging.

In the already referenced paper by Egawa et al. (32), the SCATs were measured at different body sites and for different panelist ages (6 male, 9 female). On the forearm, the SCAT tended to be higher for older skin, but at the cheek no age dependence was found. The volar forearm skin was hydrated with a wet cotton patch, and measurements were done after 15, 50, and 90 minutes of hydration. A swelling of the SC was observed of 4%, 40%, and 95%, respectively. This finding was in agreement with previously reported swelling of a corneocyte, using cryo-scanning electron microscopy (cryo-SEM) (38). In a later paper, Egawa and Tagami also addressed the effects of season on the distribution of water in the skin (39).

Infant skin is a subject in itself. A very large panelist study, comparing the barrier function and water-holding and water-transport properties of a group of infants ($N = 124$, age 3–12 months) and a group of adults ($N = 104$, age 14–73 years), was published by Nikolovski et al. (40). Capacitance, TEWL, and Raman measurement were employed in this study. The SC was found to be thinner for infants. The capacitance and TEWL values were higher for the infants, and the variations over the infant panelists were larger. Interestingly, as observed in the Raman water depth concentration profile, large differences were also observed in the amount of water that was absorbed after application for only 10 seconds with a wet-soaked paper towel. Whereas adult skin did not seem to absorb much water, for infant skin a rapid increase of 5% to –10% by mass of water in the outer 10 μm of the skin was observed. Desorption rates of water were also studied; the desorption rate for infants was high initially, followed by a slower rate. Adults only exhibited the slower desorption rate. It was concluded that the way the SC stores and transports water become adultlike only after the first year of life. (In this paper, the NMF contents were also compared; they were lower for infants.)

In the paper by Chrit et al. (41), an in vitro study on skin models was combined with an in vivo study on human volunteer skin, using the Raman technology. The hydration capacities of 2-methacryloyloxethylphosphorylcholine polymer (pMPC), native or microencapsulated and with or without hyaluronic acid, were investigated. The in vitro experiments on the skin models showed the best hydrating properties for the encapsulated-with hyaluronic acid formulation, which also exhibited the longest lasting efficacy. In a 26-volunteer in vivo study, using confocal Raman spectroscopy, the encapsulated-with hyaluronic acid formulation was tested and a statistically significant hydration effect was observed.

Hydration Effects in Dysfunctional Skin

The fact that too much exposure of the skin to water may have unbeneficial effects is long known, it is said to "dry out" the skin. This is already an example of dysfunctional skin. Van der Pol et al. demonstrated in 2005 the effects of hot bathing on the composition and distribution of components (among which water) in the skin (42). In this work, an interesting experiment was carried out. Following soaking the forearm in hot water for 30 minutes, Raman water depth concentration profiles were recorded at the same site (the volunteer did not move his arm) every 30 seconds, after the soaking. First of all, a relatively large swelling of the SC was observed, but more interestingly, within the first 30 minutes after soaking, the water redistributed over the SC. The water concentration decreased 5% to 10% by mass around a depth of 25 μm and increased a similar amount at a depth of about 10 μm. This phenomenon reflects *dynamically* the reduction of the barrier function (the barrier function is thought to reside at the stratum granulosum) as a result of the intense treatment with hot water.

Another way to arrive at dysfunctional skin is removal of the top part of the SC by sequential tape stripping, thereby disrupting the barrier function. In 2005, Hellemans et al. presented in vivo results on four volunteers using this approach for volar forearm and facial skin (43). The results illustrated clearly that for untreated skin, facial SC is thinner than SC of the forearm. Moreover, after tape stripping, the remaining thinner SC could be observed easily from the profiles. Remarkably, the remaining thickness of the SC for face and arm after tape stripping until TEWL $= 18\ g/m^2/hr$ is nearly identical. Right after disrupting the barrier, the water concentration over the SC was observed to be higher (as was expected). However, the recovery response after tape stripping between the external water fluxes, determined with TEWL, and the internal water content of the SC differed. A fast initial (4 hours) recovery of the TEWL was observed, whereas the internal water content stayed high, even 24 hours after

stripping. This effect may be explained by the release of the lipid content of the lamellar bodies immediately after barrier disruption. Such a "film of lipids" might keep the internal water content of the SC elevated, which in turn may facilitate the enzymatic processing required for the barrier recovery response.

Another way of compromising the integrity of the skin is to simply wipe it with acetone. The acetone will take away much of the skin lipids present at the outer surface of the SC. Initial results were obtained by River Diagnostics researchers (unpublished data). Immediately after wiping, a clear increase in water concentration over the entire SC can be observed. This indicated a reduced barrier function; the water apparently is leaking out of the epidermal layers.

It is expected that confocal Raman microspectroscopy will also find useful applications in the study of diseased skin and its treatment (e.g., atopic dermatitis and psoriasis).

DISCUSSION, CONCLUSION, AND OUTLOOK

In vivo confocal Raman microspectroscopy is a novel method that provides detailed information about the molecular composition of the skin. In this chapter, its application on the study of hydration was reviewed. Many applications so far have focused on the SC. However, the method is readily capable of measurements to a depth of greater than 150 μm into the skin–well into the dermis.

In the past decade, in vivo confocal Raman spectroscopy has made a major leap forward in sensitivity, speed of measurement, and ease of use. Raman technology has now reached a level of refinement where it can be applied in routine clinical studies. It has become fast enough to perform measurements on numbers of subjects ranging from several to several dozen per day, depending on the complexity of the study. The user interface has reached a stage of development where routine operation of the equipment by a laboratory technician is practical. Although the Raman technique has now been shown to be routinely useful in clinical settings, it is, like all measurement techniques, subject to certain limitations. It involves many measurements being made at a single location to generate composition depth profiles, whereas other techniques, such as electrical conductivity, for example, normally take only a single data point at a given location. This means that even with fast instrumentation, Raman measurements may be time consuming compared to other commonly used methods of in vivo skin analysis. That is, however, simply the price paid to obtain much greater information content. A related general issue is that in vivo tissue analysis normally requires considerable replication, by measurement of multiple locations and on multiple subjects, to achieve required statistical accuracy, given normal biological variability. This is, of course, a characteristic inherent in any human in vivo measurements, and not specific to Raman. Finally, Raman instrumentation for in vivo skin analysis is highly specialized and therefore expensive. However, as in vivo Raman microspectroscopy comes into more general use, the cost of the instruments eventually can be expected to drop as volume efficiencies are realized by manufacturers. These limitations are well compensated by the richness of information achievable and the unique ability to measure the same area of skin repeatedly and with microscopic spatial detail, allowing entirely new kinds of information to be gathered. It can be expected that this detailed and spatially resolved information and the ability to make these measurements in vivo will provide insights into the mode of action of skin hydration that have not been previously available.

The conclusion and outlook of the role of in vivo confocal Raman microspectroscopy of skin, in the study of the skin hydration process, is well captured in a citation from the work of Wu and Polefka (27): *"The unique and direct quantitative water content information provided by confocal Raman microspectroscopy offers a whole new perspective for fundamental skin moisturization studies and will play an important role in evaluating moisturizing profiles and the hydration potential of products designed."*

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15 The Correlation Between Transepidermal Water Loss and Percutaneous Absorption: An Overview

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INTRODUCTION

Transepidermal water loss (TEWL) is the outward diffusion of water through skin (1). TEWL measurements are used to gauge the skin's water barrier function. An increase in TEWL reflects impairment of the water barrier (2). TEWL measurements allow parametric evaluation of the effect of barrier creams against irritants and characterization of skin functionality in clinical dermatitis and in irritant and allergic patch test reactions (3). An evaporimeter determines TEWL by measuring the pressure gradient of the boundary layer, resulting from the water gradient between the skin surface and ambient air. TEWL measurements can be affected by the anatomical site, sweating, skin surface temperature, inter-and intraindividual variation, air convection, ambient air temperature and humidity, and instrument-related variables, to name a few. Although TEWL is influenced by many variables, experiments show that evaporimeter measurements are reproducible in vitro and in vivo (3,4).

Percutaneous Absorption

Percutaneous absorption refers to the rate of absorption of a topically applied chemical through the skin. A compound's absorption rate is important for determining the effectiveness and/or potential toxicity of topically applied compounds. Since many topical formulations are used on diseased skin, where the integrity of the permeability barrier is in doubt, the dose absorbed into the body could vary greatly (5). The rate of absorption in vivo through the stratum corneum (SC) cannot be described by a zero-or first-order mathematical rate equation, because the SC is a complex system variable in its penetration properties. Many factors contribute to the percutaneous absorption of a given chemical. One rate limiting the step of a compound's absorption through the skin is the rate of diffusion through the SC. This review discusses the three main categories that give rise to percutaneous absorption rate variation, namely, methodology (including the effects of application time, method of measurement, and physicochemical properties of the topical compound), interindividual variation (including the effects of skin condition, age of individual, and blood flow), and intraindividual variation (including the differences between anatomic sites) (6,7).

Why Do We Want to Correlate TEWL and Percutaneous Absorption?

The extensive procedure required to measure percutaneous absorption versus TEWL enhances the desire to find a correlation between the two measurements to more easily assess skin barrier function. Experimentation of the correlation between TEWL and percutaneous absorption has resulted in studies concluding significant quantitative correlation and a few concluding no quantitative correlation.

The majority of studies investigating TEWL and percutaneous absorption correlation observe a quantitative correlation. It is our hypothesis that the papers that did not observe a quantitative correlation (8,9) or observed a weak correlation (1,10) do so because of assumptions made in the experiment's design.

Many of the experiments investigating TEWL and percutaneous absorption make large assumptions, which could affect the results of experimentation, and hence be the source of the controversy. For example, Tsai et al. (11) and Chilcott et al. (9) assume that an in vitro

Table 1 Summary of the Permeability and Lipophilicity of all the Compounds Tested on the Barrier-Disrupted Hairless Mouse

Compound	Partition coefficient ($K_{o/w}$)	Correlation coefficient (r)
Sucrose	−3.7	0.82
Caffeine	−0.02	0.86
Hydrocortisone	1.5	0.82
Estradiol	2.7	0.72
Progesterone	3.9	0.01

Source: From Ref. 11.

measurement of TEWL and percutaneous absorption is equivalent to in vivo measurements, whereas Lamaud et al. (12) assume that animal skin may serve as a permeability model for human skin. Great sources of error and variation can also be induced depending on the measurement method and type of absorption compound used in obtaining percutaneous absorption rates. As we do not completely understand the qualitative relationship between TEWL and percutaneous absorption, it is hard to determine, which assumptions made during the experiment could be affecting the correlation results. This section investigates the probable causes that could influence the results of the correlation experiments. Provided in Table 1 is a summary of the major assumptions made by the studies discussed.

In this section, we review some major studies defining the correlation between TEWL and percutaneous absorption and discuss major assumptions made in these experiments, which could significantly affect those studies that did not conclude a quantitative correlation. Provided in Table 2 is a summary of the major assumptions made by the studies discussed.

Main Review Correlation Studies

Oestmann et al. (1) investigated the correlations between TEWL and hexyl nicotinate (HN) penetration parameters in man. HN penetration was indirectly measured by laser Doppler flowmeter (LDF), which quantifies the increase in cutaneous blood flow (CBF) caused by the penetration of HN, a vasoactive substance. Lipophilic HN was chosen over hydrophilic methyl nicotinate because HN is a slower penetrant, hence, making it easier to distinguish an intact barrier from an impaired barrier.

Table 2 A Summary of the Major Assumptions Made by the Studies Discussed in This Review

Ref.	In vivo vs. in vitro (precabs)[a]	Skin type	Percutaneous absorption measurement method	Type of absorption compound[b]	Healthy skin versus damaged skin	Correlation results
(1)	Vivo	Human	LDF	Lipophilic	Healthy	Yes
(13)	Vivo	Human	Urinary	Lipophilic	Healthy	Yes
(14)	Vivo	Human	Urinary	Hydrophilic and lipophilic	Healthy	Yes
(15)	Vivo	Human	Plasma cortisol level	Lipophilic	Damaged	Yes
(11)[c]	Vitro	Animal	Diffusion cell	Hydrophilic and lipophilic	Damaged	Yes
(11)[c]	Vitro	Animal	Diffusion cell	Highly lipophilic	Damaged	No
(8)	Vivo	Human	LDF	Lipophilic	Damaged	Yes
(9)	Vivo	Animal	Urinary	Lipophilic	Both	Yes
(9)	Vitro	Both	Diffusion cell	Hydrophilic and lipophilic	Both	No

[a]As TEWL in vivo and in vitro measurements are considered equivalent, the authors are only concerned with how percutaneous absorption measurements were taken.
[b]Type of absorption compound was determined by their octanol-water partition coefficient, $K_{o/w}$ (Table 1). Values less than one are hydrophilic and more than three is very lipophilic.
[c]Reference Tsai et al. (11) was divided into two experiments in this table, as the study found a correlation between TEWL and percutaneous absorption with some compounds and no correlation with others.
Abbreviation: LDF, laser Doppler flowmeter.

LDF parameters t_0 and t_{max} were compared with corresponding TEWL values, and a weak quantitative negative correlation was made ($r = -0.31$ and -0.32). This correlation suggests that when an individual's response time, t_0, was fast, the skin barrier was impaired. The weak negative correlation found may be because of the percutaneous absorption method used. The LDF method has some negative attributes and is not as reproducible as other methods. Further research should investigate this weak correlation between TEWL and penetration of HN.

Lamaud et al. (12) investigated whether permeability changes of hydrophilic compounds (TEWL) are correlated to those of lipophilic compounds (hydrocortisone). In the first part of the experiment, penetration of 1% hydrocortisone and TEWL rates were recorded for the hairless rats in vivo before and after UV irradiation (660 J/cm^2). Both the results, before and after UV irradiation, correlated well with the TEWL values for application periods up to one hour.

In the second part, drug penetration was evaluated by urinary excretion five days after a single 24-hour application on normal, stripped, or UV-irradiated skin of hairless rats. The quantity of drug eliminated correlated with the level of TEWL for up to two days.

These results suggest that TEWL can predict the changes of skin permeability to lipophilic drugs in normal and some damaged skin.

Lavrijsen et al. (8) characterized the SC barrier function in patients with various keratinization disorders using two noninvasive methods, namely, measuring outward transport of water through skin by evaporimetry (TEWL) and the vascular response to HN penetration into the skin determined by LDF. Three of the five types of keratinization disorders studied, autosomal dominant ichthyosis vulgaris (ADI), X-linked recessive ichthyosis (XRI), and autosomal recessive congenital ichthyosis (CI), have impaired barrier function and are a type of ichthyosis, whereas the other two keratinization disorders studied, dyskeratosis follicularis (DD) and erythrokeratoderma variabilis (EKV), have no prior information available on barrier impairment. In this experiment, the two methods of barrier function assessment, TEWL and LDF, were correlated.

TEWL measurements and the LDF parameter, t_0, showed a high negative correlation in the patient group ($r = -0.64$) and a weaker negative correlation among the control group ($r = -0.39$). As TEWL reflects the SS-flux of a compound across SC, and parameter t_0 is a function of the duration of the lag phase (non-SS), this study suggests that these two methods should not be considered as exchangeable alternatives but rather as complementary tests. Each method reflects a different aspect of the barrier function.

This paper concludes that TEWL and HN penetration injunction are suitable methods to monitor skin barrier function in keratinization disorders and are helpful in discriminating between some of these disorders.

Rougier et al. (13) attempted to establish the relationship between the barrier properties of the horny layer (percutaneous absorption and TEWL) and the surface area of the corneocytes according to anatomic site, age, and sex in man. The penetration of benzoic acid (BA) was measured in vivo at seven anatomic sites and compared with its TEWL measurement taken on the contralateral site. The amount of BA penetrated was measured through urinary extraction up to 24 hours after application. It was discovered that irrespective of anatomic site and gender, a linear relationship ($r = 0.92$, $p < 0.001$) exists between total penetration of BA and TEWL.

Comparing corneocyte surface area to permeability, the study found a general correlation of increasing permeability for both H_2O and BA with decreasing corneocyte size. The smaller the volume of the corneocyte, the greater is the intercellular space available to act as a reservoir for topically applied molecules (10). This thinking is because of other studies that have shown that the smaller the capacity of the reservoir, the less the molecule is absorbed (10,14–16). However, for certain anatomic sites where corneocyte size was similar (980–1000 mm^2), there were large differences in permeability. Therefore, showing that, when percutaneous absorption and TEWL are quantitatively correlated, corneocyte size only partially explains the difference in permeability between the different anatomic sites and age of the skin.

Lotte et al. (17) examined the relationship between the percutaneous penetration of four chemicals (acetyl-salicylic acid, BA, caffeine, and sodium salt of BA) and TEWL in man as a function of anatomic site. The amount of chemical penetrated was measured by urinary excretion for up to 24 hours after application. For a given anatomic site, the permeability varies widely with the nature of the molecule administered because of the physicochemical

interactions that occur between the molecule, vehicle, and SC. For all anatomic sites investigated, irrespective of physicochemical properties of the molecules administered, there was a linear relationship between TEWL and percutaneous absorption.

Aalto-Korte and Turpeinen (18) attempted to find the precise relationship between TEWL and percutaneous absorption of hydrocortisone in patients with active dermatitis. Percutaneous absorption of hydrocortisone and TEWL were studied in three children and six adults with dermatitis. All the subjects had widespread dermatitis covering at least 60% of the total skin area. Plasma cortisol concentrations were measured before and two and four hours after hydrocortisone application by radioimmunoassay. TEWL was measured in six standard skin areas immediately before application of the hydrocortisone cream. Each individual TEWL value was calculated as a mean of these six measurements.

The concordance between the postapplication increment in plasma cortisol and mean TEWL was highly significant, resulting in a correlation coefficient of $r = 0.991$ ($p < 0.001$). In conclusion, this study found a highly significant correlation between TEWL and percutaneous absorption of hydrocortisone.

Tsai et al. (11) investigated the relationship between permeability barrier disruption and the percutaneous absorption of various compounds with different lipophilicity values. Acetone treatment was used in vivo on hairless mice to disrupt the normal permeability barrier, and in vivo TEWL measurements were used to gauge barrier disruption. The hairless mouse skin was then excised and placed in diffusion cells for the in vitro percutaneous absorption measurements of five model compounds. The permeability and the lipophilicity of all the compounds tested on the barrier-disrupted hairless mouse are summarized in Table 1.

The permeability barrier disruption by acetone treatment and TEWL measurements significantly correlated with the percutaneous absorption of the hydrophilic and lipophilic drugs, sucrose, caffeine, and hydrocortisone. However, acetone treatment did not alter the percutaneous penetration of the highly lipophilic compounds, estradiol and progesterone, hence suggesting that there is no correlation between TEWL and the percutaneous absorption of highly lipophilic compounds. The results imply the need to use both TEWL and drug lipophilicity to predict alterations in skin permeability.

Chilcott et al. (9) investigated the relationship between TEWL and skin permeability to tritiated water (^3H_2O) and the lipophilic sulfur mustard (^{35}SM) in vitro. No correlation was found between basal TEWL rates and the permeability of human epidermal membrane to ^3H_2O ($p = 0.72$) or ^{35}SM ($p = 0.74$). Similarly, there was no correlation between TEWL rates and the ^3H_2O permeability of full-thickness pigskin ($p = 0.68$). There was no correlation between TEWL rates and ^3H_2O permeability following up to 15 tape strips ($p = 0.64$) or four needlestick punctures ($p = 0.13$). These data indicate that under these experimental circumstances TEWL cannot be used as a measure of skin's permeability to topically applied compounds.

More on Assumptions

There is no doubt that the best experimental conditions are those that are closest to reality; in our case, those are TEWL and percutaneous absorption measured in vivo, on human skin, and using the most reliable percutaneous absorption method of measurement available. It is not a coincidence that all the studies, which used these ideal experimental conditions, came up with the same result that TEWL and percutaneous absorption are quantitatively correlated. It is only the studies, which veered from these most ideal conditions by measuring in vivo or using animal skin to model human skin or using alternate and less reliable methods or percutaneous measurement that found no significant quantitative correlation between the two skin barrier indicators. In the sections below, we will discuss the possible repercussions of varying experimental conditions that form the ideal.

Using In Vitro Methods to Model In Vivo Experiments

Skin permeation can be measured in human or in vitro by using excised skin in diffusion cells. In theory, studies using excised skin are feasible models for in vivo experiments, because passage through the skin is a passive diffusion process and the SC is composed of nonliving tissue. Many studies comparing in vivo and in vitro TEWL and percutaneous absorption measurements have been conducted, and the results from those experiments support the contention that reliable measurements can be obtained from in vitro methodology (6,19–25).

Although the consensus is that in vitro experiments are reasonable models for in vivo human experiments, some experiments note significant differences between these methods for measuring skin permeation. The most significant study by Bronaugh and Stewart (23) found that the effects of UV irradiation could not be duplicated using an in vitro experimentation model, hence suggesting that in vitro experiments examining the TEWL and percutaneous absorption after barrier damage may not be an acceptable model for in vivo experimentation. In vitro damage to the SC barrier may not be an accurate model to in vivo SC damage, because in vivo exposure to skin irritants results in a cascade of reactions that do not occur in human cadaver skin (19).

Chilcott et al. (9) investigated TEWL and percutaneous absorption correlation in vitro after inducing different types of barrier damage. This was also one of the only studies reviewed, which did not observe a correlation between TEWL and percutaneous absorption. Perhaps, using in vitro methodology in the experimental design may be responsible for the lack of correlation to skin damage reported in this study.

Using Animal Skin to Model Human Skin

Comparing the skin morphology and chemical absorption of human versus animal skin, it is clear that human skin is unique in both aspects and should be used for the most meaningful results (26). Yet an experiment by Bronaugh et al. (27) found that depending on the compound of interest and the vehicle used, permeability values obtained using animal skin can be well within an order of magnitude of the permeability values for human skin.

Independently, in vitro methods and animal skin models prove to be reliable models for human in vivo absorption. Therefore, it seems logical to assume that in vitro and animal methods may be used in unison to accurately model in vivo human absorption. However, Rougier et al. (28) documented a distinct difference between animal studies done in vivo versus in vitro when compared with human absorption. This experiment compares the skin permeability of humans to the hairless rat (29) and the hairless mouse (22) using molecules of widely different physicochemical properties. The results show that, in vivo, for whatever the molecule tested the permeability ratios remained relatively constant, whereas in vitro they do not. Therefore, when application conditions are strictly identical in humans and animals, it may be possible to model human in vivo absorption by measuring in vivo animal absorption but not using in vitro animal absorption. The inaccurate results obtained when conducting experiments in vitro using animal skin may have affected the results studied by Tsai et al. (11) and Chilcott et al., (9), which were the only two papers to conclude no correlation between TEWL and percutaneous absorption, and these were the only two papers using in vitro animal methodology.

Percutaneous Absorption Measurement Methods

A major factor affecting percutaneous absorption measurements is methodology (30,31). All methods for percutaneous absorption measurements are not equal and hence can give different results. The fourth column of Table 2 summarizes the percutaneous absorption methods used in these correlation studies.

The most common method for determining percutaneous absorption in vivo is measuring the radioactivity of excreta, following topical application of a labeled compound. Determination of percutaneous absorption from urinary radioactivity does not account for metabolism by skin, but has been proven to be a reliable method for absorption measurement and is widely accepted as the ''gold standard'' when available.

The most commonly used in vitro technique involves placing a piece of excised skin in a diffusion chamber, applying radioactive compound to one side of the skin, and then assaying for radioactivity in the collection vessel on the other side (32). The advantages of using this in vitro technique are that the method is easy to use and the results are obtained quickly. The disadvantage is that the fluid in the collection bath, which bathes the skin, is saline, and though it may be appropriate for studying hydrophilic compounds, it is not so for hydrophobic compounds. If the parent compound is not adequately soluble in water, then determining in vitro permeability into a water receptor fluid will be self-limiting.

When conducting in vitro experiments, animal skin is often substituted for human skin. Because animal skin has different permeability characteristics from human skin, one should be careful, which type of animal skin is used (refer to section Using Animal Skin to Model Human

Skin). In addition, proper care should be taken in skin preparation of excised skin to make sure not to damage skin barrier integrity. Anatomical site is as important as using of many different skin samples.

The only two experiments, which did not find a correlation between TEWL and percutaneous absorption, by Tsai et al. (11) and Chilcott et al. (9), were those, which measured percutaneous absorption in vitro. Perhaps, using a diffusion cell to measure percutaneous absorption is the reason for not finding a correlation.

Oestmann et al. (1) and Lavrijsen et al. (8) used LDF to measure HN penetration. LDF measures the increase in CBF caused by the penetration of HN, a vasoactive substance. One problem with this method is that LDF measurements are on the amount of HN absorbed but also on the individual's vasoreactivity, gender, and age. This may be the reason that Oestmann et al. (1) and Lavrijsen et al. (8) obtained only a weak correlation between TEWL and percutaneous absorption of HN. Another disadvantage of this method is that LDF measurements have many sources of variation, which make it difficult to compare interlaboratory results. If an attempt should be made, note that LDF parameters t_0 and $t_{\max}$ are the function of HN concentration, the vehicle used, and the application time; the LDF parameters LDF_{base} and LDF_{max} are relative values depending on the type of LDF used.

Type of Compound Used to Measure Percutaneous Absorption

The percutaneous absorption rate and/or total absorption of a compound varies greatly depending on the compound and its lipophilicity. Yet, many of the papers reviewed did not consider how lipophilicity of the test compound would affect percutaneous absorption and hence affect correlation results. Feldmann and Maibach (20) measured both the total absorption and maximum absorption rate for 20 different compounds of different lipophilicities. The range for total absorption for the 20 compounds tested was >250 times, whereas the difference in maximum absorption rate was >1000-fold (20). Because of the extreme range of absorption for topically applied compounds, it seems reasonable to assume that the correlation between TEWL and percutaneous absorption may not be independent of the physicochemical properties of the compound applied. Namely, can TEWL measurements predict the skin barrier's permeability changes to both hydrophilic and very lipophilic compounds?

Correlation results from many studies, Oestmann et al. (1), Lamaud et al. (12), Lavrijsen et al. (8), Lotte et al. (17), Aalto-Korte and Turpeinen (18), and Tsai et al. (11), suggest that TEWL can predict the changes in skin permeability to hydrophilic and slightly lipophilic topical drugs. Tsai et al. (11) also discovered that the percutaneous absorption of highly lipophilic compounds does not correlate with TEWL.

The highly lipophilic compounds are the compounds that did not show evidence of a correlation between percutaneous absorption and TEWL, whereas the moderately lipophilic compounds, such as hydrocortisone and BA, did. This should be further investigated. In the future, it may be necessary to use both TEWL and drug lipophilicity to predict alterations in skin permeability.

EXPLORING THE QUALITATIVE REASONING FOR THE CORRELATION BETWEEN PERCUTANEOUS ABSORPTION AND TEWL

Yet, despite the significant quantitative correlation demonstrated in some experiments, the precise qualitative relationship between percutaneous absorption and TEWL remains unsettled. Is the quantitative correlation just a coincidence or have we not discovered the link between the two indicators?

Experiments investigating the correlation between TEWL and percutaneous absorption have found a quantitative correlation between the two skin barrier indicators, yet have failed to find their precise qualitative relationship. Most experiments looking for an explanation of skin permeability examine and compare trends in physical aspects of the skin such as SC membrane thickness, corneocyte size, area of the horny layer, transcorneal routes, sebum lipid film, intercellular volume, to name a few. Yet we remain clueless about the structure-function relationship of the SC, because there is no morphological aspect that explains the permeability of the SC. Skin has particular features, which combine together in varying degrees to produce

different experimental values of TEWL and percutaneous absorption (17). Further investigation needs to be done regarding the relationship between TEWL and percutaneous absorption in skin structure and morphology.

CONCLUSION

Although it is not certain why studies by Tsai et al. (11) and Chilcott et al. (9) showed no quantitative correlation, we can postulate some estimations.

The study by Tsai et al. (11) is the only paper demonstrating a clear distinction between highly lipophilic compounds and slightly lipophilic compounds, when correlating percutaneous absorption and TEWL. Acetone treatment could affect a certain aspect of the skin barrier that mostly affects and interacts with hydrophilic compounds, hence having no affect on the highly lipophilic compounds such as estradiol and progesterone. It would be interesting to ascertain if the same results were obtained when selecting a different form of barrier damage such as physical tape stripping. Or it could be the fact that the lipophilic compounds chosen were even more hydrophobic than those used in other experiments, and indeed, TEWL and percutaneous absorption of highly lipophilic compounds are not correlated.

It is difficult to understand why Chilcott et al. (9) found no correlation between TEWL and percutaneous absorption. The results could have been affected, because the experiment was done in vitro, partly on animal skin, using an extremely lipophilic compound, ^{35}SM. It would be interesting to ascertain if TEWL and percutaneous absorption of ^{35}SM correlated with the results up to one hour after application.

Taken together, the weight of evidence confirms a relationship between TEWL (water transport) and percutaneous penetration, yet much remains before this can fully be generalized and the mechanism understood. Future experiments should take into consideration the effects of modeling realistic situations using alternative methods to the ideal.

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16 Role of Calcium in the Regulation of Skin Barrier Homeostasis

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INTRODUCTION

Some natural products have been shown to benefit the skin, especially for the restoration of skin barrier. Dead Sea mud and water, balneotherapeutic water preparations, deep sea sponges, milk, and pearl, for examples, have been used in ancient to modern formulations for topical application to provide healthy ageless skin. Results were not always well documented, but the effects have been observed and triggered many investigations. Among many components within these materials, calcium is one notable ingredient in common.

Calcium is important for human body and involved in many life processes. For instance, this element plays a crucial role in the growth, death, differentiation, and function of immune cells. Calcium is also important in the regulation of skin barrier homeostasis, as calcium is involved in the regeneration process of skin barrier components (1). The role of calcium in skin is more complex than previously assumed. The elucidation of calcium regulation mechanism in skin could be useful to understand and solve skin problems.

MECHANISM OF CALCIUM CELL SIGNALING IN SKIN

Calcium is the most abundant metal ion and fifth (after H, O, C, and N) most abundant element in human body on both an atom and weight basis. Over 98% of body calcium resides in bones and tooth enamel. The rest, in form of ion Ca^{2+}, is found throughout body fluids and takes part in various processes, including muscle contraction, blood clotting, nerve excitability, intercellular communication, membrane transport of molecules, hormonal responses, exocytosis, and cell fusion, adhesion, and growth (2).

Calcium ion is used as a universal messenger for living things, even in simple organisms and plants. The unique combination of its ionic radius and double charge allows Ca^{2+} to be specifically recognized and to yield tighter binding to receptors to the exclusion of other ions, leading to strong, specific binding (3). The specificity enables cells to form special receptors to assess signals from calcium. For many parts of the body, Ca^{2+} often acts as a second messenger in a manner similar to cyclic adenosine monophosphate (cAMP). Transient increases in cytosolic Ca^{2+} concentration trigger numerous cellular responses, including muscle contraction, release of neurotransmitters, and glycogen breakdown (glycogenolysis), also as an important activator of oxidative metabolism (4). Ca^{2+} does not need to be synthesized and degraded with each message transmission, so it is an energy-efficient signal for the cell (5).

In skin, calcium can provide signals for the cells, either extracellular or intracellular (in the cytosol). The extra- and intracellular signals are connected to each other, but may also act separately. In keratinocytes, extracellular Ca^{2+} levels influence growth and differentiation (6,7). At low extracellular Ca^{2+} levels (<0.1 mM), keratinocytes proliferate as a monolayer, rapidly becoming confluent (6,7). In this condition, keratinocytes never stratify, but show an undifferentiated, basal cell-like phenotype (8). The cells synthesize keratin proteins and are connected by occasional gap junctions but not by desmosomes. The cells also synthesize mainly ceramide type 2 (Cer-NS) and a small amount of ceramide type 3 (Cer-NP) (9). Keratinocytes grown in low-calcium medium (0.02 mM) maintained intracellular Ca^{2+} levels adequate for arachidonic acid metabolism and actually showed increased prostaglandin (PGE_2 and PGF_2) production up to 4.5 times compared with cells grown at normal Ca^{2+} level

(1.2 mM) (10). If this is true for the in vivo condition, a low level of extracellular Ca^{2+}, for instance, due to a defective skin barrier may cause an increase in prostaglandin synthesis, leading to hyperproliferative epidermal disorders, such as psoriasis, which are often associated with abnormalities in prostaglandin production (11).

Extracellular Ca^{2+} levels at equal or more than 0.1 mM trigger the differentiation of keratinocytes and synthesis of a complex pattern of free and covalently bound ceramides (12). The mRNA levels of keratin 10 (K10) and profilaggrin as well as those of ceramide glucosyltransferase and glucosylceramide-β-glucosidase increased (9). The early differentiation markers, K1 and K10, are observed within 8 to 24 hours, then the late markers, loricrin and filaggrin, are shown after 24 to 48 hours (8). Keratinocytes rapidly flatten, form desmosomes, and differentiate with stratification, while cornified envelopes form in cells of the uppermost layers (6,7).

The response to signaling is also shown in a progressive way. Keratinocytes grown in a low-calcium media proliferate. Increased extracellular Ca^{2+} inhibits proliferation, while it induces differentiation (13). With the increase, K14 expression is downregulated (8). On the other hand, differentiation of keratinocytes caused a decrease in responsiveness to extracellular Ca^{2+}, which may facilitate the maintenance of the high level of intracellular Ca^{2+} required for differentiation (14).

Raised extracellular Ca^{2+} increases intracellular Ca^{2+} (15–17). This implies that increased intracellular Ca^{2+} is the actual signal to trigger keratinocyte differentiation. Intracellular Ca^{2+} signals are assessed through calcium-binding proteins to induce responses. The major calcium-binding protein in skin is calmodulin. Calmodulin regulates target protein by modulating protein–protein interactions in a calcium-dependent way. Calmodulin regulates many enzymes, for example, adenyl and guanyl cyclase, phosphodiesterase, ornithine decarboxylase, calcium-calmodulin–dependent protein kinase, transglutaminase, and phospholipase, which are also found in skin (5).

Both intracellular release and transmembrane flux contribute to the rise in intracellular Ca^{2+} (16,17). The rise in keratinocyte intracellular Ca^{2+} in response to raised extracellular Ca^{2+} has two phases: (*i*) an initial peak, not dependent on extracellular Ca^{2+}, and (*ii*) a later phase that requires extracellular Ca^{2+} (16). An early response of human keratinocytes to increases in extracellular Ca^{2+} is an acute increase in intracellular Ca^{2+}. Stepwise addition of extracellular Ca^{2+} to neonatal human keratinocytes is followed by a progressive increase in intracellular Ca^{2+}, where the initial spike of increased intracellular Ca^{2+} is followed by a prolonged plateau of higher intracellular Ca^{2+} (18). The response of intracellular Ca^{2+} to increased extracellular Ca^{2+} in keratinocytes is saturated at 2-mM extracellular Ca^{2+} (18,19). The response of intracellular Ca^{2+} to increased extracellular Ca^{2+} in keratinocytes resembles the response in parathyroid cells, in that a rapid and transient increase in intracellular Ca^{2+} is followed by a sustained increase in intracellular Ca^{2+} above basal level. This multiphasic response is attributed to an initial release of Ca^{2+} from intracellular stores followed by an increased influx of Ca^{2+} through voltage-independent cation channels. The keratinocyte and parathyroid cell contains a similar cell membrane calcium receptor thought to mediate this response to extracellular Ca^{2+}. This receptor can activate the phospholipase-C pathway, leading to an increase in the levels of inositol 1,4,5-triphosphate (IP_3) and *sn*-l,2-diacylglycerol (DAG)—both of which are important messengers—as well as stimulating Ca^{2+} influx and chloride currents (20,21). IP_3 causes release of Ca^{2+} from internal stores, such as endoplasmic reticulum, further increasing intracellular level to precede a number of calcium-stimulated cellular events (22). DAG forms a quarternary complex with phosphatidylserine, calcium, and protein kinase C to activate the kinase, which will accelerate terminal differentiation (13). The signal transduction mediated through calmodulin induces other proteins, for example, desmocalmin, which is associated with the formation of desmosomes (23).

REGULATION OF CALCIUM GRADIENT

The regulation of calcium in skin shows an ingenious adaptation of living organisms to the presence of this ion. As Ca^{2+} cannot be metabolized like other second-messenger molecules, cells tightly regulate intracellular levels through numerous binding and specialized extrusion proteins (24). The concentration of calcium in extracellular spaces (generally $\sim$1.5 mM) is four orders of magnitude higher than in the cytosol ($\sim$0.1 μM). In excitable cells, for example,

muscle cells, the extracellular concentration of calcium must be closely regulated to keep it at its normal level of ~1.5 mM, so that it cannot accidentally trigger the muscle contraction, the transmission of nerve impulses, and blood clotting (4). In other cells, including keratinocytes, the extracellular level is similarly maintained in a specific equilibrium with the intracellular concentration.

Ca^{2+} also regulates melanin production in melanocyte; one way is through its ability to act as a cofactor for phenylalanine hydroxylase, which catalases the conversion of L-phenylalanine to L-tyrosine, the precursor of melanin (25). As with keratinocytes, low extracellular Ca^{2+} concentrations increase the proliferation of melanocytes, whereas high concentration does not show effect (26). Elevations in intracellular Ca^{2+} concentration have an inhibitory effect on the melanin production (27), but if coupled with the increase of cAMP, elevated intracellular Ca^{2+} level stimulates melanogenesis (28).

It is important for the cells to keep the intracellular calcium level low. A low-calcium concentration makes the use of the ion as an intracellular messenger energetically inexpensive. The movement of calcium ions across membranes requires energy, usually supplied by adenosine triphosphate (ATP). If the resting level of calcium in the cell were high, a large number of ions would need to be transported into the cytoplasm to raise the concentration by the factor of 10, which is ordinarily needed to activate an enzyme; afterward the excess calcium would have to be expelled from the cell. The normally low-calcium level means that relatively few ions need to be moved, with a relatively small expenditure of energy, to regulate an enzyme. In contrast, energetic cost of regulation by the other important intracellular messenger, cAMP, is high; it must be synthesized and broken down each time it carries a message, and both steps requires a significant investment of energy (3). Furthermore, low intracellular calcium is a necessary condition for the phosphate-driven metabolism characteristic of higher organisms. The energy-rich fuel for most cellular processes is ATP. Its breakdown releases inorganic phosphate. If the intracellular concentration of Ca^{2+} were high, the phosphate and the calcium would combine to form a precipitate of hydroxyapatite crystals, the same stony substance found in bone, and the calcification would ultimately doom the cell (3).

The large concentration gradient between extracellular spaces and cytosol is maintained by the active transport of Ca^{2+} across the plasma membrane, the endoplasmic reticulum (or the sarcoplasmic reticulum in muscle), and the mitochondrial inner membrane. Generally, plasma membrane and endoplasmic reticulum each contain a Ca^{2+}-ATPase that actively pumps Ca^{2+} out of the cytosol at the expense of ATP hydrolysis (4). Mitochondria act as a "buffer" for cytosolic Ca^{2+}. If cytosolic concentration of calcium rises, the rate of mitochondrial Ca^{2+} influx increases while that of Ca^{2+} efflux remains constant, causing the mitochondrial concentration of Ca^{2+} to increase, while the cytosolic concentration of Ca^{2+} decreases to its original level (its set point). Conversely, a decrease in cytosolic concentration of Ca^{2+} reduces the influx rate, causing net efflux of concentration of Ca^{2+} and an increase of cytosolic concentration of Ca^{2+} back to the set point (4). In melanocytes, Ca^{2+} homeostasis is regulated by melanin (29). Addition of high Ca^{2+} concentration to melanocytes kept in Ca^{2+}-free medium shows different type of increase between poorly and well-melanized melanocytes. This may be the result of the different content of melanin, which provides clearance of cytoplasmic Ca^{2+} into melanosomes (29). The strong Ca^{2+}-binding capacity of melanin (particularly inside melanosomes) is evident in its protective characteristic against DNA damage induced by reactive oxygen species (ROS) in both melanocytes and keratinocytes (30). It was reported that H_2O_2 and other reactive oxygen compounds induce increases in intracellular Ca^{2+} concentration and disrupt intracellular Ca^{2+} homeostasis, causing DNA strand breaks (31). On the other hand, the presence of melanin reduces intracellular Ca^{2+} level and stabilizes intracellular Ca^{2+} homeostasis (29).

Besides the already mentioned Ca^{2+}-ATPase, the transport of Ca^{2+} is regulated by a series of calcium pumps, transport systems, and ion channels. The availability of certain regulatory systems is dependent on the activity of the cells. In excitable cells such as cardiac muscle, the influx of Ca^{2+} to cytosol is regulated by voltage- (or potential-) dependent channels, while the efflux (out of cytosol) is regulated by cation exchanger, such as Na^{+}-Ca^{2+} exchanger (5). Undifferentiated keratinocytes in the basal layer have different sets of Ca^{2+} transport system than differentiated cells in the upper layers. In basal layer, the system consists of 14-pS nonspecific cation channels (NSCC) (32) and does not possess functional voltage-sensitive Ca^{2+} channels (17). Differentiated keratinocytes are likely to possess at least two and possibly three pathways of Ca^{2+} influx: (*i*) nicotinic channel (nicotinic acetylcholine receptor or

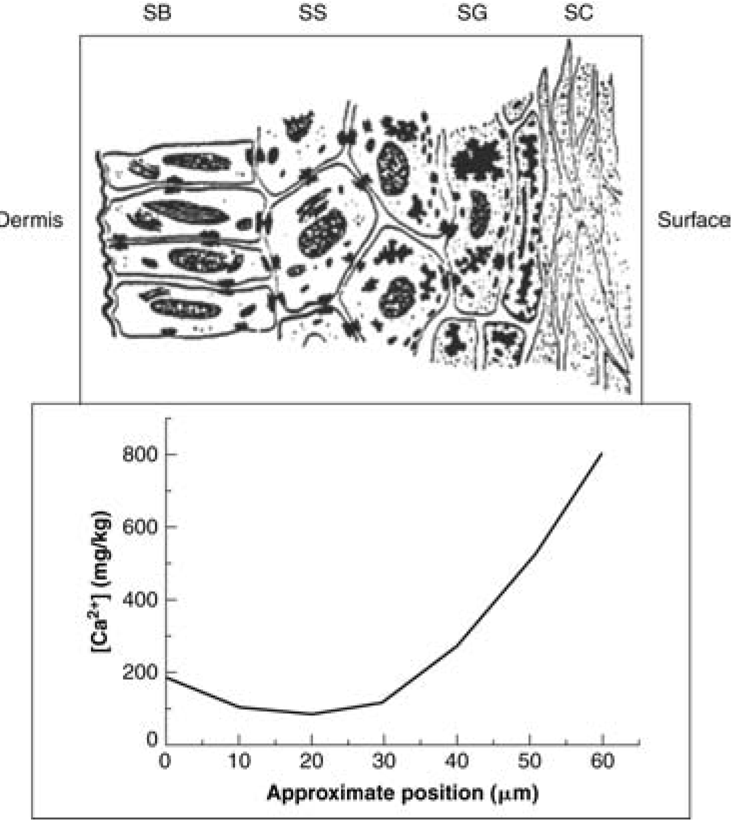

Figure 1 Illustration of calcium gradient in epidermis based on literature data. *Abbreviations*: SB, stratum basale/basal layer; SS, stratum spinosum; SG, stratum granulosum; SC, stratum corneum. *Source*: From Ref. 36.

nAChR); (*ii*) voltage-sensitive Ca^{2+} channels (VSCC) which can be blocked by nifedipine or verapamil; and (*iii*) NSCC, which is not activated by nicotine (33).

Other than the high-calcium gradient between extra- and intracellular domains of keratinocytes, a calcium gradient is present within the epidermis, with higher quantities of Ca^{2+} in the upper than in the lower epidermis, as the cell moves from the basal layer to the stratum granulosum (SG) (34). Ca^{2+} concentration increases steadily from the dermal-epidermal junction to the region just below the stratum corneum (SC), while this is not the case with other ions (35). Figure 1 illustrates the calcium gradient in human skin in comparison with an actual literature data (36). Such a gradient is not observed in skin abnormalities related to the formation of abnormal barrier function, such as psoriasis (37). Studies in mice and rats showed that this gradient exists at the same time as the formation of a maturing skin barrier at the end of gestation. The gradient is then maintained from the newborn throughout the adult life (38), although it tends to change with aging. It is not yet clear whether the calcium gradient leads to the formation of a mature barrier or the barrier caused the gradient. It may even be both if the regulation uses a feedback mechanism, as the differentiation will eventually form a barrier leading to the accumulation of Ca^{2+} in the upper epidermis. This high level of Ca^{2+} will, in turn, guarantee the ongoing process of differentiation toward the formation of corneocytes, fully differentiated keratinocytes in SC. The mechanism is thus almost completely autonomous and perpetual and, if it runs smoothly, requires little correction from the body.

SKIN BARRIER HOMEOSTASIS AND REPAIR

The skin barrier function is connected to the chemical and physical condition of SC, the uppermost layer of the epidermis, where the final phase of keratinocyte differentiation into corneocytes takes place. Skin barrier gives protection against desiccation and environmental

challenge by regulating water flux and retention (39). The optimal level of hydration maintained in skin barrier layer is largely dependent on three components, which are constantly regenerated particularly in SC, namely, (*i*) intercellular lamellar lipids, as an effective barrier to the passage of water; (*ii*) corneocytes, which provide the tortuous diffusion path created by the SC layers and corneocyte envelopes that retard water loss, and (*iii*) natural moisturizing factor (NMF), a complex mixture of low–molecular weight, water-soluble compounds first formed within the corneocytes by degradation of the histidine-rich protein known as filaggrin. Disturbance to the regeneration processes of these components, in which calcium plays a significant role as mentioned above, results in dry, flaky skin conditions (40). At normal calcium gradient condition, Ca^{2+} induces synthesis of intercellular lipid (41), full terminal differentiation into corneocytes (42), and the formation of the cornified envelope (43). Abnormal calcium distribution in aging people has been linked to fragile skin barrier in elderly (44).

Disruption of the barrier with acetone treatment or tape stripping depletes Ca^{2+} from the upper epidermis, resulting in the loss of the Ca^{2+} gradient (45–47). This is due to accelerated water transit that leads to the increased passive loss of Ca^{2+} into and through SC (45,47), because the permeability of SC to Ca^{2+} dramatically increased after SC was pretreated with acetone or sodium lauryl sulfate solution (48). The permeability of skin to Ca^{2+} ions has been known from some dermatoses, such as calcinosis cutis (49–51) and perforating verruciform collagenoma (52). In a shorter term, calcinosis cutis developed after a 24-hour (at least) topical application of an electrode paste containing saturated calcium chloride solution, bentonite, and glycerin, which are used for examination by electroencephalography or electromyography (53,54). The permeability of human skin to Ca^{2+} ions in vitro shows a marked dependence on anatomic site. In agreement with the data observed for nonelectrolytes, permeation decreased in the following order: foreskin > mammary > scalp > thigh. Mouse and guinea pig skin show comparable permeability to that of human scalp. Ca^{2+} transport from dermis across epidermis is higher than that from epidermis to dermis (55,56). Using a technique to continuously monitor the low level of Ca^{2+} flux across human SC in vitro, the flux through untreated human SC was shown to be sigmoidal. After SC was pretreated with acetone or sodium lauryl sulfate, the shape of the curve was similar, but the Ca^{2+} flux was significantly higher (48).

The decrease in Ca^{2+} levels in the outer epidermis is associated with enhanced lamellar body secretion and lipid synthesis (important components in repair responses) (45,57). Experiment in mice shows that after the calcium gradient disappears following acute permeability barrier disruption, the gradient returns after six hours in parallel with barrier recovery. This indicates that skin barrier formation (through restriction of transcutaneous water movement) could regulate the formation of the epidermal calcium gradient (58). Note that the barrier repair in response to the skin barrier disruption is not the same as the normal barrier regeneration process. The response is an emergency step to quickly reduce the transepidermal water loss to its set point and thereby returning the calcium gradient to its natural condition (45). Once the calcium gradient is normalized, the normal skin barrier regeneration takes place. The process of barrier repair in connection with transepidermal water loss and calcium gradient is illustrated in Figure 2.

Addition of high calcium concentration during the barrier disruption process will induce higher influx of calcium into epidermal keratinocytes, which delays the emergency skin barrier repair process (59). Also, if Ca^{2+} gradient can be preserved after skin barrier disruption by the addition of Ca^{2+} into the media, or occlusion of barrier-disrupted skin with water vapor-impermeable membrane, lamellar body secretion, lipid synthesis, and emergency barrier recovery are inhibited (57,60). The inhibition raised by high extracellular concentration of Ca^{2+} is potentiated by high extracellular potassium (K^{+}) (61). However, during this delay, and if the applied calcium concentration is within the right physiological range, the normal skin regeneration process can take place and the normal barrier function is restored without the formation of intermediate emergency barrier. This is indicated in a study on the cultured keratinocytes that extracellular calcium in physiological range of concentration is not a sufficient signal for growth arrest when other growth conditions are optimized (62).

Another study confirmed that barrier recovery is accelerated by the decreased level of Ca^{2+} and also K^{+} during an increased water loss, since water loss may induce a decrease in the Ca^{2+} concentration in the upper epidermis, which, in turn, may stimulate lamellar body secretion and barrier repair (63). Furthermore, the inhibition raised by high extracellular Ca^{2+} concentration is reversed by nifedipine or verapamil, which are specific calcium channel

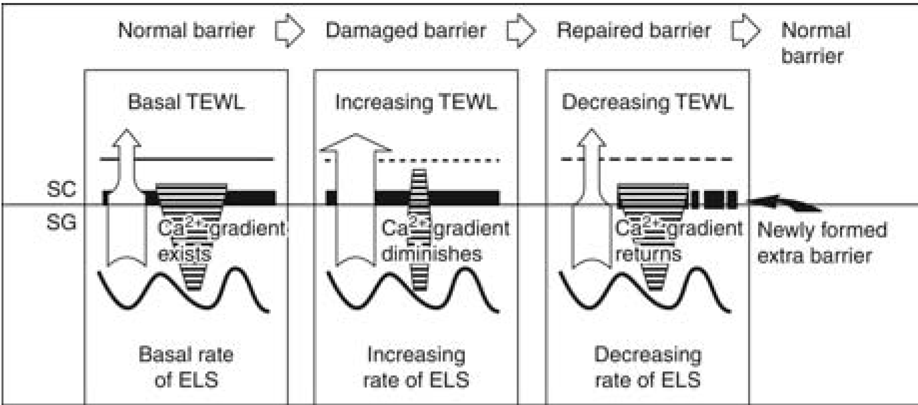

Figure 2 Illustration of skin barrier repair in epidermis. *Abbreviations*: SC, stratum corneum; SG, stratum granulosum; TEWL, transepidermal water loss; ELS, epidermal lipid synthesis.

blockers (61). In another study, administration of Ca^{2+}-free solutions by sonophoresis resulted in a marked decrease in Ca^{2+} content in the upper epidermis, and subsequently the loss of the Ca^{2+} gradient was accompanied by accelerated lamellar body secretion (a sign of emergency skin barrier repair) (64).

Dry, itchy, and scaly skin symptoms are frequently linked to an impaired skin barrier function, as observed in psoriasis, ichtyosis, atopic skin, and contact eczemas (65). Psoriatic lesions have been directly related to the loss of the normal calcium gradient in epidermis (37). The abnormal calcium gradient is shown in the people with atopic skin (66). In chronic hemodialysis patients, the commonly incident of uremic pruritus is found linked to the disrupted calcium gradient, especially with higher Ca^{2+} deposition in the extracellular fluid and cytoplasm of basal cells, and in the extracellular fluid, nuclei and cytoplasm of spinous cells compared with the non-pruritus group (67). On the other hand, studies on reconstructive epidermis have clearly demonstrated that once Ca^{2+} distribution profile is restored to normal, the terminal differentiation and SC barrier formation is improved (68). These facts indicate that restoration of Ca^{2+} gradient may lead to alleviation of dry, itchy, scaly, and other adverse skin symptoms related to skin barrier function.

TOPICAL APPLICATION OF CALCIUM

With the understanding that decreased Ca^{2+} level at the suprabasal cell layers results in abnormal differentiation, it is logical to attempt calcium supplementation by topical application. However, there are two difficulties in this approach. Topical application of high level of calcium alone is not recommended, because it may lead to calcitosis cutis, as seen in long-term occupational exposure to high levels of dissolved calcium, for example, in miners (49), agricultural laborers (50), and oil field workers (51). Secondly, if Ca^{2+} level in the basal cell layer increases after such application, then it causes disturbance of keratinocyte proliferation, reducing epidermal growth rate, and also may cause symptoms such as detected in uremic pruritus patients (67). The normalization of distribution of calcium ion requires high concentration below SG and SC interface (68), thus requires delivery of calcium below the skin barrier region in SC. As learned from the therapy using natural resources, topical application of calcium apparently should be accompanied in certain balance with other ions, such as sodium, potassium, magnesium, chlorides, and bromides, and also the delivery of calcium should be targeted only to the suprabasal cell layers (69).

As mentioned earlier, Dead Sea mud and water, balneotherapeutic water preparations, deep sea sponges, milk, and pearl are among natural products that contain high-calcium level in balance with other ions and demonstrate beneficial effects for skin barrier–related disorders. The restoration of normal barrier function during the application of high concentration of calcium is evident from the effect of bathing in the calcium-rich Dead Sea water to improve skin diseases related to skin barrier impairment (70) as well as to enhance skin hydration and

reduce inflammation in atopic dry skin (71). Other products such as milk and pearl have been used for specialty cosmetics for centuries in many countries. Although many components in milk may also contribute to the effects on skin, such as its biopeptide (72), milk is generally known as natural resource for calcium. In China, pearl powder has been investigated for various treatments (73).

Skin therapy with natural mineral waters has been intensively studied. The analysis of various water sources with clear benefits revealed unanimously high content of Ca^{2+}, compared with other natural water springs (74). One study of spa therapy has been reported on the basis of well-documented records on spa treatment in the 18th and 19th century in Bath, England. One of the factors that contributed to the success of this spa therapy is attributed to the large quantities of water rich in calcium found in the area (75).

It is possible that the effect of other ions also contributes to the positive outcome of the therapy. Magnesium, another divalent cation abundantly found in the body and in beneficial mineral waters, provides vasodilation, thereby lowering blood pressure effect, supposedly through its competition with cellular calcium (76). Bromides found in the thick haze overhanging the Dead Sea are also cited to have particularly improved psoriatic conditions (74). Sodium and potassium can also contribute to the ionic balance in the epidermis, as shown in the beneficial study of seawater to skin disorders (77). Some other elements such as selenium, zinc, rubidium, and sulfur may also provide additional effects, although their concentrations in mineral waters are generally low (74).

Specific topical formulations containing calcium in a mixture with other ions, sodium, potassium, magnesium, chloride, and bromide, have been used as adjunctive treatment for skin barrier restoration, which is also applicable for post treatment of cosmetical procedure, such as microdermabrasion or photothermolysis. The formulation is shown to accelerate the restoration of a quality skin barrier and alleviate scaly skin symptoms related to skin barrier disruption in relatively short time because of its ability to restore epidermal calcium gradient (78). This type of therapy might be considered safer than the application of calcium channel modulators or growth factors because of the additional adverse effects.

CONCLUSION

Calcium ions play an important role in the homeostasis of skin barrier. A change in the barrier will change the calcium ion gradient in skin and lead to disturbance in the skin barrier regeneration process. A severe change might lead into a high degree of calcium signaling, which may induce the activation of various processes, from increased synthesis of skin components or messengers to the inflammatory reactions. All these are important factors leading to impaired skin conditions. The regulation of calcium in skin is therefore necessary to maintain a good skin barrier function and to avoid abnormal skin symptoms. Application of topical preparations containing relatively high level of calcium in balance with other ions and targeted delivery to suprabasal cell layers has been shown to help the skin barrier recovery and homeostasis. Ranging from natural products to laboratory compositions, the preparations are getting more acknowledgments from dermatological experts, not only because of the safe but effective results for therapy but also for more understanding on the effects of calcium on skin health in general.

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17 Percutaneous Penetration Enhancers: An Overview[a]

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INTRODUCTION

Skin is an optimal interface for systemic drug administration. Transdermal drug delivery (TDD) is the controlled release of drugs through intact and/or altered skin to obtain therapeutic levels systematically and to affect specified targets for the purpose of, for example, blood pressure control, pain management, and others. Dermal drug delivery (DDD) is similar to TDD except that the specified target is the skin itself (1). TDD has the advantages of bypassing gastrointestinal incompatibility and hepatic "first pass" effect; reduction of side effects due to the optimization of the blood concentration time profile; predictable and extended duration of activity; patient-activated/patient-modulated delivery; elimination of multiple dosing schedules, thus enhancing patient compliance; minimization of inter- and intrapatient variability; reversibility of drug delivery allowing the removal of drug source; and relatively large area of application compared with the mucosal surfaces (1).

After nearly four decades of extensive study, the success of this technology remains limited, with many problems waiting to be solved, one of which is the challenge of low skin permeability hindering the development of TDD for macromolecules. To overcome the skin barrier safely and reversibly while enabling the penetration of macromolecules is a fundamental problem in the field of TDD and DDD.

Several technological advances have been made in the recent decades to overcome skin barrier properties (2). Examples include physical means such as iontophoresis, sonophoresis, and microneedles; chemical means such as penetration enhancers (PEs); and biochemical means such as liposomal vesicles and enzyme inhibition.

We overview physical and biochemical means of penetration enhancement, and focus on the common chemical PEs. We discuss the classification and mechanisms of chemical PEs, its applications in TDD, and trends and development in penetration enhancement.

PHYSICAL PENETRATION ENHANCEMENT

Physical means of penetration enhancement mainly incorporate mechanisms to transiently circumvent the normal barrier function of SC and to allow the passage of macromolecules. Although the mechanisms are different, these methods share the common goal to disrupt SC structure to create "holes" big enough for molecules to permeate. Table 1 summarizes the commonly investigated technologies of physical penetration enhancement. Two of the better-known technologies are iontophoresis and sonophoresis, and the holes created by these methods are generally believed to be of nanometer dimensions, permissive of transport of small drugs (3). A new and exciting technology for macromolecule delivery is microneedle-enhanced delivery. Theses systems use arrays of tiny needlelike structures to create transport pathways of microns' dimensions, and should be able to permit transport of macromolecules, possibly supramolecular complexes and microparticles. These systems have greatly enhanced (up to 100,000 fold) the penetration of macromolecules through skin (4), while also offering painless drug delivery (5,6).

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Table 1 Physical Methods of Penetration Enhancement

Method	Definition	Mechanism(s)	Examples of drugs	Reference
Ionotophoresis	The electrical driving of charged molecules into tissue by passing a small direct current through a drug-containing electrode in contact with skin	1. Electrical repulsion from the driving electrode drives charged molecules	Calcitonin, trans-nail delivery of salicylic acid, transdermal delivery of peptides, proteins, and oligonucleotides	(4,7–10)
		2. The flow of electric current enhances skin permeability		
		3. Electroosmosis affect uncharged and large polar molecules		
Electroporation	A method of reversibly permeabilizing lipid bilayers by the application of an electric pulse	Application of short (micro- to millisecond) electrical pulses of ~100–1000 V/cm creates transient aqueous pores in the lipid bilayers	Methotrexate, timolol, fentanyl, tetracaine, nalbuphine, cyclosporin-A	(11–17)
Sonoporation	Ultrasound-mediated delivery of therapeutic agents into biological cells	1. (Low-energy frequency): disturbs the lipid packing in SC by cavitation	Insulin, cutaneous vaccination, transdermal heparin delivery, transdermal glucose monitoring, delivery of acetyl cholinesterase inhibitors for the treatment of Alzheimer's disease, treatment of bone diseases and Peyronie's disease and dermal exposure assessment	(4,18,19)
		2. (Shock waves): increase free volume space in bimolecular leaflets thus enhancing permeation		
Microneedle-enhanced delivery systems	A method using arrays of microscopic needles to open pores in SC thus facilitating drug permeation	Bypasses the SC and delivers drugs directly to the skin capillaries. Also has the advantage of being too short to stimulate the pain fibers.	Oligonucleotide, insulin, protein vaccine, DNA vaccine, methyl nicotinate	(3,6)

Abbreviation: SC, stratum corneum.

BIOCHEMICAL PENETRATION ENHANCEMENT

Biochemical means of penetration enhancement include using prodrug molecules (20), chemical modification (21), enzyme inhibition (22), and the usage of vesicular systems or colloidal particles (23). Among these strategies, special formulation approaches, based mainly on the usage of colloidal carriers, are most promising. Liposomes (phospholipids-based artificial vesicles) and niosomes (nonionic surfactant vesicles) are widely used to enhance drug delivery across the skin. In addition, proliposomes and proniosomes, which are converted to liposomes and niosomes upon simple hydration are also used in TDD (24). Generally, these colloidal carriers are not expected to penetrate into viable skin. Most reports cite a localizing effect whereby the carriers accumulate in SC or other upper skin layers (4).

More recently, a new type of liposomes called transferosomes has been introduced (25,26). Transferosomes consist of phospholipids, cholesterol and additional "edge activators"—surfactant molecules such as sodium cholate. The inventors claim that 200- to 300-nm sized transferosomes are ultradeformable and squeeze through pores less than one-tenth of their diameter, and are thus able to penetrate intact skin. Penetration of these colloidal particles works best under in vivo conditions and requires a hydration gradient from the skin surface toward the viable tissues to encourage skin penetration under non-occluded conditions.

In addition, ethosomes, which are liposomes high in ethanol content (up to 45%), penetrate skin and enhance compound delivery to deep skin strata or systematically. The mechanism suggested is that ethanol fluidizes both ethosomal lipids and lipid bilayers in the SC, allowing the soft, malleable vesicles to penetrate through the disorganized lipid bilayers (27).

In general, six potential mechanisms of actions of these colloidal carriers were proposed (4):

1. Penetration of SC by a free drug process—drug releases from vesicle and then penetrates skin independently
2. Penetration of SC by intact liposomes
3. Enhancement due to release of lipids from carriers and interaction with SC lipids
4. Improved drug uptake by skin
5. Different enhancement efficiencies control drug input
6. The role of protein requires elaboration

CHEMICAL PENETRATION ENHANCERS

Substances that help promote drug diffusion through the stratum corneum (SC) and epidermis are referred to as PEs, accelerants, adjuvants, or sorption promoters (28). PEs have been extensively studied given its advantages such as design flexibility with formulation chemistry and patch application over large area. PEs improve drug transport by reducing the resistance of SC to drug permeation. To date, none of the existing chemical penetration enhancers (CPEs) have proven to be ideal. In particular, the efficacy of PEs toward the delivery of high–molecular weight drugs remains limited. Attempts to improve enhancement by increasing the potency of enhancers inevitably lead to a compromise on safety issues. Achieving sufficient potency without irritancy has proved challenging.

CLASSIFICATION OF CPEs

The diverse physicochemical properties and variation in mechanisms of action of compounds investigated for their penetration enhancement effects made a simple classification scheme for PEs difficult to set up. Hori et al. (29) proposed a conceptual diagrammatic approach based on Fujita's data (30) for the classification of PEs. In this approach, they determined organic and inorganic values for PEs, and the resultant plot of organic versus inorganic characteristics grouped PEs into distinct areas on the diagram—area I encloses enhancers, which are solvents; area II designates PEs for hydrophilic drugs; and area III contains PEs for lipophilic compounds. On the other hand, Lambert et al. (31) grouped most PEs into three classes: solvents and hydrogen bond acceptors (e.g., dimethylsulfoxide, dimethylacetamide, and dimethylformamide), simple fatty acids and alcohols, and weak surfactants containing a moderately sized polar group (e.g., Azone®, 1-dodecylazacycloheptan-2-one); whereas Pfister et al. (28) classified PEs as either polar or nonpolar. To date, there is no consensus as to which classification to adopt. Table 2 classifies commonly

(*text continues on page 191*)

Table 2 Chemical Penetration Enhancers

Category and examples	Cosolvent/vehicle	Mechanism	Examples of drugs (33)	Comment	Reference
Sulfoxides					
DMSO		1. Increase lipid fluidity 2. Promote drug partitioning	DMSO: theophylline, salicylic acid, hydrocortisone, testosterone, scopolamine, antimycotics, fluocinolone acetonide, flufenamic acid		(34,35)
DCMS		Protein-DCMS interactions, resulting in a change in protein conformation, creating aqueous channels	DCMS: methotrexate, naloxone, pyridostigmine bromide, hydrocortisone, progesterone	DCMS enhance polar drug more effectively	
Alkanones					
N-heptane, n-octane, n-nonane, n-decane, n-undecane, n-dodecane, n-tridecane, n-tetradecane, n-hexadecane		Extensive barrier alteration of SC	Propanolol, diazepam		(36)
Alcohols					
Alkanol: E, propanol, butanol, 2-butanol, pentanol, 2-pentanol, hexanol, octanol, nonanol, decanol, BA		1. Low–molecular weight alkanols ($C \leq 6$) may act as solubilizing agents 2. More hydrophobic alkanols may extract lipids from SC[a], leading to increased diffusion	E: tacrine, metrifonate, dichlorvos, ketolorac, nitroglycerin, tazifylline, betahistine, cyclosporin A		(37–40)
Fatty alcohol: caprylic, decyl, LA, 2-lauryl, myristyl, cetyl, stearyl, oleyl, linoleyl, linoleyl alcohol			LA: buprenorphine		(41)
Polyols					
PG, PEG, ethylene glycol, diethylene glycol, triethylene glycol, dipropylene glycol, G, propanediol, butanediol, pentanediol, hexanetriol	43× enhancement of diazepam and 86× enhancement of midazolam maleate seen in PG and 5% Azone in a PG:ethanol: water (2:2:1) vehicle.	PG may solvate α-keratin and occupy hydrogen bonding sites, reducing drug-tissue binding	PG: 5-fluorouracil, tacrine, ketorolac, isosorbide dinitrate, clonazapem, albuterol, verapamil, betahistine, estradiol, dihydroergotamine, methotrexate, steroids, midazolam maleate, diazepam PEG: terbutaline G: diazepam, terbutaline, 5-fluorouracil	Inclusion of 2% Azone or 5% oleic acid to PG produced a more bioactive formulation	(42,43)

Amides				
Urea, DMA, diethyltoluamide, DMF, dimethyloctamide, dimethyldecamide	Urea: hydration of SC, keratolytic, creating hydrophilic diffusion channels DMA/DMF: (low conc.): partition to keratin, (high conc.): increase lipid fluidity, disrupt lipid packaging	Urea: ketoprofen, 5-fluorouracil DMA/DMF: griseofluvin, betamethasone 17-benzoate, caffeine	Urea analogues in PG enhanced permeability of 5-fluorouracil 6×	(41,44)
Biodegradable cyclic urea:				
1-alkyl-4-imidazolin-2-one		Indomethacin	Comparable to or better than Azone	(45)
Pyrrolidone derivatives: 1M2P, 2-pyrrolidone, 1-lauryl-2-pyrrolidone, 1-methyl-4-carboxy-2-pyrrolidone, 1-hexyl-4-carboxy-2-pyrrolidone, 1-lauryl-4-carboxy-2-pyrrolidone, 1-methyl-4-methoxycarbonyl-2-pyrrolidone, 1-hexyl-4-methoxycarbonyl-2-pyrrolidone, 1-lauryl-4-methoxycarbonyl-2-pyrrolidone, NMP, *N*-cyclohexylpyrrolidone, *N*-dimethylaminopropylpyrrolidone, *N*-cocoalkypyrrolidone, *N*-tallowalkylpyrrolidone	Interact with both keratin in the SC and with lipids in the skin structure	1M2P: griseofulvin, theophylline, tetracycline, ibuprofen, betamethasone 17-benzoate NMP: prazosin		(41,46)
Biodegradable pyrrolidone derivatives: Fatty acid esters of *N*-(2-hydroxyethyl)-2-pyrrolidone				(31)

(*Continued*)

Table 2 Chemical Penetration Enhancers (*Continued*)

Category and examples	Cosolvent/vehicle	Mechanism	Examples of drugs (33)	Comment	Reference
Cyclic amides:					
1-dodecylazacycloheptane-2-one(Azone), 1-geranylazacycloheptan-2-one, 1-farnesylazacycloheptan-2-one, 1-geranylgeranylazacyclo-heptan-2-one, 1-(3,7-dimethyloctyl) azacycloheptan-2-one, 1-(3,7,11-trimethyldodecyl) azacycloheptan-2-one, 1-geranylazacyclohexane-2-one, 1-geranylazacyclopentan-2,5-dione, 1-farnesylazacyclopentan-2-one	Azone: enhancer effect can be increased by use of a cosolvent such as PG.	Azone: 1. Affects lipid structure of SC 2. Increases partitioning 3. Increases membrane fluidity	Azone: 5-fluorouracil, antibiotics, glucocorticoids, peptites, clonazepam, albuterol, estradiol, levonorgestrel, HIV protease inhibitor (LB-71148), betahistine, dihydroergotamine	Azone: significant accelerant effects at low conc. (1–5%), can be applied undiluted to skin without significant discomfort, effective for both hydrophilic and hydrophobic drugs	(47–49)
Hexamethylenelauramide and its derivatives					(50)
Diethanolamine, triethanolamine					(42)
Fatty acids					
Linear: LIA, valeric, heptanoic, pelagonic, caproic, CA, LAA, myristic, stearic, OA, caprylic		Selective perturbation of the intercellular lipid bilayers OA: decreases the phase transition temperatures of the lipid, increasing motional freedom or fluidity of lipids	Naloxone, mannitol, betamethasone 17-benzoate, hydrocortisone, acyclovir, nitroglycerin OA: galanthamine, estradiol, levonorgestrel CA: buprenorphine, albiterol LAA: buprenorphine, betahistine	Among stearic, oleic, and linoleic acids, maximum enhancement was observed with linoleic acid	(41,51,52)
Branched: isovaleric, neopentanoic, neoheptanoic, neononanoic, trimethyl hexanoic, neodecanoic, isostearic					

Fatty acid esters					
Aliphatic: isopropyl n-butyrate, isopropyl n-hexanoate, isopropyl n-decanoate, IPM, isopropyl palmitate, octyldodecyl myristate		IPM: direct action on SC, permeating into liposome bilayers, increasing fluidity Aliphatic: increase diffusivity in the SC and/or the partition coefficient Alkyl: increase lipid fluidity (similar to DMSO)	IPM: galanthamine, ketorolac, chlorpheniramine, dexbrompheniramine, diphenhydramine, theophylline, pilocarpine, verapamil EA: levonorgestrel, 17β-estradiol, hydrocortisone, 5-fluorouracil, nefedipine		(53,54)
Alkyl: EA, butyl acetate, methyl acetate, methylvalerate, methylpropionate, diethyl sebacate, ethyl oleate					
Surfactants					
Anionic: sodium laurate, sodium lauryl sulfate, sodium octyl sulfate		Alter the barrier function of SC, allowing removal of water-soluble agents that normally act as plasticizers		Greater damage and permeation enhancement with anionic surfactants than with nonionic surfactants	(55,56)
Cationic: Cetyltrimethylammonium bromide, tetradecyltrimethylammonium bromide, octyltrimethylammonium bromide, benzalkonium chloride, octadecyltrimethylammonium chloride, cetylpyridinium chloride, dodecyltrimethylammonium chloride, hexadecyltrimethylammonium chloride	Significant increases in the flux of lidocaine from saturated systems in PG-water mixtures	Adsorb at interfaces and interact with biological membranes, causing damage to skin		Cationic surfactants are more destructive to skin than anionic surfactants.	(56–58)
zwitterionic surfactants hexadecyl trimethyl ammoniopropane sulfonate, oleyl betaine, cocamidopropyl betaine, cocamidopropyl hydroxysultaine, cocamidopropyl betaine					(59)
Nonionics: Polyxamer (231, 182, 184), Polysorbate (20, 60), Brij (30, 93, 96, 99), Span (20, 40, 60, 80, 85), Tween (20, 40, 60, 80), Myrj (45, 51, 52), Miglyol 840	Polysorbate 20 and 60 increased lidocaine flux in the presence of PG	Emulsify sebum, enhancing the thermodynamic activity of coefficients of drugs	Tween 80: ketoprofen Polysorbate 20, 60: lidocaine		(41,60,61)

(Continued)

Table 2 Chemical Penetration Enhancers (*Continued*)

Category and examples	Cosolvent/vehicle	Mechanism	Examples of drugs (33)	Comment	Reference
Bile salts:					
sodium cholate, sodium salts of TC, glycolic, desoxycholic acids			TC: elcatonin and vit. D_3, estradiol and vit. D_3,		(62)
Lecithin					(63)
Terpenes					
Hydrocarbons: D-Limonene, α-pinene, β-carene		1. Increases diffusivity of drugs within SC due to disruption of intercellular lipid barrier 2. Opens new polar pathways within and across the SC	5-Fluorouracil, aspirin, haloperidol	Hydrocarbon terpenoids were least effective, oxides moderately effective, and the alcohols, ketones, and cyclic ethers most effective accelerants of 5-fluorouracil permeation	(36,64,65)
Alcohols: α-Terpineol, terpinen-4-ol, carvol					
Ketones: Carvone, pulegone, piperitone, menthone					
Oxides: Cyclohexene oxide, limonene oxide, α-pinene oxide, cyclopentene oxide, 1,8-cineole					
Oils: Ylang ylang, anise, chenopodium, eucalyptus					
Organic acids					
Salicylic acid and salicylates (including their methyl, ethyl, and propyl glycol derivatives), citric and succinic acid					(66)
Cyclodextrins					
HPβCD DIMEB	Higher penetration of liarzole in DIMEB with PG/oleic acid compared with HPβCD	Form inclusion complexes with lipophilic drugs and increase their solubility of in aqueous solutions	Liarzole		(67,68)
Proprietary chemical enhancers					(69)
Alkyl-2-(N,N-disubstituted amino)-alkanoate ester (NexAct®)					
2-(n-nonyl)-1,3-dioxolane (SEPA®)			Ibuprofen, ketoprofen, alprostadil, testoterone		

[a]SC: stratum corneum.
Abbreviations: DMSO, dimethylsulfoxide; DCMS, decylmethylsulfoxide; BA, benzyl alcohol; LA, lauryl; PG, propylene glicol; E, ethanol; PEG, polyethylene glicol; G, glicerol; DMA, dimethylacetamide; DMF, dimethyformamide; 1M2P, 1-methyl-2-pyrrolidone; NMP, *N*-methyl-pyrrolidone; LIA, linoleic acid; CA, capric acid; LAA, lauric acid; OA, oleic acid; IPM, isopropyl myristate; EA, ethyl acetate; TC, taurocholic; HPβCD, 2-hydroxypropyl-β-cyclodextrin; DIMEB, 2,6-dimethyl-β-cyclodextrin; conc., concentration; vit., vitamin.
Source: From Ref. 9.

investigated PEs based on the chemical classes to which the compounds belong (32). Only representative compounds are listed to avoid an exhaustive list. Note that a perfect classification is yet to be developed, and the key lies in a comprehensive understanding of the mechanisms and the physicochemical parameters of CPEs.

MECHANISM OF CPEs

The mechanisms of action proposed for commonly seen CPEs are listed in Table 2. Basically, transdermal penetration of most drugs is a passive diffusion process (70). There are three major potential routes for penetration—appendageal (through sweat ducts and/or hair follicles with associated sebaceous glands), transcellular permeation through the SC, or intercellular permeation through the SC (4). The appendageal route usually contributes negligibly to steady-state drug flux given its small available fractional area of 0.1%. This route may be important for short diffusional times and for ions and large polar molecules, which have low penetration across SC. The intact SC thus comprises the predominant route through which most molecules penetrate.

Kanikkannan et al. (71) suggested three pathways for drug penetration through the skin: polar, nonpolar, and both. The mechanism of penetration through the polar pathway is to cause protein conformational change or solvent swelling; whereas the key to penetrate via the nonpolar pathway is to alter the rigidity of the lipid structure and fluidize the crystalline pathway. Some enhancers may act on both polar and nonpolar pathways by dissolving the skin lipids or denaturing skin proteins. On the other hand, Ogiso and Tanino (72) proposed the following mechanisms for the enhancement effect: (*i*) an increase in the fluidity of the SC lipids and reduction in the diffusional resistance to permeants, (*ii*) the removal of intercellular lipids and dilation between adherent cornified cells, (*iii*) an increase in the thermodynamic activity of drugs in vehicles, (*iv*) the exfoliation of SC cell membranes, the dissociation of adherent cornified cells, and elimination of the barrier function.

Ogiso et al. (73) also proposed examples of PEs with different relative enhancement capabilities due to differences in the chemical structure and other parameters. In their study, the relative ability to enhance transdermal penetration of indomethacin into hairless rat skin was studied. The results were summarized in Table 3 (69).

Furthermore, Kanikkannan et al. (71) proposed that on the basis of the chemical structure of PEs (such as chain length, polarity, level of unsaturation, and presence of specific chemical groups such as ketones), the interaction between the SC and PEs may vary, contributing to the different mechanisms in penetration enhancement. A comprehensive understanding of the mechanisms of action and a judicious selection of CPE would be helpful in the successful development of TDD and DDD products.

FDA-APPROVED TDD

There has been an increased focus on the potential of TDD as evident from the increase in the number of patents as well as scientific publications on TDD systems. Many drugs have been evaluated for TDD in prototype patches, either in vitro permeation studies using mouse, rat, or

Table 3 Examples of Penetration Enhancers with Different Relative Enhancement Capabilities due to Differences in the Chemical Structure and other Parameters

Mechanisms	Comparison
Extraction of intercellular lipids and dilations between cornified cells, permitting percutaneous passage of polar substances	1-dodecylazacycloheptane-2-one (Azone) > n-octanol > d-limonen > oleic acid > cíñelo
Increase in partitioning into skin	1-dodecylazacycloheptane-2-one > n-octanol > cineol > d-limonen > oleic acid > isopropyl myristate > monooleate
Increase in the fluidity of SC lipids and reduction in diffusional resistance	1-dodecylazacycloheptane-2-one > isopropyl myristate > monoolein > oleic acid > cineol, sodium oleate
Increase in thermodynamic activity in vehicles	n-octanol > sodium oleate > d-limonen > monoolein > cineol > oleyl oleate > isopropyl myristate

human skin or have reached varying stages of clinical testing. Examples are listed in Table 2. Despite a wide array of TDD systems undergoing research and development, only a small percentage of the drugs reach the market successfully because of three limitations: difficulty of penetration through human skin, skin irritation and allergenicity, and clinical need. In addition, it is generally accepted that the best drug candidates for passive adhesive transdermal patches must be nonionic; must have low molecular weight (<500 Da), adequate solubility in oil and water (log P in the range 1–3), and a low melting point (<200°C); and must be potent (dose <50 mg/day, and ideally <10 mg/day) (74–76). Given these operating parameters, the number of drug candidates, which fits the criteria, may seem low. Nevertheless, with the development of novel technologies, such constraint may be overcome.

Since the introduction of a TDD for scopolamine in 1981, several new products have been introduced. The U.S. TDD market approached $1.2 billion in 2001 and was based on 11 drug molecules: fentanyl, lidocaine, prilocaine, nitroglycerin, estradiol, ethinyl estradiol, norethindrone acetate, testosterone, clonidine, nicotine, and scopolamine (77). Barry (4) reported that 40% of drug delivery candidate products that were under clinical evaluation and 30% of those in preclinical development in the United States were TDD or DDD systems.

Examples of Food and Drug Administration (FDA)-approved transdermal patches and their applications are given in Table 4. Despite a plethora of candidate CPEs to choose from, all currently available TDD products adopt skin occlusion as the primary mechanism for penetration enhancement, perhaps due to its simplicity and convenience, and the following effects on SC (78,79): an increase in SC hydration and a reservoir effect in penetration rates of the drug due to hydration, an increase in skin temperature from 32°C to 37°C, and the prevention of accidental wiping or evaporation (volatile compound) of the applied compound.

Table 4 Examples of FDA-Approved Transdermal Patches, Their Applications, and the Mechanisms/Compounds Used for Penetration Enhancement

Drug	Application(s)	Example of commercially available product(s)	Penetration enhancement effect and PEs
Scopolamine	Motion sickness	Transderm Scop	Occlusive effect
Fentanyl	Moderate-to-severe chronic pain	Duragesic	Occlusive effect
Lidocaine	Anesthesia	Lidoderm	Occlusive effect, urea, propylene glycol
Prilocaine	Anesthesia	EML anesthetic disc	Occlusive effect, polyoxyethylene fatty acid esters
Testosterone	Hormone replacement therapy	Androderm	Occlusive effect, glycerol monooleate
Estradiol/norethindrone acetate	Hormone replacement therapy	Combipatch	Occlusive effect, silicone, oleic acid, dipropylene glycol
Estradiol	Symptomatic relief of postmenopausal symptoms and prevention of osteoporosis	Alora, Climera, Esclim, Vivelle, Vivelle-dot	Occlusive effect; Climera: fatty acid esters; Vivelle: 1,3-butylene glycerol, oleic acid, lecithin, propylene glycol, dipropylene glycol; Vivelle-dot: oleyl alcohol, dipropylene glycol
Norelgestromin/ethinyl estradiol	Contraception	Ortho Evra	Occlusive effect, lauryl lactate
Nitroglycerin	Angina pectoris	Nitro-Dur, Nitrodisc, Transderm-Nitro	Occlusive effect, fatty acid esters
Clonidine	Hypertension	Catapres-TTS	Occlusive effect
Nicotine	Smoking cessation	Nicoderm CQ	Occlusive effect
Methyphenidate	Attention deficit hyperactive disorder	Daytrana	Occlusive effect
Selegiline	Depression	Emsam	Occlusive effect
Oxybutynin	Urge/urinary incontinence	Oxytrol	Occlusive effect

Abbreviations: PEs, penetration enhancers; EMLA, eutectic mixture of local anesthetic.

FUTURE TRENDS

The protective function of human SC imposes physicochemical limitations to the type of molecules that can traverse the barrier. As a result, commercially available products based on TDD or DDD have been limited. Various strategies have emerged over the last decade to optimize delivery. Approaches such as the optimization of formulation or of drug-carrying vehicle to increase skin permeability do not greatly improve the permeation of macromolecules.

On the contrary, physical or mechanical methods of enhancing delivery have been more promising. Improved delivery has been shown for drugs of differing lipophilicity and molecular weight, including proteins, peptides, and oligonucleotides, using electrical methods (iontophoresis and electroporation), mechanical (abrasion, ablation, and perforation), and other energy-related techniques such as ultrasound and needleless injection (80).

Another strategy for penetration enhancement is to exploit the synergistic effects offered by combined techniques. Karande et al. (81) reported the discovery of synergistic combinations of penetration enhancers (SCOPE), which allow permeation of 10-kDa macromolecules with minimal skin irritation using high-throughput screening method. Kogan and Garti (51) also showed that the combination of several enhancement techniques led to synergetic drug penetration and decrease in skin toxicity. In essence, the possibilities seem endless in the field of TDD and DDD.

CONCLUSION

TDD would avoid problems associated with the oral route as well as the inconvenience and pain associated with needle delivery and has thus competed with oral and injection therapy for the accolade of the innovative research area for drug delivery. Yet there remains a paucity of candidates for TDD or DDD to be marketed. The reasons are twofold: (*i*) most candidate drug molecules have low permeation rates through the skin to ever reach clinically satisfactory plasma level; (*ii*) risk of skin irritation and allergic contact dermatitis may be increased by skin occlusion (79,82) and/or the application of potent PEs (81). The ideal characteristics of PEs include the following (28):

- Be both pharmacologically and chemically inert
- Be chemically stable
- A high degree of potency with specific activity, rapid onset, predictable duration of activity, and reversible effects on skin properties
- Show chemical and physical compatibility with formulation and system components
- Be nonirritant, nonallergenic, nonphototoxic, and noncomedogenic
- Be odorless, tasteless, colorless, cosmetically acceptable, and inexpensive
- Be readily formulated into dermatological preparations, transdermal patches, and skin adhesives
- Have a solubility parameter approximating that of skin (83)

Future studies on the mechanisms of penetration enhancement, the metabolic processes of chemicals within the skin, skin toxicity, as well as the development of novel technologies will improve our knowledge on penetration enhancement. While the current TDD and DDD technologies still offer significant potential for growth, next-generation technologies will enable a much broader application of TDD to the biopharmaceutical industry.

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18 Tests for Skin Protection: Barrier Effect

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INTRODUCTION

One important skin function is protecting us from environmental toxicity. This is evident in certain occupations where there is constant exposure to hazardous substances. Precautionary measures such as glove use minimizes the risk of incurring contact dermatitis (CD), though at times the gloves themselves may cause this skin disease. Barrier creams (BCs) may play an important role in the prevention of CD (1–6), and various in vitro and in vivo methods have been developed to evaluate their efficacy. In practice, their utilization remains the subject of a lively debate; some suggest that the inappropriate BC application may exacerbate rather than prevent irritation (1–3,6–9). The accuracy of measurements depends on the use of appropriate methodology.

This chapter provides the investigative details of pertinent scientific literature and summarizes methodology and efficacy of BC.

IN VITRO METHODS

In 1946, Sadler and Marriott (10) introduced facile tests to evaluate the efficiency of BC. One method used the fluorescence of a dyestuff and eosin as an indicator to measure penetration and the rates of penetration of water through BC; this is rapid and simple, but provides only a qualitative estimate.

Suskind (11) performed a simple method to measure the relative efficacy or repellency of several formulations with film immersion test in a specific exposure. Formulation containing 52.5% silicone in bentonite and 30% silicone in petrolatum were effective against a range of aqueous irritants and sensitizers.

Langford (12) conducted in vitro studies to determine that the efficacy of the formulated fluorochemical (FC)-resin complex included solvent penetration through treated filter paper, solvent repellency on treated pigskin, and penetration of radio-tagged sodium lauryl sulfate (SLS) through treated hairless mouse skin. He also conducted an in vivo study on 75 persons who had previously experienced irritation on their hands because of continued contact with solvents. Eighty-three percent of the panelists stated the cream was effective in protecting their hands.

Reiner et al. (13) examined the protective effect of ointments on guinea pig skin in vitro and in vivo. The permeation values of a toxic agent through unprotected and protected skin within 10 hours as a function of time was determined radiologically and enzymatically. Permeation of the toxic agent was markedly reduced by polyethylene glycol ointment base and ointments containing active substance. In in vivo experiments on guinea pigs, mortality was greater after applying the toxic agent to unprotected skin. All formulations with nucleophilic substances markedly reduced the mortality rate.

Loden (14) evaluated the effect of BC on the absorption of (^{3}H) water (^{14}C)-benzene and (^{14}C)-formaldehyde into excised human skin. The control and the BC-treated skins were exposed to the test substance for 30 minutes, whereupon absorption was determined. The experimental "water barrier" cream reduced the absorption of water and benzene, but not formaldehyde. One cream slightly reduced benzene and formaldehyde absorption. Two other creams did not affect the absorption of the substances studied.

Treffel et al. (15) measured in vitro on human skin the effectiveness of BC against three dyes (eosin, methylviolet, and oil red O) with varying n-octanol/water partition coefficients (0.19, 29.8, and 165, respectively). BC efficacy was assayed by measurements of the dyes in the epidermis of protected skin samples after 30 minutes' application. The efficacy of BC against the three dyes showed in several cases data contrary to manufacturer's information. There was

no correlation between the galenic parameters of the assayed products and the protection level, indicating that neither the water content nor the consistency of the formulations influenced the protection effectiveness.

Fullerton and Menne (16) tested the protective effect of various ethylenediaminetetraacetate (EDTA) barrier gels against nickel contact allergy using in vitro and in vivo methods. In an in vitro study, about 30 mg of barrier gel was applied on the epidermal side of the skin, and a nickel disc was placed above the gel. After 24-hour application, the nickel disc was removed and the epidermis separated from the dermis. Nickel content in epidermis and dermis was quantified by adsorption differential pulse voltametry (ADPV). The amount of nickel in the epidermal skin layer on barrier gel–treated skin samples was significantly reduced compared with the untreated control. In vivo patch testing of nickel-sensitive patients was performed using nickel discs with and without barrier gels. Test preparations and nickel discs were removed one day post application, and the test sites were evaluated. Reduction in positive test reactions was highly significant on barrier gel–treated sites.

Zhai et al. (17) used an in vitro diffusion system to measure the protective effect of quaternium-18 bentonite (Q18B) gels to prevent 1% concentration of [^{35}S] SLS penetration by human cadaver skin. The accumulated amount of [^{35}S]-SLS in receptor cell fluid was measured to evaluate the efficacy of the Q-18B gels over a 24-hour period. These test gels significantly decreased SLS absorption when compared with the unprotected skin control samples. The percentages of protection effect of three test gels against SLS percutaneous absorption were 88%, 81% and 65%, respectively.

IN VIVO METHODS

Schwartz et al. (18) introduced an in vivo method to evaluate the efficacy of a vanishing cream against poison ivy extract using visual erythema on human skin. The test cream was an effective prophylaxis against poison ivy dermatitis as compared to unprotected skin.

Lupulescu and Birmingham (19) observed the ultrastructural and relief changes of human epidermis following exposure to a protective gel and acetone and kerosene on humans. Unprotected skin showed cell damage and a disorganized pattern in the upper layers of epidermis. Protective agent prior to solvent exposure substantially reduced the ultrastructural and relief changes of epidermis cells.

Lachapelle et al. (3,20–23) used a guinea pig model to evaluate the protective value of BC and/or gels by laser Doppler flowmetry and histological assessment. The histopathological damage after 10 minutes of contact with toluene was mostly confined to the epidermis, while the dermis was almost normal. The dermal blood flow changes were relatively high on the control site compared with the gel-pretreated sites.

Frosch et al. (1,8,9,24,25) developed the repetitive irritation test (RIT) in the guinea pig and in humans to evaluate the efficacy of BC using bioengineering techniques. The cream-pretreated and untreated test skin (guinea pig or humans) were exposed daily to the irritants for two weeks. The resulting irritation was scored on a clinical scale and assessed by biophysical techniques' parameters. Some test creams suppressed irritation with all test parameters; some failed to show such an effect or even exacerbated (9).

Zhai (2) used an in vivo human model to measure the effectiveness of BC against dye indicator solutions: methylene blue in water and oil red O in ethanol, representative of model hydrophilic and lipophilic compounds. Solutions of 5% methylene blue and 5% oil red O were applied to untreated and BC-pretreated skin with the aid of aluminum occlusive chambers for zero and four hours. At the end of the application time, the materials were removed, and consecutive skin surface biopsies (SSBs) obtained. The amount of dye penetrating into each strip was determined by colorimetry. Two creams exhibited effectiveness, but one cream enhanced cumulative amount of dye.

Zhai et al. (5) introduced a facile approach to screening protectants in vivo in human subjects. Two acute irritants and one allergen were selected: SLS representative of irritant household and occupational CD, the combination of ammonium hydroxide (NH_4OH) and urea to simulate diaper dermatitis, and Rhus to evaluate the effect of model-protective materials. Test materials were spread over onto the test area, massaged, allowed to dry for

30 minutes, and reapplied with another 30-minute drying period. The model irritants and allergen were applied with an occlusive patch for 24 hours. Inflammation was scored with an expanded 10-point scale at 72 hours post application. Most test materials statistically suppressed the SLS irritation and Rhus allergic reaction and not NH_4OH- and urea-induced irritation.

Wigger-Alberti et al. (26) determined which areas of the hands were likely to be skipped on self-application of BC by fluorescence technique at workplace. Results showed that the application of BC was incomplete, especially on the dorsal aspects of the hands.

Draelos (27) conducted a randomized, double-blind, split-body study in 80 men, women, and children (neonate–80 years) with the following dermatological conditions: household dermatitis (21), occupational hand dermatitis (18), latex glove irritant CD (9), diaper dermatitis (5), cutaneous wounds (17), and allergic CD (10). The subjects were given two identical jars (1 jar containing petrolatum-based cream, and the other contained hydrogel-based barrier/repair cream) and were instructed to apply one cream to half of their bodies, while the other cream to the other half for four weeks. Results showed that 62% of the subjects preferred hydrogel-based barrier/repair cream over the petrolatum-based cream ($p \leq 0.005$) as well as the investigator's assessment ($p \leq 0.00001$) in terms of the overall skin appearance.

McCormick et al. (28) performed a double-blind, randomized trial comparing a novel BC versus an oil-containing lotion in 54 health care workers for two months. Results showed that both creams substantially protected the health care workers against drying and chemical irritation, preventing skin breakdown and promoting more frequent hand washing.

The skin protection efficacy of dexapanthenol was investigated by Biro et al. (29) in a double-blind, randomized, placebo-controlled study design in 25 healthy volunteers (18–45 years). They compared a cream containing 5% dexapanthenol with its vehicle-moisturizing base and applied to the flexor forearms twice daily for 26 days—one arm treated by the test product, while the other treated with placebo. In days 15 to 25, 2% SLS was applied on both forearms. Measures of skin physiology included sebumetry, corneometry, pH values, and clinical appearance (photographs). Results showed, though not significantly, a decreasing trend of the pH values and sebum content during SLS treatment but normalized when SLS was discontinued. Hydration of the stratum corneum remained stable throughout the study in the dexapanthenol group, while corneometry for the placebo group showed a significant ($p < 0.05$) decrease at the end of the SLS treatment on day 23. This study demonstrates the capability of dexapanthenol to protect skin from experimentally induced skin irritation.

Perrenoud et al. (30) conducted a double-blind crossover study comparing a new registered BC containing 5% aluminum chlorhydrate as active ingredient with its vehicle in 21 apprentice hairdressers who are frequently exposed to repeated shampooing and hair care products for a period of two months. The subjects were randomly assigned two groups; then, each subject was given identical 50-g tubes at the onset of the study, after two weeks, and at the start of the second phase. The contents of the tubes were unknown to the investigators and subjects. The participants recorded their daily comments. Evaluation of the creams' efficacy included: (*i*) clinical scores (dryness, redness, and breaks rated as 0 = none−3 = maximum) assessed by the researchers; (*ii*) biometric measurements using evaporimetry, corneometry, and chromametry; and (*iii*) recording of subjective opinions. Result for clinical evaluation showed low scores—nearly everyone had a "0" or "1" score. Only corneometric values showed a significant difference, i.e., the scores for the control group were significantly ($p < 0.01$) higher than the test product.

De Paepe et al. (31) investigated the beneficial effects of a skin tolerance−tested moisturizing cream on the barrier function in experimentally elicited irritant and allergic CD in 24 white female volunteers. Skin compatibility tests with the raw cosmetic materials and the final test product were initially performed in a large population to verify that the test product was well tolerated. Irritant CD was elicited using 1.25% SLS patch tested for 24 hours on the volar forearms of 12 white female volunteers in two sites (1 site for treatment with the test cream, while the other site left untreated). A third site was patch tested with filter paper soaked in pure water. Following patch removal, the forearms were washed, and application of 0.03-mL test cream was initiated the next day, twice daily for 14 consecutive days. There was a significant ($p < 0.05$) decrease in transepidermal water loss (TEWL) values of the treated site

on days 3, 8, and 15 as compared with the untreated site. Allergic CD was elicited using nickel-mediated contact allergy patch (CAP) test in another 12 white female volunteers with well-established histories of nickel (Ni)-contact allergies. Two patches contained 0.3 mL of 5% nickel sulfate in petrolatum and a third patch contained 0.3 mL of physiological serum (0.9% NaCl) to serve as control. Patches were removed after 48 hours, and test sites were cleaned with dry tissue, then 0.3 mL of the test cream was applied on the test sites twice a day for four consecutive days. Results revealed a significant ($p < 0.05$) decrease in TEWL values of the treated site when compared with the untreated site on days 3, 8, and 15.

Diepgen et al. (32) investigated six skin care products (Locobase® Pro cream, Debba® Wet, Taktosan®, Pluctect® Dual, Locobase® fatty cream, and Kerodex® 71) for their compatibility with normal and diseased skin, as well as their efficacy as protective skin barriers in 40 healthy volunteers in a double-blind study. The chamber scarification test (33) was used to compare the test products with known positive (aqueous SLS 0.5%) and negative (paraffin oil) controls and to rank the irritancy potential of products in 20 healthy volunteers. Approximately 0.1 mL of each product was applied to the scarified normal skin of the flexor forearms of the participants using Finn Chambers®. Patches were removed after 23 hours (day 1) and read an hour later and immediately before reapplication of the samples for days 2 and 3. Reactions were scored visually using a 5-point scale ("0" = no reaction to "4" = confluent, severe redness with edema or bullae). Results revealed that out of the eight samples applied, Debba Wet had the highest sum of scores ("12") in five subjects, while positive control only reached a maximum score of "10" in three subjects. Both Debba Wet and SLS 0.5% were considerably more irritating ($p < 0.0001$, $x^2 = 87$, df = 7) than the other test products. The ranking of the test products were: Debba Wet (score average = 11) $\geq$ aqueous SLS 0.5% (score average = 7.4) $\geq$ Taktosan cream (score average = 3.7) $\geq$ Locobase fatty cream (score average = 3.3) $\geq$ Kerodex 71, Pluctect Dual, Locobase Pro cream, and paraffin oil (score average = 2.2−3.0). On the other hand, the short-time repeated exposure occlusive irritation test (ROIT) was used to assess the efficacy of the six products and yellow Vaseline® as protective skin barriers in another 20 healthy volunteers. ROIT involved multiple short application times using low concentration of irritants. Aqueous SLS 0.5% was used as the irritant and was patch tested using Large Finn Chambers on the volar forearms of the subjects. For each site, the following were applied: irritant alone and water alone; one site was left blank, while the rest of the sites were first pretreated with the seven test creams 10 minutes before irritant application. The placing of the test products was changed from person to person according to a rotation system. The whole procedure was done every 3 to 3.5 hours for three consecutive days. Parameters used were TEWL (measured by Tewameter® TM 210), erythema (measured by ChromaMeter® CR 300), and clinical visual scoring (numerical scale: "0" = no reaction to "3" = pronounced erythema and edema, extensive scaling, possibly vesicles, bullae, pustules, and/or pronounced crusting). The comparison of the differential TEWL values between the test areas and the untreated sites showed significantly ($p < 0.05$) increased values for Vaseline, Taktosan, and Debba Wet. There was no significant difference among the TEWL values for Locobase Pro Cream, Plutect Dual, Locobase fatty cream, and Kerodex 71 when compared to normal skin. The increase in TEWL values was not significant ($p > 0.05$) between SLS-exposed sites and pretreated sites. Clinically, treatment with the SLS increased the visual scores. Likewise, Vaseline, Taktosan, and Debba Wet did not offer protection from skin irritation.

Modak et al. (33) demonstrated that the use of topical formulation with zinc gel delayed or prevented latex sensitivity in 22 volunteers known to have mild-to-moderate latex intolerance. Three centiliters of both zinc gel formulation and placebo creams were applied to the subjects divided into three groups: group A (zinc gel formulation applied on the right hand and placebo cream on the left hand in 10 subjects who used powdered latex gloves); group B (no cream on the right hand and zinc gel formulation on the left hand in another 10 subjects who used powdered latex gloves); and group C (no cream on the right hand and zinc gel formulation on the left hand in 2 volunteers who used powder-free latex gloves). Latex gloves were then worn by the subjects until they perceived discomfort or until three to four hours had passed without symptoms. Investigators rated the subjects using numerical scale: "0" = no visible reaction to "3" = severe itching, redness, and papules all over the hand within 30 minutes. Results showed that zinc gel formulation protected 21 out of 22 volunteers

from latex sensitivity. Only one subject had a score of "1" belonging to group A. Additionally, the investigators extracted latex proteins from the gloves and treated with zinc gel formulation diluted in distilled water. Results revealed that zinc gel formulation–treated latex proteins decreased (mean = 0.28) as compared with the untreated ones (mean = 1.14) by ~74%. Lastly, zinc gel formulation was compared with three other creams and a control (no cream applied) to evaluate its barrier efficacy. Zinc gel formulation proved superior among the three creams.

IN VITRO AND IN VIVO METHODS

Teichmann et al. (34) investigated the reservoir and barrier functions of the skin in two study designs because the former function is dependent on the latter function. Study design A was carried out in six healthy volunteers according to the method described by Teichman (35) and in pigskin to quantify stratum corneum penetration. Patent Blue V ($C_{54}H_{26}CaN_4O_{14}S_4$) in water (the penetrant) was applied to the human skin in increasing amounts—10 and 40 $\mu g/cm^2$ of the 0.5% concentration and 40 $\mu g/cm^2$ of the 2% concentration. After one hour, substances were wiped to avoid occlusion, and then tape stripping was performed on the fifth hour. Results for the 10 $\mu g/cm^2$ of the 0.5% concentration revealed that the amount of stratum corneum extracted was 5.10 ± 1.25 $\mu g/cm^2$—no penetrant was recovered, i.e., no excess amount developed. However, after the applications of 40 $\mu g/cm^2$ of the 0.5% concentration and 40 $\mu g/cm^2$ of the 2% concentration (21.5 ± 1.0 $\mu g/cm^2$ and 27.7 ± 1.5 $\mu g/cm^2$ of extracted stratum corneum, respectively), excess amounts of penetrants were recovered (6.7 ± 2.8 $\mu g/cm^2$ and 27.7 ± 1.5 $\mu g/cm^2$, respectively). The same procedure was performed on the porcine skin to obtain a histological diagnosis and showed that a large amount of Patent Blue V was located on the skin surface and the upper parts of the stratum corneum, and greater amounts were also found in the furrows.

Study B was performed in another six healthy volunteers and the three BCs—commercial BC, beeswax, Vaseline—were investigated using the penetration behavior of Patent Blue V in water in the different BC-pretreated skin, and one untreated site by tape stripping. Results revealed that the commercial BC did not demonstrate barrier function—similar to the untreated site ($p > 0.05$), while beeswax and Vaseline were significant ($p < 0.05$) in their efficacy of barrier function.

Chilcott et al. (36) conducted an in vivo and in vitro study evaluating the efficacy of a BC (70% w/w Fomblin™ HC/R and 30% w/w lubricant grade polytetrafluoroethylene) versus chemical warfare agent in domestic white pigs. The in vivo study involved 18 pigs, prepared as previously described (37), and divided into three groups: control group (no agent, no BC), positive control group (with agent, no BC), and pretreated group (application of agent 15 minutes post application of the BC). An amount of 40 μL of the BC and ^{14}C-VX (~6-hour $2LD_{50}$) was applied over the inner ear of the animals. Indicators of mortality included a decrease in serum acetylcholinesterase (AchE) and a large pupil diameter. Animals in the control and BC-treated groups survived the three-hour exposure period, while five of the six animals in the positive control group died after a mean time of 65 ± 13 minutes. Correspondingly, there was a significant ($p < 0.05$) decline in serum AchE, while there was no significant ($p > 0.05$) change in pupil diameter. On the other hand, the in vitro study involved the contralateral (unexposed) ears of the postmortem pigs from the in vivo study. Twelve pigskins were placed in Franz-type glass diffusion cells filled with phosphate-buffered saline (PBS) receptor chamber fluid. An amount of 25.4 μL of the BC was applied onto the skin using a 25-μL positive displacement pipette and spread using a piston from a 1-mL syringe, as previously described (37), to give a nominal thickness of 0.1 mm. Each diffusion cell was subjected to the same decontamination procedure similar to the in vivo study. Pretreatment with the BC significantly ($p < 0.05$) decreased skin surface spreading of ^{14}C-VX and lowered the total amount penetrated, similar to the in vivo study results. On the other hand, the three-dose parameters (i.e., unabsorbed, skin, and receptor/systemic) were significantly ($p < 0.05$) different except for one parameter (i.e., total amount absorbed) between the two systems.

Recent BC experiments are summarized in Table 1.

Table 1 Brief Data of Recent Experiments of Barrier Creams

Models						
In vitro	In vivo					
Animals or humans		Irritants or allergens or penetrants	Barrier creams	Evaluations by	Efficacy	Reference
Human skin		Dyes (eosin, methyl violet, oil red O)	16 BCs	Amount of dyes in the epidermis	Various % protection effects	Treffel et al. (15)
Human skin	Nickel-sensitive patients	Nickel disc	Ethylenediaminetetraacetate (EDTA) gels	Nickel content	Significantly reduced the amount of nickel in the epidermis in vitro, and significantly reduced positive reactions in vivo	Fullerton and Menne (16)
Human skin		[^{35}S]-SLS	3 quaternium-18 bentonite (Q-18B) gels	Amount of [^{35}S]-SLS	% protection effect was 88%, 81%, and 65%, respectively	Zhai et al. (17)
	Guinea pigs	n-Hexane, trichlorethylene, toluene	3 water-miscible creams	Morphological assessment	Limited protective effects	Lachapelle et al. (23)
	Guinea pigs and humans	SLS, sodium hydroxide, toluene, lactic acid	Several BCs	Various bioengineering techniques	Some of them suppressed irritation, some failed	Frosch et al. (1,8,24,25)
	Humans	Dyes (methylene blue and oil red O)	3 BCs	Amount of dye penetrating into strips	Two of them exhibited effectiveness, one enhanced cumulative amount of dye	Zhai and Maibach (2)
	Humans	SLS, ammonium hydroxide (NH_4OH) and urea, Rhus	Several protectants	Clinical scores	Most of them suppressed the SLS irritation and Rhus-allergic reaction, failed to suppress NH_4OH and urea irritation	Zhai et al. (5)
	Humans	Self-application of BC	An oil-in-water emulsion	Fluorescence technique	Self-application of BC was incomplete	Wigger-Alberti et al. (26)
	Humans	Skin with dermatitis	Hydrogel Barrier/repair creams	Questionnaire	62% of the subjects' and 75% of the investigators' assessments favored the BC	Draelos (27)

	Humans	Antiseptics, gloves	Novel barrier cream and cream with oil-containing lotion	Clinical scores	Both creams offered protection	McCormick et al. (28)
	Humans	SLS on days 15–25	5% dexpanthenol	Various bioengineering techniques and photography	Capable to protect skin in experimentally elicited irritation	Biro et al. (29)
	Humans	Shampoos and other hair care products	5% aluminum chlorhydrate	Clinical scores, bioengineering techniques, subjects' personal assessment	Very little difference between BC and its vehicle	Perrenoud et al. (30)
	Humans	SLS and nickel	Skin tolerance–tested moisturizing cream	TEWL	Significant decrease in TEWL values of treated sites	Paepe et al. (31)
Ears of the domestic white pigs	Domestic white pigs	VX chemical warfare	AG-7 (70% w/w Fomblin™ HC/R plus 30% w/w lubricant grade polytetrafluoroethylene)	Acetylcholinesterase, pupil diameter;	Treated groups survived the 3-hr exposure; Pretreatment of BC lowered the amount of VX penetration	Chilcott et al. (36)
	Humans	SLS	6 skin care products	Chamber scarification test and ROIT	Debba Wet and SLS were more irritating; Vaseline, Takstosan, and Debba Wett did not offer protection from skin irritation.	Diepgen et al. (32)
Dissolved latex proteins	Humans	Latex gloves	Topical formulation with zinc	Clinical Scores	Protected 95% of subjects; Decreased dissolved latex proteins by ~74 %	Modak et al. (33)
Pigskin	Humans	Patent Blue V	Quantify stratum corneum penetration; and comparison of 3 BCs for efficacy	Penetration behavior of Patent Blue V	Higher concentrations of the penetrant yielded excess amount recovered; and Vaseline and beeswax are effective BCs	Teichman (34)

Abbreviations: SLS, sodium lauryl sulfate; BC, barrier cream; TEWL, transepidermal water loss; ROIT, repeated occlusive irritation test.

CONCLUSIONS

Some BCs reduce CD under experimental conditions. But, inappropriate BC application may enhance irritation rather than benefit. To achieve the optimal protective effects, BC should be used with careful consideration based on a specific exposure conditions; also, the proper use of BC should be instructed.

In vitro methods are simple, rapid, and safe and are recommended in screening procedures for BC candidates. With radiolabeled methods, we may determine the accurate protective and penetration results even in the lower levels of chemicals because of the sensitivity of radiolabeled counting when BCs are to be evaluated. Animal experiments may be used to generate kinetic data because of a similarity between humans and some animals (pigs, monkeys, etc.) in percutaneous absorption and penetration for some compounds. But no one animal, with its complex anatomy and biology, will simulate the penetration in humans for all compounds. Therefore, the best estimate of human percutaneous absorption is determined by in vivo studies in humans. The histological assessments may define what layers of skin are damaged or protected and may provide the insight mechanism of BC. Noninvasive bioengineering techniques may provide accurate, highly reproducible, and objective observations in quantifying the inflammation response to various irritants and allergens when BCs are to be evaluated that could assess subtle differences to supplement traditional clinical studies.

To validate these models, well-controlled field trials are required to define the relationship of the model to the occupational setting. Finally, the clinical efficacy of BC should be assessed in the workplace rather than in experimental circumstance. A recent review of evaluating the efficacy of BC provides additional insights (38).

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19 Electron Paramagnetic Resonance Studies of Skin Lipid Structure

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INTRODUCTION

Stratum corneum (SC) is the outermost layer of skin and the skin barrier against chemicals, surfactants, UV irradiation, and environmental stresses.The SC has a heterogeneous structure composed of corneocytes embedded in the intercellar lipid lamellae, as illustrated in Figure 1. The morphology of the SC lipids is closely associated with the main epidermal barrier. Knowledge of the lipid structure is important in understanding the mechanism of irritant dermatitis and other SC diseases. The structural properties of the SC lipid are obtained by the analyses of aliphatic spin probes incorporated into intercellular lamella lipids using electron paramagnetic resonance (EPR) (1–7). The EPR spin probe method measures nondistractively the ordering of the lipid bilayer of SC.

EPR (or electron spin resonance, ESR) utilizes spectroscopy, which measures the freedom of an unpaired electron in an atom or molecule. The principles behind magnetic resonance are common to both EPR and nuclear magnetic resonance (NMR), but there are differences in the magnitudes and signs of the magnetic interactions involved. EPR probes an unpaired electron spin, while NMR probes a nuclear spin. EPR can measure 10^{-9} molar concentration of the probe and is one of the most sensitive spectroscopic tools. Therefore, EPR is able to elucidate skin lipid structures as well as dynamics.

EPR in conjunction with the spin probe (or label) method has considerable advantages in the study of lipid structures as well as behaviors. The macroscopic and local viscosity of the environment profoundly influences the rate of lipid molecular reorientation. The physicochemical properties of intercellar lipids of SC as a function of various surfactants (1,2), water contents (3), various kinds of spin probes (4), and ordering (or fluidity) change of the SC lipid (5) were investigated. These studies provided the fluidity-related behaviors of SC at the different conditions by measuring EPR signal intensities and hyperfine coupling values. Furthermore, quantitative analysis of the experimental spectra can be achieved by a modern slow-tumbling simulation, which showed that the spectral simulation provided insight into the quantitative ordering of human lipid structure (6,7). In this chapter, the quantitative evaluations of SC lipid structure as a function of skin depth are described.

EPR APPARATUS

EPR apparatus consists of a klystron to generate microwaves, electromagnet, resonant cavity, microwave detector, amplifier, A/D converter, and PC (Fig. 2). The microwaves from the klystron have a constant frequency, and those microwaves reflected from the resonant cavity are detected, changed to an electronic signal, amplified, and then recorded. In contrast to NMR, substances that contain unpaired spin can be observed by EPR. Paramagnetic substances including transition metal complexes, free radicals, and photochemical intermediates are observed. Approximately 10^{-13} mole of a substance gives an observable signal, thus EPR has great sensitivity.

EPR OF SPIN PROBES (or SPIN LABELS)

Nitroxide Probes for EPR

Momentum of electron spin in a magnetic field orients only two quantum states: $m_s = ½$ and $m_s = -½$. Application of an oscillating field perpendicular to a steady magnetic field (H) induces transitions between the two states, provided the frequency (ν) of the oscillating field

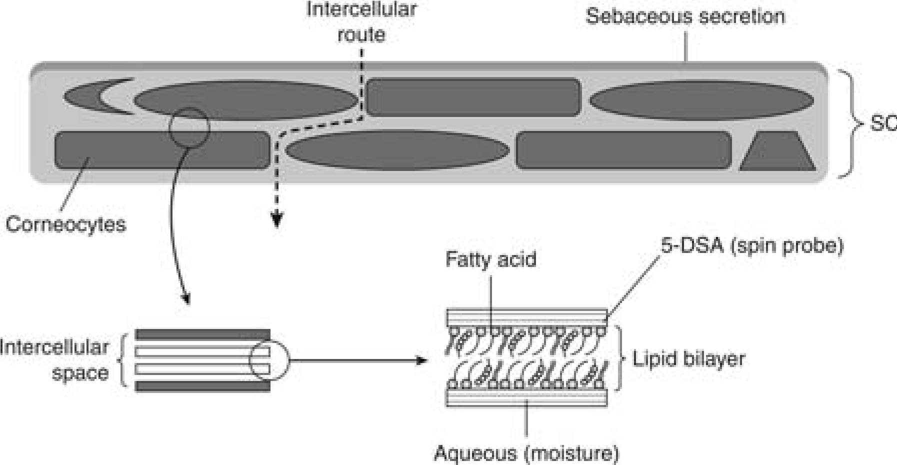

Figure 1 Schematic representation of the "Brick and Mortar" model of the stratum corneum is shown. Also, the most likely probe location in the lipid bilayer and pathways of drug permeation through intact stratum corneum is shown.

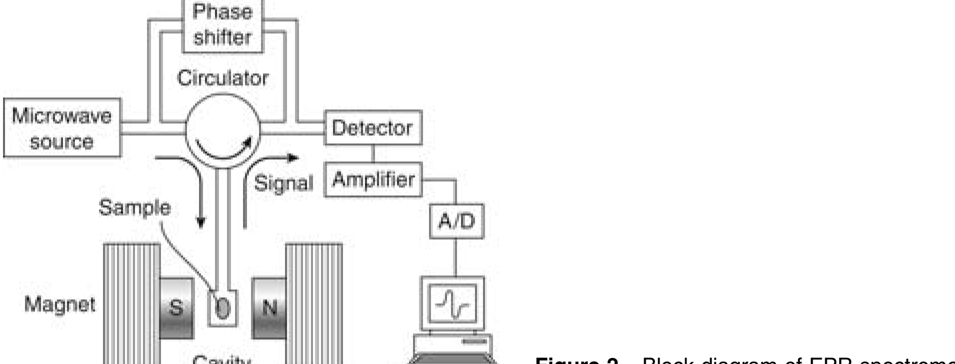

Figure 2 Block diagram of EPR spectrometer.

satisfies the resonance condition:

$$\Delta E = hv = g\beta H \tag{1}$$

where ΔE is the energy-level separation, h is Planck's constant, g is a dimensionless constant called the g-value, β is the electron Bohr magneton, and H is the applied magnetic field.

The interaction of an electron spin in resonance with a neighboring nuclear spin in a molecule is called hyperfine coupling. In the case of nitroxide spin probe, ^{14}N of the probe has three quantum states: +1, 0, and −1. Each quantum state interacts with an electron spin and further splits into two sets of energy states (Fig. 3). The selection rules for transitions in hyperfine coupling are $\Delta m_s = 1$ and $\Delta m_I = 0$. Thus, one can observe three transition (resonance) lines for fast-tumbling nitroxide spin probe in a spectrum. The interval of the resonance lines is called the hyperfine coupling constant. The EPR spectra are usually recorded as the first derivative of the absorption spectrum as shown in lower part of Figure 3.

Single-Chain Aliphatic Spin Probes

The ordering (or fluidity) of the lipid bilayer is obtained with doxyl stearic acid (DSA), which is most commonly used. The chemical structures of DSAs are depicted in Figure 4. Changes of lipid chain ordering are able to monitor using the probes. The orientation of spin probe reflects the local molecular environment and should serve as indicator of conformational changes in lipid bilayers.

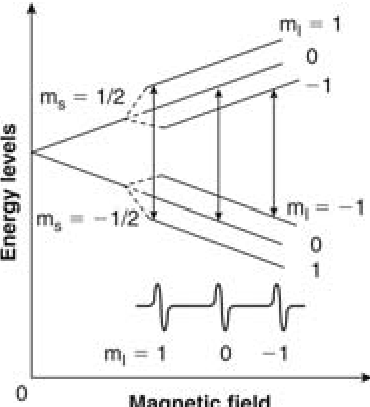

Figure 3 Hyperfine levels and transitions for a nitroxide nitrogen nucleus (^{14}N) of I = 1 with positive coupling constant.

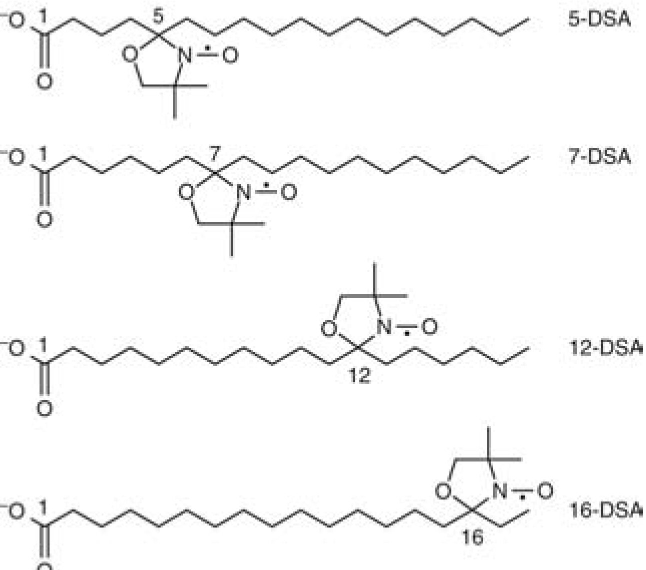

Figure 4 Chemical structures of various doxyl stearic acid (DSA) spin probes.

The ordering at different position of the lipid bilayer is obtained with 5-, 7-, 12-, and 16-DSA. The 5-DSA is usually used for extraction of information near surface region in a lipid membrane. The 16-DSA is for near the end of the lipid chain. It is notable that other spin probes are also commercially available.

EPR Line Shapes Due to Spin Probe Motion

The line widths can vary under certain spin probe environments. When line broadening arises from incomplete averaging of the *g*-value and the hyperfine coupling interactions within the limit of rapid tumbling in a medium, EPR line shape starts changing from the triplet pattern. EPR spectra of nitroxide radicals for different tumbling times as well as different order parameters are presented in Figure 5. If a spin probe is oriented (immobilized) in a membrane, EPR spectrum is an anisotropic pattern, which clearly shows parallel and perpendicular hyperfine coupling structures (the top spectrum in Fig. 5). The order parameter is approximately 0.7 or higher. If a spin probe tumbles relatively fast (weakly immobilized) in a membrane, EPR spectrum is a triplet pattern with unequal intensities. The order parameter is usually very small (~0.1).

	Description of spectra	Approx. tumbling time (ns)	Approx. order parameter
$2A_{\parallel}$ / $2A_{\perp}$	Immobilized	0.5	0.7
	Moderately immobilized	2.5	0.3
	Weakly immobilized	5.0	0.1

Figure 5 Nitroxide EPR line shape as a function of tumbling time and order parameter. Parallel and perpendicular hyperfine couplings, $2A_{\parallel}$ and $2A_{\perp}$, are also indicated for an anisotropic (immobilized) EPR spectrum.

CALCULATION OF ORDER PARAMETER

Conventional Order Parameter (*S*)

The order parameter indicates the lipid fluidity and microenvironment of the medium in which the spin probe is incorporated. The conventional order parameter (S) is determined by the hyperfine coupling (A) of the EPR spectrum according to the following relations (8):

$$S = \frac{A_{II} - A_{\perp}}{A_{ZZ} - (1/2)(A_{XX} + A_{YY})} \frac{a}{a'}, \tag{2}$$

$$a' = \frac{A_{II} + 2A_{\perp}}{3}, \tag{3}$$

where a is the isotropic hyperfine coupling value, $(A_{XX} + A_{YY} + A_{ZZ})/3$; A_{XX}, A_{YY}, and A_{ZZ} are the principal values of the spin probe. The experimental hyperfine couplings of $2A_{\parallel}$ and $2A_{\perp}$ are obtained from the EPR spectrum. In a calculation of the order parameter, the principal components of $A_{XX}, A_{YY}, A_{ZZ} = (0.66, 0.55, 3.45)$ mT and $g_{XX}, g_{YY}, g_{ZZ} = (2.0086, 2.0063, 2.0025)$ were used for 5-DSA (9).

Note that the conventional analysis measuring $2A_{\parallel}$ and $2A_{\perp}$ gives limited information concerning the probe moiety in the lipid. Changes in the probe behavior are reflected in the EPR line width as well as the line shape, besides hyperfine values. In some cases, S-values do not represent the subtle difference in overall EPR spectral changes related to the lipid chain ordering (6). Thus, the conventional calculation is qualitative analysis.

Order Parameter (S_0) by Slow-Motional EPR Simulation

In general, the large ordering value indicates the anisotropy of the probe site in the lipid (Fig. 5). For example, the spin probe is incorporated in the highly oriented intercellular lipid bilayer in normal skin; the probe cannot move freely because of the rigid lipid structure. Once the normal lipid structure is completely destroyed by chemical and/or physical stress, the clear triplet spectrum yields the small ordering value.

The slow-tumbling motions of the spin probes can be exactly calculated using a nonlinear least square–fitting program called NLLS, which analyzes the experimental EPR spectra on the basis of stochastic Liouville's equation (10–12). The simulation of the EPR spectra for spin probes incorporated into multilamella lipids is carried out using a microscopically ordered but macroscopically disordered (MOMD) model introduced by Meirovitch et al. (13). This model is based on the characteristics of the dynamic structure of lipid dispersions.

The order parameter, S_0, is defined as (14,15):

$$S_0 = \langle D_{00}^2 \rangle = \left\langle \frac{1}{2}(3\cos^2\gamma - 1) \right\rangle = \frac{\int d\Omega \exp(-U/kT) D_{00}^2}{\int d\Omega \exp(-U/kT)}, \tag{4}$$

which measures the angular extent of the rotational diffusion of the nitroxide moiety. Gamma (γ) is the angle between the rotational diffusion symmetry axis and the z-axis of the nitroxide axis

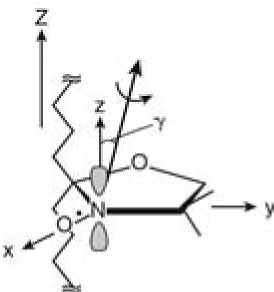

Figure 6 A schematic representation of a conformation of DSA spin probe in the SC membrane, where z-axis of the acyl chain is parallel to z-axis of the nitrogen $2P_z$ orbital.

system; z is the axis of the nitrogen $2p_z$ orbital, and x-axis is along the N–O bond (Fig. 6). The local or microscopic ordering of the nitroxide spin probe in the membrane is characterized by the S_0 value. A larger S_0 value indicates very restricted motion in the membrane. It is notable that the angle in relation with the S_0 value is discussed later in the next section.

Conventional (S) and Simulated Order Parameter (S_0)

The "Brick and Mortar" model of the SC is illustrated in Figure 1. SC intercellular lipids arrange themselves into bilayer and pack into lamellae. The single-chain 5-DSA normally dissolves into lipids and fat phases. The most likely location of the single-chain probe in the SC is shown in Figure 1. The aliphatic probe will be located in the lipid phase and fat-like sebaceous secretion of the SC.

Stripped SC was examined to characterize the lipid chain ordering using two methods: conventional order parameter and simulated order parameter (5,6). One piece of stripped SC (~7×37 mm^2) was incubated in ~50 μm 5-DSA aqueous solution for about 1 hour at 37°C. After rinsing with deionized water to remove excess spin probe, the SC sample was mounted on an EPR cell. A commercially available X-band (9 GHz), EPR spectrometer, was used to measure the ordering of the SC sample. The typical spectrometer settings were the following: microwave power, 10 mW; time constant, 1 second; sweep time, 480 seconds; modulation, 0.2 mT; and sweep width, 15 mT. The detailed sample preparations are described elsewhere (7).

Figure 7 shows the experimental and simulated EPR spectra of 5-DSA in the SC. The reasonable agreement of the experimental and simulated spectra suggests that simulation analysis can provide detailed information regarding the SC lipids. The S_0 value changes from 0.61 to 0.96, while the S value is in the range of 0.56 to 0.59. The conventional S value was obtained by Eq. (2) measuring the hyperfine values from the observed spectrum.

There are significant differences between the conventional and simulated order parameters. Because the slow-tumbling simulation calculates the total line shape of the

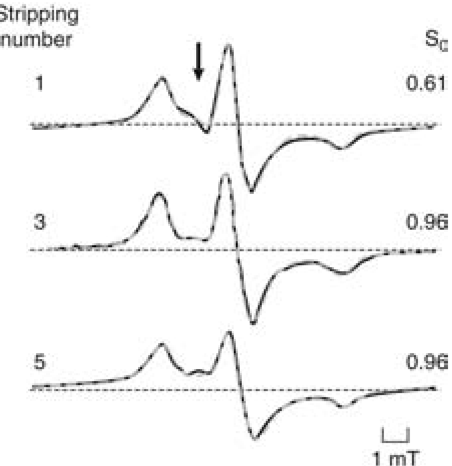

Figure 7 Experimental (*solid line*) and simulated (*dashed line*) EPR spectra of 5-DSA probe. Stripping numbers show consecutively stripped SC from the surface downward. The arrow of stripping number 1 indicates the characteristic peak.

spectrum, it is able to extract more detailed information about the SC structure than the conventional analysis, which is normally ambiguous in distinguishing the two hyperfine components (parallel and perpendicular) from the experimental spectrum because of the presence of weak and broad signals (6). Thus, the S_0 values (0.2–0.5) obtained by the simulation suggest that the outermost SC layers are less rigid (or more mobile), while the deeper lipid layers (S_0~0.9) have more rigid and oriented structures.

The arrow in the spectrum indicates the characteristic peak, which is prominent only for the first stripping (Fig. 7). This peak diminishes in intensity with increasing depth in the SC. The marked peak appears near the center of the spectrum because the probe embedded in the first stripping sample has greater freedom of motion. The other two lines of the nitroxide probe overlaid the central region of the spectrum. The results imply that signals can originate from sebaceous secretion.

Further investigation of the characteristic peak was performed. Figure 8A shows the EPR spectrum of the first stripping from SC. The strong and broad peak observed for the SC sheet from the human forehead is shown in Figure 8B. The peak intensity decreases after washing the SC with soap (Fig. 8C). Thus, the signal can be attributed to sebaceous secretion (7). The strength of the signal is considered to reflect the abundant sebaceous secretion at the forehead compared with that of the forearm.

Furthermore, one can calculate the angle (γ in Fig. 6) between the rotational diffusion symmetry axis (the lipid in SC) and the z-axis of the nitroxide axis system. Figure 9 represents the schematic illustration of the bilayer distance in relation to the angle. The simulated S_0 value of 0.61 can be the angle of 30. The value of 0.96 is the angle of 9.4. The angle suggests that the SC lipids align nearly perpendicularly to the bilayer surface. The larger S_0 value yields larger distance between the lipid bilayer. The analysis implies that the long distance of the lipid bilayer can be related to the well-oriented SC structure.

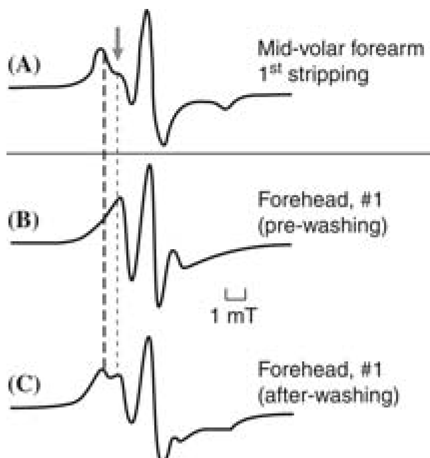

Figure 8 Experimental EPR spectra of 5-DSA in (**A**) the first stripped SC from human mid-volar forearm, (**B**) the first stripping SC from human forehead prewashing, and (**C**) the first stripping SC from human forehead after-washing. The short dashed line corresponds to the characteristic signal. The long dashed line corresponds to the probe incorporated into the SC lipids.

Upper layer

Simulated value

S_0 = 0.61 (γ = 30°)

Middle layer

Simulated value

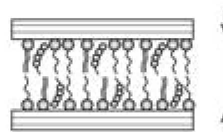

Figure 9 The bilayer distances and the values of simulated order parameter related to the angles between the bilayer surface and the single-chain probe.

OTHER APPLICATIONS OF THE EPR METHOD

Effects of Surfactants

Different types as well as mixtures of surfactants change the fluid structure of lipid bilayer differently. Kawasaki et al. examined the influence of anionic surfactants, sodium lauryl sulfate (SLS) and sodium lauroyl glutamate (SLG), on human SC by the EPR spin label method (1). The order parameter obtained by 1.0% wt SLS-treated cadaver SC (C-SC) was 0.52. On the other hand, the high S value of 0.73 for 1.0% wt SLG was obtained. The results suggest clear surfactant effects on the structure of lipid bilayer. In addition, a reasonable correlation between order parameters and human clinical data (visual scores and transepidermal water loss values) was shown.

Effects of Skin Penetration Enhancers

Interaction of skin penetration enhancer correlates with the fluidity of the intercellular lipid bilayers. Quan and Maibach investigated the effects on a C-SC at three concentrations of laurocapram (1-dodecylazacyclo-heptan-2-one) utilizing the EPR spin probe method (16). The EPR spectra of laurocapram-treated human SC were totally different from those of untreated C-SC. The results suggest that laurocapram causes an increase in the flexibility and polarity of local bilayers surrounding 5-DSA.

CONCLUSION

EPR along with a modern computational analysis provides quantitative insight into the SC structure as a function of the depth. The EPR spectral pattern contains important information regarding the probe moiety as well as the SC structure. Satisfactory agreement between the experimental and calculated spectrum can provide a quantitative S_0, which gives the microscopic lipid ordering in the SC lipid. The spectral simulation offers a reliable value of the lipid ordering where conventional order parameter cannot reveal the detailed ordering (6). In addition, the EPR method recognizes sebaceous exudates (7). Therefore, EPR together with a computational analysis is a powerful method to investigate various SC.

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20 Human Skin Buffering Capacity: An Overview

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INTRODUCTION

When dilute aqueous acid or alkaline solutions come into contact with the skin, the change in pH is generally temporary, and the original skin pH (a measure of the hydronium ion concentration) is rapidly restored indicating that the skin has significant buffering capacity.

A buffer is a chemical system that can limit changes in pH when an acid or a base is added. Buffer solutions consist of a weak acid and its conjugated base. The system has its optimum buffering capacity when about 50% of the buffer is dissociated or, in other words, at a pH about equal to its pKa (1). The pKa is the negative of the common logarithm of the acid dissociation constant (Ka) and is a measure for the strength of the acid. The buffer capacity is further dependent on the concentration of the system.

SKIN'S ACID CHARACTER AND BUFFER CAPACITY

The acidic character of the skin was first mentioned by Heuss (2) and later by Schade and Marchionini (3) who introduced the term "acid mantle" for the skin's acidic outer surface pH. The importance of the skin's acidic character has more recently been recognized as playing a crucial role in barrier homeostasis and immune function (4–6). The skin was further shown to partially resist acidic/alkaline aggression to some extent (7).

This article provides a review of studies investigating the skin's buffering capacity, specifically the epidermis, via alkali/acidic aggression tests. This review tries to discern which components of the epidermis are most likely responsible for the skin's buffering capacity.

Alkali/Acidic Aggression Tests

An acid/alkali aggression test is a way to measure the acid/alkali resistance (i.e., buffering capacity) of the skin. Alkali/acidic resistance tests were commonly used in the 1960s to detect workers who may likely develop occupational diseases in certain chemical work environments (7). A mild variation of the alkali/acidic resistance tests, also called acid/alkali neutralization test, assesses how quickly the skin is able to buffer applied acids/bases without the occurrence of skin corrosion. Repetitive applications of acid or base demonstrate that the skin's buffering capacity is limited and may be overcome; as illustrated by the long time required for neutralization (8–11).

The next section focuses on the aggression tests aiming to study which components of the epidermis are responsible for the skin's buffering capacity.

Free Fatty Acids/Sebum

Early experimentation hypothesized that the sebum contributes to the buffering capacity of the skin in two ways: first, it protects the epidermis against the influence of alkali by slowing down the exposure and penetration of acids or alkalis applied to the skin (12–14), and second, the fatty acids in sebum may act as a buffer system (15,16).

Later experiments by Lincke et al. (17) refuted the second hypothesis by demonstrating that the sebum had no relevant acid and a negligible alkali-buffering capacity of around pH 9. Further challenging the hypothesis, a quicker neutralization was observed on the delipidized skin than the untreated skin (12,14).

Vermeer concluded similarly when comparing the neutralization on soles and forearm with and without sebum removal (16). However, when comparing these skin regions,

differences in the sebum content and stratum corneum (SC) thickness may have also contributed to the observed effect.

Vermeer (14) and Neuhaus (18) believe that the increased rate of neutralization is due to a higher carbon dioxide (CO_2) diffusion. This theory, discussed later in detail, is generally not accepted but is also not clearly substantiated either way. After lipid removal, the skin starts to increase acid production, which may account for the faster neutralization. The same investigators also found that the increase in neutralization after lipid removal is temporary and limited to the first few minutes, which is probably related to the activity of sebaceous glands to produce relevant amounts of sebum.

Because of the negligible buffering capacity of sebum and to standardize the experiment (limit inter- and intra-individual variability), today most neutralization experiments are performed after cleansing the skin with solvents, which remove most of the sebum, including fatty acids.

Epidermal Water-Soluble Constituents

Vermeer et al. (16) first demonstrated the importance of water-soluble constituents to the skin's buffering ability. Water-soaked skin, where the water-soluble constituents were extracted, demonstrated a significantly reduced neutralization capacity, indicating that water-soluble substance constituent(s) of the skin is a major contributor to the buffering capacity (10,19,20). Water soaking may have induced also other changes to the skin, altering buffering capacity.

The significance of water-soluble constituents of the epidermis to the buffering capacity of the skin further supports the theory of minimal contribution from the sebum of the skin due to its lipid-soluble nature (16).

Sweat

Eccrine sweat initially accelerates the neutralization of alkalis (8–11,16,19,21,22). Spier and Pascher (23) suggest that the main buffering agents of sweat are lactic acid and amino acids (AAs). The lactic acid-lactate system in sweat has a highly efficient buffering capacity between pH 4 and 5 (13). However, it has not been completely demonstrated that lactic acid is the main buffering agent in sweat or at the surface of the skin. Conversely, the contribution of AAs to the buffering capacity of sweat and of the horny layer surface has been investigated thoroughly (16,19,22).

By comparing sweating and non-sweating persons, Vermeer et al. (16) found that AAs play a significant role in neutralization during the first five minutes while lactic acid does not. This confirms that AAs are key elements contributing to the buffering capacity of the skin.

Keratin

The contribution of keratin to the buffering capacity of the skin remains questionable. Keratin is an amphoteric protein with the ability to neutralize acids and alkalis in vitro (8–11,17,24–26) and hence may participate in the skin's buffering capacity. Scales scraped from the normal skin bind small amounts of alkali in vitro (27,28). However, Vermeer and coworkers showed that water-soluble constituents of the epidermis participate more in the skin's buffering capacity than the insoluble constituents of the skin such as keratin.

While insoluble keratin filaments on the skin may have only little buffering capacity (16,29), keratin hydrolysates and free AAs might contribute to the water-soluble portion of the epidermis. However, AA's composition of keratin (30,31) does not correspond with AA found in the water-soluble portion of the SC (23), which implies that keratin is not a major contributor to the pool of free AA.

Despite little evidence of keratin's role in the buffering capacity, a modifying action of keratin is assumed (17). Without an intact keratin layer, neither a physiological surface pH nor normal neutralization capacity can be maintained (32). Further research remains to be conducted to determine keratin's role in the buffering capacity of the epidermis.

Stratum Corneum Thickness

Differences in thickness of the SC may explain the interindividual differences in buffering capacity (33). The thicker the SC, the better the buffering (10,19), which is likely related to a better barrier for acids/alkalis within a thicker SC (33). In addition, as the skin ages, its

thickness diminishes and its buffering ability diminishes (34). Current technology, allowing more accurate SC measurement, may help in clarifying this point (35).

CO_2

Little is known about the role of CO_2/HCO_3^- participating in the skin's buffering capacity. Burckhardt's studies were the first to suggest that the CO_2 diffusing from the epidermal layer may be responsible for neutralizing alkali in contact with the skin (8–11). He demonstrated (8,9) that when a five-minute alkali neutralization experiment was repeated subsequently several times on the same skin area, the neutralization times were becoming longer, but finally reached an approximately constant time. He suggested that the shorter neutralization times at the beginning were due to acids present on the skin surface rapidly neutralizing the alkali. He further suggested that, after successive alkali exposure, the endogenous acids were not anymore present on the skin surface, which resulted in longer neutralization times, and that through skin diffusing, CO_2 took over the role in neutralizing the alkali. At this time, Burckhardt's hypothesis of the role of CO_2 as a buffering agent was accepted by others despite the rather weak experimental evidence (17,25,36,37).

For instance, the increased neutralization time after lipid removal of the skin surface with the help of soaps or neutral detergents was believed to be the consequence of a greater diffusion of CO_2, although this has never been quantified (13,36,37). It was also postulated that the hydrated SC retains CO_2 and limits its diffusion, whereas a moderate hydration level was regarded best for an effective alkali neutralization, although this has also never been analyzed in further details (37).

Clearly, the above studies fail to provide quantitative support for their conclusions concerning CO_2 as a relevant buffering agent. More likely, the constant neutralization time after successive alkali exposure may be related to the destruction of the "skin barrier" and unlimited penetration of the applied alkali as suggested by others (18,19).

Knowing that several authors considered CO_2 a relevant contributor in alkali neutralization without having quantitative data to sustain their hypothesis, Vermeer et al. (19) demonstrated that CO_2 is unlikely of great importance for alkali neutralization on the skin.

His experiment was focused on the first minutes of the neutralization process in contrast to the previous experiments mentioned (18,25,36,37), which paid attention to the later neutralization process. For example, Piper (25) analyzed the neutralization process for up to one hour and concluded that for the first half an hour alkalis are neutralized on the skin by the skin's own amphoteric substances (such as AAs) but in the second half hour diffusing CO_2 takes over. Piper's conclusions are not necessarily contradictory to the results obtained by Vermeer above and may actually be in agreement. According to Piper, "the longer the contact between the skin and alkali, the greater the importance of CO_2." Supported by the recent discoveries of relatively low level of CO_2 production in the epidermis and the limited activity of the Kreb's cycle suggesting that a minimal amount of CO_2 would be available for neutralization (30), it seems likely that CO_2 does not significantly contribute in the alkali neutralization process. Further studies should help to clarify the relevance of CO_2 in the skin's buffering capacity.

Free AAs

The free AAs in the water-soluble portion of the epidermis seem to play a significant role in the neutralization of alkalis within the first five minutes of experimentation (16,25,26).

Piper (25) found a good buffering capacity of the skin between pH 4 and pH 8, with an optimum at 6.5 well corresponding to the pKa of AA. This observation further indicates that lactic may be less relevant in alkali neutralization of the skin.

Despite the general agreement about the role of AAs in the neutralization of alkalis, which AAs are the key buffering agents remains an open question. The AAs' composition of the upper SC was reported by Spier and Pascher (23).

Spier and Pascher reported that the free AAs of the SC account for 40% of the water-soluble substances extracted from the SC removed by tape stripping (23,29). From the AAs present, 20% to 32% was serine and 9% to 16% was citrulline. Aspartic acid, glycine, threonine, and alanine were 6% to 10%. The smallest concentration of AAs accounted for glutamic acid at 0.5% to 2%.

The water-soluble, free AAs on the skin surface may originate from three possible sources.

1. Eccrine sweat

   Sweat contains 0.05% AAs, which remain on the surface of the skin after evaporation. The specific AA found in sweat was not investigated.
2. Degradation of skin proteins

   Degradation of skin proteins, including proteins constituting the desmosomes, may be a source for AAs such as serine, glycine, and alanine.
3. Hair follicle

   Citrulline is recognized as a constituent of protein synthesized in the inner root sheath and medulla cells of the hair follicle. Specific proteases release citrulline. Citrulline was also found in proteins in the membrane of the corneocytes as well as free floating (30).

   Further research needs to be completed to identify which AAs contribute to the buffering capacity of the skin and what is their main source.

DISCUSSION

The buffering capacity depends on many factors such as the following.

Alkali/Acid Agression

The nature and content of epidermal constituents available for buffering is likely a function of the acid/alkali aggression. For example, the more corrosive the compounds, the more the destruction of the skin, which results in an increased level of skin substances potentially available for buffering. In addition, a corrosive compound damages the skin's barrier, leading to increased penetration of the acid/alkali, which may further influence the skin's buffering capacity.

Skin Condition

Skin conditions were shown to greatly influence buffering capacity. Besides the skin's barrier properties, which were shown to vary between subjects and depend on the skin conditions and health, the presence of free AAs participating in the neutralization process may also play a role in the buffering capacity of the skin. Subjects with low buffering capacity are especially susceptible to the irritating effect of acids and/or bases, and predisposed to contact occupational eczema and dermatitis (29).

CONCLUSION

Whereas the skin's exquisite buffering capacity has been widely studied in vitro and in vivo, further research needs to be completed to better understand the exact mechanisms.

Experimentation reviewed here suggests that AAs are primarily responsible for the neutralization capacity of the skin. The exact sources of the AAs as well as the types of AAs that are primarily responsible for the neutralization capacity remain still rather speculative. In addition, it seems that a sweat component increases the neutralization capacity of the epidermis. Whether the buffering component of sweat is additional AA or lactic acid remains unknown.

While additional components of the epidermis such as sebum, keratin, and CO_2 seem not to significantly participate as buffering agents of the epidermis, they still do seemto play a role in the protection of the skin from the harm of acids and bases. Sebum may slow down the initial penetration of applied substances. Keratin is important for the hydration of the skin and may contribute some of the free AAs responsible for buffering of applied acids/alkalis. Finally, CO_2 may play a role in the buffering capacity of certain compounds under certain circumstances such as after prolonged or repetitive exposure to an alkali.

When the buffering capacity is exhausted, the pH of the skin becomes significantly altered; repair mechanism similar to wound healing may step in to restore the normal skin's pH (38). After thorough review of studies investigating the buffering capacity of the skin and

other studies investigating the endogenous mechanisms for restoring and maintaining the skin pH, it is interesting to note that the two topics have been investigated separately without looking for a commonality. It would not be surprising if the mechanisms responsible for maintaining the skin pH influence the processes responsible for maintaining the skin's buffering capacity. The above rationale may shed light on skin diseases in persons with diminished buffering capacity, an increased sensitivity to acids and/or bases, and an increased skin surface pH.

Taken together, we interpret this rich experimental literature, even if often quite old, as leading the way to the use of contemporary methods to further refine our insight into the skin's buffering capacity. This capacity, when fully understood, may lead not only to the potential for decreasing threat to the skin of exogenous acids and bases, but for establishing an experimental base for optimal pH in many pharmacologic, metabolic, and toxicologic situations.

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21 Skin pH and Skin Flora

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INTRODUCTION

The skin being the largest organ covers the entire exterior of the body and thus forms a protective barrier in between the human body and its environment. This tough and dry exterior signifies the physical character of the skin. The uppermost layer of the skin is a multilayered structure called the stratum corneum. The top three to five layers of stratum corneum undergo progressive desquamation. The morphology and thickness of stratum corneum is different at various body sites (1–3). The skin maintains characteristic physicochemical features such as structure, hydration, temperature, pH, and oxygen and carbon dioxide gradients. Changes in any of these factors impact the overall physiology of the skin. The acidic nature of the skin was discovered by Heuss in 1892 (4) and was later validated by Schade and Marchonini in 1928, (5) who underlined acidity as its protective feature and called it the "acid mantle." Current literature indicates that the skin surface pH is largely acidic between 5.4 and 5.9 (6).

The skin surface pH plays an important role in skin physiology and directly or indirectly influences various other factors such as composition of stratum corneum lipids, stratum corneum hydration, barrier function of the skin, and the skin's microbiota (7–15). The acidic pH of skin provides optimal pH for enzymes, e.g., glucocerebrosidase (16) and phospholipases (17), to work on extracellular lipids and a vitamin A–esterifying enzyme (18). Conversely, acidic pH of the skin has also been shown to accelerate the repair process of barrier function when damaged with acetone or extensive tapestripping (19). Also, the acidic skin pH has been shown to correlate with enhanced resistance against sodium lauryl sulfate (SLS)-induced irritant dermatitis (15,20).

An intraday variation (circadian rhythm) of skin pH was reported at some body sites, e.g., shin, forearms, and axilla (21,22). The skin pH was higher (pH 5.3) in the afternoon and lower (pH 4.9) at night (21–23). Investigations on seasonal differences in skin surface pH are limited (24). During summer, the pH of the skin surface is usually 0.5 units below pH values during the rest of the year (25).

Acidic pH of the skin is the result of the physiology of human body, which in turn regulates endogenous skin flora (15,26,27). The skin further provides a habitat for resident microbiota, which under normal conditions protects the skin from pathogenic organisms. Soon after birth, bacteria start to colonize the skin and other body sites. Despite wide variations in environmental conditions, the skin is capable of maintaining a stable microbial ecosystem (28). The skin temperature tends to be cooler than normal body temperature, slightly acidic, and mostly dry, whereas most bacteria prefer neutral pH, 37°C temperature, and moisture for optimal growth. Therefore, skin's microenvironment greatly dictates the microbial spectrum and population density. Some of the resident bacteria play an active role in maintaining acidic pH of the skin and preventing colonization by pathogenic bacteria.

THE ORIGIN OF THE SKIN pH

It is now well accepted that the acid mantle of the stratum corneum is very important for normal skin physiology and its bacterial flora. What makes the skin surface "acidic" is still not fully understood (14). Many endogenous and exogenous factors have been proposed, which influence the skin pH, e.g., eccrine and sebaceous secretions, anatomic sites, moisture, proton pumps, genetic predispositions, and age (10,11,14). Active and passive energy, bioenergetic processes, have also been suggested as sources for the acidic pH of the skin (11,29,30). For

example, lactic acid produced by passive process acidifies the superficial layers of skin (31). Other important components of passive metabolic processes include free fatty acids, cholesterol sulfate, urocanic acid, pyrrolidone carboxylic acid, which also contribute to the skin's acidic pH (32). Active proton pumps (e.g., the sodium/hydrogen anion exchanger proteins or NHE1) present in the membranes of the lamellar bodies are responsible for acidification of the intracellular space in the lower layers of stratum corneum (33). Free fatty acids generated by lipases of bacterial and/or pilosebaceous origin are partly implicated in the genesis of acid mantle (34).

The pores of the skin are made up of a combination of sebaceous and sudoriferous glands. When in balance, the combined excretion of oil and sweat from these pores has a pH of about 5.5. However, occlusive dressing has been shown to significantly increase the skin surface pH, moisture content, and bacterial density (35), indicating the role of endogenous factors in these changes. Exogenous factors such as skin cleansers, cosmetics, occlusive treatments, and topical antibiotics/antiseptics have been shown to alter the skin surface pH (36–39). Altered skin pH has been associated with dermatological conditions such as irritant contact dermatitis, atopic dermatitis, ichthyosis, acne vulgaris, and *Candida albicans* infections (31,40–42).

There could be many factors affecting the overall pH of the skin depending on the subject, body sites, and other biochemical factors. The skin of newborns and small infants differ from adults in some characteristics (43,44). The pH of infant skin is higher (e.g., 6.6 $\pm$ 0.25) than the adults (45–48). The pH of the skin differs at various anatomical sites, the superficial pH on the nose was the lowest among the regions tested (49). Regions with higher *Staphylococcus epidermidis* concentrations are slightly more acidic. In general, the skin surface pH is relatively similar at different body sites; however, slightly higher pH was reported in areas with higher moisture such as intertriginous areas (axillae, inguinal and submammary folds, and finger webs) (6,14,50).

A slight person-to-person variation in skin pH occurs because not everyone's skin is exposed to the same conditions such as weather and harsh detergents. Recently, in a large multicenter study, the skin surface pH of the volar forearm was assessed before and after refraining from shower and cosmetic product application for 24 hours (15). The average pH dropped from 5.12 $\pm$ 0.56 to 4.93 $\pm$ 0.45. The authors concluded that the "natural" skin pH is on average 4.7, which is below the generally reported pH range between 5.0 and 6.0. Interestingly, the study also suggested that showering with plain tap water in Europe, which has a pH around 8.0, could increase the skin pH for >four hours. The skin surface pH not only varied at different locations (Table 1), but also the lipid composition in the stratum corneum differs as a function of skin region and could influence the pH profile across the stratum corneum (51,52). Other reports (11,29,31,53,54) suggest that the pH of the skin follows a sharp gradient across the stratum corneum, which is possibly involved in controlling enzymatic actives and skin renewal (55).

Table 1 The pH Values on Human Skin at Various Locations as Reported in Selected Literature

Skin surface pH	Location	Reference
4.0–5.5	Forehead	50,59
4.0–5.5	Forehead and cheek	57
4.1–4.2	Forearm	62
4.4	Volar forearm	63
4.4–5.1	Volar forearm	64
4.5–5.6	Forehead	65
4.2–4.5	Forearm	65
5.5–5.8	Forehead	66
5.56–5.96	Back of the wrist	66
4.8–5.0	Volar forearm	67
4.93–5.12	Volar forearm	15
5.0–5.4	Volar forearm	68
5.0–5.5	Ventral forearm	30
5.4–5.9	Lower arm	6
5.5–5.8	Forearm	61

AGE, RACE, AND GENDER DIFFERENCES

Reports on the differences and/or similarities in the skin surface pH among various age, race, and gender are scanty. The newborn baby's skin pH recorded to be neutral soon becomes acidic within a month (56). The higher skin pH in infants is well documented (45–48) and may be associated with different chemical composition of the skin lipids (44); however, within a month, the baby's skin attains an acidic pH similar to adult skin. The available literature on skin surface pH indicates that the pH remains constant between 18 and 60 years of age (21,57,58). Men and women older than 80 years showed increased pH values (57,59). In the older age group of over 70 years, the mean pH value of the forehead was measured to be 5.6 as opposed to 5.3 in the younger age group (59). Anatomical differences in pH have also been reported (Table 1), which also influence the microbial composition and density (see later in the text and Table 3). In one of the studies (57), among 89% of the subjects, the skin surface pH on the cheek was higher than that on the forehead. In subjects younger than 80 years, the average pH is ranged between 4.0 and 5.5 on the forehead and 4.2 to 5.9 on the cheek (57). In another study, facial pH at different sites did not differ significantly between subjects with and without acne (60). Unlike in women, in men, the area close to the wrist had significantly lower pH values compared with the proximal sites (61).

Skin pH has been reported to vary with race, gender, and genetic background. Black people have a lower skin surface pH when compared with Caucasians (58,68), which has been attributed to pigmentation effects (29). Gupta et al. 1987 (65) measured the skin surface pH of 55 brown-skinned Indians comprising of 30 males and 25 females in the age range 12 to 58 years. Indian skin was slightly more alkaline, though the data are not definitive because the groups tested were small (65). The average pH values on the forehead of male and female were 5.51 and 5.73, respectively.

The differences between male and female skin surface pH have not been fully established. Studies published to date show contradictory results (Table 2). In most studies, significantly more acidic skin pH was found in men when compared with women (60,64,68–71), while other studies (61) showed the reverse situation, i.e., more acidic pH for women rather than men, while others showed no gender differences (21,57,58,61,72). A study conducted in India found that the male skin was slightly but significantly more acidic than the female. The same study (65) reported that the pH values at the axilla, umbilicus, palm, foot, sole, and cheek were consistently higher than those at scalp, forehead, retroauricular and popliteal fossae, anterior arm, anterior forearm, posterior neck, back, dorsum of hand, anterior leg, and anterior thigh. The highest pH was recorded in axilla (5.98 for male and 6.00 for female). The study notes that high density of both sweat glands and bacterial flora leads to a high skin pH, whereas lower pH was observed in area with high concentration of sebaceous glands and bacterial flora.

In the underarm region, the skin surface pH is significantly different between men and women, more acidic pH values were found in women than in men (71). The baseline pH value before washing was 6.58 ± 0.63 (right armpit) and 6.67 ± 0.65 (left armpit) in men versus 5.8 ± 0.53 (right armpit) and 5.94 ± 0.62 (left armpit) in women. Interestingly, washing of armpits with pure tap water further increased the difference between male and female pH values (71). The pH difference between right and left armpit was not statistically significant or (71) similar to some earlier reports of no difference in skin pH between the dominant and nondominant forearms or hands (61,62).

Table 2 Gender Differences in Skin pH

	pH		
Anatomical sites	Female	Male	Reference
Forehead	5.4–5.8	5.1–5.5	60
Forehead	5.73	5.51	66
Axilla	5.80–5.94	6.58–6.67	72
Volar forearm	4.8–5.8	4.3–4.7	53
Volar forearm	5.60–5.88	4.76–4.93	68
Volar forearm	4.97–6.09	5.44–6.16	61
Back of wrist	5.84	5.56	66
Back skin surface	5.43–5.73	4.96–5.12	22

One of the prevalent hypotheses about the role of skin pH is its putative importance in antimicrobial defense (63,73). Possible explanations are that (*i*) the top layer of the skin is very dry and densely packed, which makes this first line of defense inhospitable to many bacteria; (*ii*) salty secretions from sweat glands create an environment that is hyperosmotic and thus unfavorable for bacteria; and (*iii*) normal flora grow best at a more acidic pH, whereas pathogenic bacteria, such as *Staphylococcus aureus*, grow best at neutral pH (74). A more acidic pH helps to protect skin against colonization by nonresident and pathogenic bacteria because many of them survive well in a narrow pH range near neutral.

The acidic condition of the skin is caused by secretions from sweat glands, skin oil, and the breakdown of fatty acids by *S. epidermidis*. Thus, a resident microflora is partly responsible for the acidic pH of skin. A multicenter study also found that the acidic pH of the skin surface (4.0–4.5) keeps the resident bacteria attached to the skin, whereas an alkaline pH (8,9) increased the dispersal of bacteria from the skin (11,15,27). The importance of pH for antimicrobial function is further supported by neonatal eczematous and atopic skin, which displays a neutral pH (41,75,76).

SKIN FLORA

The skin provides the largest organ (about 2 m^2 skin surface in average human adult) and an intricate habitat for a complex microbial ecosystem comprising resident and transient microflora, mainly bacteria (77,78), to a lesser extent fungal and possibly viral agents. Bacteria-skin relationship can be commensal, symbiotic, or parasitic relative to the host's overall physical and immune status. Persistant colonization is the result of alterations in the host's immune status, resulting in a significant impact on the balance of the bacteria-skin relationship.

The acid mantle, level of mineral and moisture, and use of skin cleansers and cosmetics influence the growth and maintenance of resident flora; and the state of resident flora influence the acquisition of transient bacteria (77). This acid mantle, a fine film with a slightly acidic pH on the surface of the skin, provides a protective barrier to the skin. The microbial population dynamics on various parts of the skin is determined by the anatomical location, the amount of sebum and sweat production, local pH, humidity, temperature, light exposure, etc. (71,79). Host factors such as age, immune status, hormonal status, and other habits also influence the composition and density of the skin flora (80,81). The development of bacterial flora on skin from birth to adulthood has not been systematically studied. During the prenatal stage, the skin remains sterile but soon becomes colonized by bacteria after birth. Not all bacteria are welcomed onto the skin. The skin allows the colonization and growth of those bacteria, which protect the host from pathogenic bacteria both directly and indirectly. These bacteria can act by producing antibiotics (e.g., bacteriocin), toxic metabolites, inducing a low reduction-oxidation potential, depleting essential nutrients, preventing attachment of competing bacteria, inhibiting translocation, by degrading toxins, etc (81,82).

Microbial status on skin can be temporary or transient, short-term resident and long-term resident biota. Establishment of a resident status depends on the ability of the bacteria to adhere to the skin epithelium, grow in a relatively dry and acidic environment, and establish a relationship that is more mutualistic than commensalistic (11,15,82). Bacterial adhesion or detachment from the skin could be mediated by (*i*) specific interactions via lectin or sugar binding; (*ii*) hydrophobic interactions; and (*iii*) electrostatic interactions (83,84). Hand washing with a skin cleanser containing microbial anti-attachment ingredients has also been shown to prevent bacterial adherence to skin, which may be working via electrostatic interaction (85, unpublished data). Recently, using 1% lactic acid (pH 3.0) and 1% sodium carbonate decahydrate (pH 11.0) under acidic conditions, the dispersal rate of the resident bacteria from volar forearm was much lower than under alkaline conditions, suggesting the role of electrostatic interaction between bacteria and positive charges of the skin under acidic pH. The differences in dispersal rate under acidic and alkaline pH have not been fully understood. Various explanations (15) are put forward for high dispersal rate under alkaline condition: (*i*) Under alkaline conditions both keratines and the bacterial surfaces are negatively charged resulting in repulsion. (*ii*) Net negative charge of the keratins created by alkaline treatment would lead to the swelling of the skin, which may open up the sponge-like corneocytes, allowing the bacteria to diffuse to the surface. A laboratory-based study has shown that washing hands with plain soap spreads bacteria on the entire hands (personal observation). It has also been reported that repeated washing could not diminish numbers of bacteria (86); therefore, the practice of rigorous preoperative washing of the hands in hospitals has been questioned (87,88). Because of the inefficacy

of washing regimens, especially in health care settings, selection of an effective skin cleanser for routine hand hygiene is very important (88,89).

Bacterial species commonly isolated from normal skin include *Staphylococcus, Micrococcus, Corynebacterium, Brevibacteria, Propionibacteria*, and *Acinetobacter* (79,81,90,91). *S. aureus, Streptococcus pyogenes, Escherichia coli*, and *Pseudomonas aeruginosa* are transient colonizers (91,92). The gram-negative bacteria are the minor constituents of the normal skin flora, and *Acinetobacter* is one of the few gram-negative bacteria commonly found on skin. The presence of *E. coli* on the skin surface is indicative of fecal contamination. Yeasts are uncommon on the skin surface, but the lipophilic yeast *Pityrosporum ovalis* is occasionally found on the scalp. Racial and gender differences in skin microflora are not fully examined (93). A more recent study using molecular techniques has provided better understanding on microbial ecology of the skin (94). Gao et al. (94), using molecular techniques, have identified 182 species of bacteria on human forearm skin, of which 8% were unknown species that had never been described before (94). This study also shed some light on the gender differences of skin microbiota, the microbial mix, and the possible role of pH (61). Roughly, half of the bacteria identified in the samples represented the genera of *Propionibacteria, Corynebacteria, Staphylococcus*, and *Streptococcus*, which are generally considered as the resident flora of human skin. Among the six individuals sampled, only four species of bacteria were in common: *Propionibacterium acnes, Corynebacterium tuberculostearicum, Streptococcus mitis*, and *Finegoldia AB109769*. Interestingly, three bacterial species were found only in the male subjects: *Propionibacterium granulosum, Corynebacterium singulare*, and *Corynebacterium appendixes* (95).

The skin surface pH influences various factors for the growth of resident and pathological microorganisms (7,11,71,95). The acidic pH of the skin is regarded as one of the major factors in making the skin a less favorable habitat for bacteria (96). A high density of bacteria was found in skin area with less acidic pH such as genitocrural area, anal regions, toe webs, submammary fold, and axillae (55,71). Those areas of the skin, which are relatively dry and exposed, have lower pH and lower microbial population density as well. For example, volar forearm skin has bacterial population about 10^2 to 10^3 cfu/cm^2 (colonies forming units/cm^2) (63), compared with 10^5 cfu/cm^2 in relatively moist underarms area (78). Artificial occlusion of the forearm skin leads to significant changes in skin pH and in the composition and density of bacterial species (35,63). For example, before occlusion, the skin pH value was 4.38, and after five days of occlusion, the pH increased to 7.05 (63). Similarly, the average bacterial count before occlusion was 1.8×10^2 cfu/cm^2, which increased to 4.5×10^6 cfu/cm^2 on the fifth day (63). It is evident that moist skin environment promotes bacterial growth and colonization. The distribution and composition of bacterial species on the skin vary at different body sites (Table 3). In intratrigenous area, the skin surface pH is somewhat higher, which in turn favors higher bacterial density (90,91).

Table 3 Normal Skin Microflora in Areas with High Density

Bacteria	Area
S. epidermidis	Upper trunk
S. hominis	Glaborous skin
S. capitis	Head
S. saccharolyticus	Forehead/antecubital
S. saprophyticus	Perineum
M. crococcus luteus	Forearm
Corynebacterium xerosis	Axilla, conjuctiva
C. minutissimum	Intertriginous (e.g., axilla)
C. jeikeium	Intertriginous (e.g., axilla)
P. acnes	Sebaceous gland, forehead
P. granulosum	Sebaceous gland, forehead, axilla
P. avidum	Axilla
Brevibacterium spp.	Axilla, toe webs
Dermabacter spp.	Forearm
Acinetobacter spp.	Dry area
Pityrosporum spp.	Uppermost part of sebaceous gland follicle

Abbreviations: *S. epidermidis*, Staphylococcus epidermidis; *S. hominis*, Staphylococcus hominis; *S. capitis*, Staphylococcus capitis; *S. saccharolyticus*, Staphylococcus saccharolyticus; *S. Saprophyticus*, Staphylococcus saprophyticus; *C. minutissimum*, Corynebacterium minutissimum; *C. jeikeium*, Corynebacterium jeikeium; *P. acnes*, Propionibacterium acnes; *P. granulosum*, Propionibacterium granulosum; *P. avidum*, Propionibacterium avidum.

Table 4 Effects of Skin pH on Skin Microflora

Effects	Reference
Acidic pH (4–4.5) keeps the resident flora attached to the skin.	15
Alkaline pH (8,9) promotes dispersal of bacteria from the skin.	
Less acidic pH promotes bacterial growth, especially gram-negative bacteria and propionibacteria.	63,74
Skin candidal infection was more inflammatory when the SC was buffered to pH 6.0 versus 4.5, indicating that pH may mediate immune reaction to infections.	113
High pH in the axilla promotes high bacterial growth and malodor.	114
Acidic pH boosts the activity of antibacterial lipids and peptides.	26,104,107,108
Acidic pH facilitates production of natural antimicrobial peptides, wound healing, and regulating keratinization and desquamation processes.	9,53,105,106,107

Abbreviation: SC, stratum corneum

The normal flora also acts as a barrier to prevent invasion and growth of pathogenic bacteria (34,97). The relevance of normal skin flora as a defensive barrier can be articulated with the finding that intensive use of antimicrobial skin cleansers could lead to an increased susceptibility to skin infections by gram-negative bacteria (98–100). A healthy growth and maintenance of the resident bacteria effectively deny the colonization by transient bacteria (e.g., *E. coli*, *Pseudomonas*, coagulase positive *S. aureus*, *C. albicans*). The skin's antimicrobial defenses include the mechanical rigidity of the stratum corneum, its low moisture content, stratum corneum lipids, lysozyme, acidity (pH 5), and defensins (29,101–103). Recent studies suggest that increased enzyme activity of phospholipase A2 is related to the formation of the acid mantle in the stratum corneum (29,31).

PROTECTIVE ROLE OF ACIDIC pH OF THE SKIN

Besides other physicochemical roles of the skin pH, it is now generally accepted that the normal skin surface pH has a beneficial role in relation to skin microflora (Table 4). Acidic pH of the skin (pH 4.0–4.5) helps the resident bacterial flora to remain attached to the skin and prevents cutaneous invasion by pathogenic microorganisms (7,8,15), whereas alkaline pH (8.0–9.0) is reported to promote dispersal of the bacteria (15). Acids produced by bacteria also contribute to the local protective mechanisms. For example, *S. epidermidis*, *P. acnes*, *Pityrosporum ovale*, and *Corynebacteria* produce lipases and esterases that break triglycerides to free fatty acids, leading to a lower skin surface pH and thereby creating an unfavorable environment for skin pathogens. Acidic pH of the skin also facilitates the production of natural antimicrobial peptides, attributes to the wound healing, and regulates the keratinization and desquamation processes (9,53,104–108). The skin flora also produces proteinaceous or lipidic antibacterial compounds termed "bacteriocins." These bacteriocins are involved in controlling/regulating bacterial competition for survival in this microenvironment. For example, a bacteriocin-Pep 5 produced by *S. epidermidis* is particularly active against other staphylococci, specifically *S. aureus* (109). Interestingly, the acidic pH of the skin boosts the activity of these antibacterial lipids and peptides possibly by enhancing the interaction with the bacterial membrane (26,105,108,109).

EFFECTS OF THE SKIN pH ON SKIN FLORA AND PATHOLOGY

Cutaneous pH plays an important role in maintaining the normal bacterial flora of the skin and preventing cutaneous invasion by pathogens (26,110). The acidic pH of the skin surface has long been regarded as the result of exocrine secretions of the skin glands, which in turn is involved in regulating the skin flora. Furthermore, a number of recent investigations published on the pH gradients in deeper layers of skin indicate a close relationship among the barrier function, a normal maturation of stratum corneum, and desquamation. Initially, work done in test tubes clearly demonstrated the effect of pH on bacterial growth (111,112). The study found that *S. aureus* grew equally well at pH 5, 6, and 7; normal micrococci showed somewhat, but

not significantly, better growth at pH 6 and 7 than at pH 5. On the other hand, aerobic diphtheroids grew significantly better at pH 7 than at lower pH levels (113). The acidic pH of skin provides a balanced environment for the resident bacteria. Changes in the skin pH and other organic factors play a role in certain skin pathogenesis and in their prevention and treatment (Table 4).

P. acnes is a classical example of how a slight increase in the skin pH can facilitate the resident bacteria to become pathogenic. Under normal pH of 5.5, growth of *P. acnes* is at its minimum; however, a slight shift toward alkaline pH would make it a more favorable environment, resulting in increased growth of this organism (112,115). As mentioned earlier, prolonged occlusion of skin significantly affects the growth of the normal skin flora, skin pH, and the rate of transepidermal water loss (TEWL) and carbon dioxide emission (35,63).

Recent studies have shown the relationship between a change in skin pH and its consequences in atopic dermatitis, particularly disturbances in skin barrier function and increased colonization with *S. aureus* (116). However, other studies (105,117) have suggested that in atopic dermatitis, increased colonization by *S. aureus* and other bacteria could be associated with a decrease in sphingosine and ceramide production. In atopic eczema, not only the skin surface pH was significantly higher than in normal healthy skin (41,118) but also the growth of *S. aureus* and exotoxin production were increased, which have been shown to induce eczema on intact skin (119).

Changes in the skin pH from acidic to alkaline could also be a risk factor for the development of candidal infections (113). A laboratory-based study, where right and left forearms were respectively buffered at pH 6.0 and 4.5, inoculated with a suspension of *C. albicans* and occluded for 24 hours showed more pronounced skin lesions with the higher pH suggesting that the higher pH may increase yeast virulence and/or modulate the host's defence capability (66). Yosipovitch et al. (67) found that the pH values in the intertrigenous skin among 50 noninsulin-dependent diabetic patients were significantly higher than in normal healthy volunteers and attributed the higher pH as a risk factor for candidal infection (66). Patients on dialysis also showed significant increase in their skin surface pH.

In a moist intertrigenous area, such as axilla, the pH is physiologically higher than in other skin regions (78,90,114), which promotes the growth of local flora. It has been established that underarm odor is created by the action of indigenous bacteria on axillary apocrine gland secretions (78, personal observations). Application of a deodorant product showed significant reduction in axillary pH, which in turn inhibited the growth of underarm bacteria (114).

EFFECTS OF SKIN CLEANSERS AND COSMETICS ON SKIN pH AND FLORA

As mentioned earlier, there are many external factors that influence the skin surface pH. Some of the external factors include the use of soap, detergents, and cosmetic products. Long-term use of these agents has been shown to alter the skin surface pH and to some extent affect the skin microflora at least for a short duration (37,114,120). Alterations in skin pH could cause irritation or interfere with the keratinization process as well (11,121).

Frequent hand washing with soap may damage the skin and facilitate more bacterial colonization. In fact, water and soap washing of damaged skin were not effective in reducing the bacterial contamination (122). Use of an alkaline soap with pH 10.5 to 11.0 resulted in higher skin surface pH and marked increase in the number of *Propionibacteria*, but the counts of coagulase negative *Staphylococci* were not much changed (48,96). In acne-prone young adults, washing of facial skin with an alkaline cleansing agent was reported to cause more inflammatory reaction than the acidic syndet bar (42). On the other hand, washing with an acidic skin cleanser (pH of 5.5) similar to the normal skin pH in adults increased the skin surface pH but significantly less than the alkaline soap (48,74,98,123). At the forehead, there was a clear correlation between bacterial counts and the skin pH, both with *Propionibacteria* and *Staphylococci*, but on the forearm only *Propionibacteria* count was higher with higher pH. The skin surface pH was significantly higher when neutral preparations were used. The number of *Propionibacteria* was significantly linked to the skin pH (74). The use of synthetic detergents with pH similar to the skin surface pH led to a rise in the skin surface pH for a shorter duration (36,42), and such temporary changes in skin pH were limited to the top layers of the stratum corneum (55).

Korting et al. (96) were among the first to examine the effect of different skin cleansing treatments on the bacterial flora and the skin surface pH in healthy volunteers (37,96) using a crossover clinical design. Essentially, volunteers in one group washed their foreheads and forearm with an alkaline soap twice daily for one minute and those in the other group used an acidic soap (syndet). After four weeks, both groups switch their soaps, respectively, in a crossover fashion. The skin pH and bacterial density were determined at the beginning of the study and at the end of every week (96). The pH was increased when alkaline soap was used first and the pH dropped with the changeover to syndet. When syndet was used first, the pH dropped slightly, and then increased when alkaline soap was used. Long-term use of syndet lowered the skin pH by about 0.3 units. In general, washing with alkaline soap resulted in an increase in skin pH and a marked increase in *Propionibacteria* without any significant change in counts of coagulase negative *Staphylococci*.

A more recent study found the natural skin surface pH below 5, which is on the lower end of many studies reported to date in the literature (15). They assessed the pH on volar forearm before and after refraining from shower and using any cosmetic products on skin for 24 hours. The baseline pH before taking shower was 5.12 ± 0.56. After 24 hours without any product application or contact with water, the pH value dropped to 4.93 ± 0.45. On average, the authors estimated that the natural pH value of the volar forearm skin to be 4.7 (15), which is in contrast to general assumption that the average skin pH ranges between 5.0 and 6.0. Interestingly, the study also found that plain tap water with pH around 8.0 as generally found in Europe could increase the skin pH up to six hours after application.

CONCLUSION

Since the first report in 1892 by Heuss (4) on the acidic nature of the skin, significant progress has been made in the field of skin biochemistry/microbiology, yet a number of areas remain to be fully explored. The exact origin of the skin acidity is still being investigated, but recent studies appear to indicate that several endogenous factors, including the presence of lactic acid, free fatty acids, urocanic acid, pyrrolidone carboxylic acid in sweat and sebaceous secretions are involved. The skin is the primary organ protecting the human body from external physical and chemical assaults. Overall, the skin surface is acidic with subtle differences between race and gender. It is not yet clear whether the skin has an inherently acidic pH that provides a hospitable environment for certain organisms or whether the organisms are attracted by other factors to colonize the skin. A recent study on human forearm superficial bacterial flora using molecular techniques highlighted the subtle differences in skin bacteria between men and women and possible relation with the skin pH (94), and suggested the need for further studies including host-and site specificities of the bacterial species on skin and their role (if any) in the pathogenesis of skin diseases. The acidic pH of the skin provides an optimal environment for resident bacteria and their enzymatic activities. Together, the acidic pH of the skin and the resident flora of the skin play an important role in maintaining skin health. The acidic pH of the skin is a key factor in the barrier function (14,19) and plays a key role in the mutualistic relationship with resident microflora (80,124–126). It is well recognized that an increased skin surface pH may be associated with the pathogenesis or the severity of many skin disorders, including acute eczema, irritant contact dermatitis, atopic dermatitis, ichthyosis, acne vulgaris, and *C. albicans* infections (11,20,26). It is becoming more evident that the repeated use of alkaline skin cleansing products, detergents, and even hard water (pH 8.0) can adversely affect the natural skin pH and disturb the normal flora. To maintain the normal physiology and microflora of the skin, use of cosmetics and skin cleansing products, which do not alter the skin pH or adversely affect the skin flora should be considered. Additionally, more research on pre- and probiotics in regulating healthy skin flora and maintaining optimal skin biochemistry is needed.

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22 Skin Ageprint: The Causative Factors

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INTRODUCTION

The pace of change in the demography of westernized populations has been rapid over the past century. In the corresponding affluent societies, an extraordinary shift has taken place in the age profile of the population, with older people representing a progressively growing segment. The aging "baby boomers" are now a major demographical force partly driven by the West's cultural obsession with the prevention of aging and the desire to maintain a youthful appearance. Thus, the current demographical evolution has enormous social implications. Indeed, the aging process is increasingly one of the daring topics of both the media and medical community. Any new antiaging treatment modality is avidly watched by the population. Middle-aged and even younger subjects show a craze for cosmetic dermatology when their once youthful bodies exhibit the early signs of wear and tear. In this field, breakthroughs and novel treatments fulfil some of the promises. No longer is this search a uniquely female characteristic, men also disdain an elderly demeanor. In addition to new technological advances, the future prospect of the scientific approach of skin aging relies on a better understanding of the relationships between skin biology and physiology and the ultimate clinical appearance.

The aging problem is even more complex and severe in cases where the skin has lost its protective mechanical function. The atrophy is such that the aspect of "transparent skin" is reached. Such chronic cutaneous insufficiency/fragility syndrome has been coined "dermatoporosis" (1). The clinical manifestations of dermatoporosis comprise morphological markers of fragility such as senile purpura, stellate pseudoscars, and skin atrophy. In addition, functional expression of skin fragility results from minor traumas such as frequent skin laceration, delayed wound healing, nonhealing atrophic ulcers, and subcutaneous bleeding with the formation of dissecting hematomas leading to large zones of necrosis. Dissecting hematomas bear significant morbidity that requires hospitalization and urgent surgical procedures.

FROM GLOBAL TO MOLECULAR AGING AND BACK AGAIN

All living organisms are subjected to aging. However, this event results from a multifaceted process, which is not the same in all of them. The limitation to any definition of aging lies in the diversity of life histories of the organisms. Two distinct classifications of life histories are of major importance. The first classification distinguishes between species that have a clear distinction between germ cells and somatic tissues from those that do not. The second classification makes a distinction between the semelparous species, which reproduce only once in their lifetime, and the iteroparous species, which reproduce repeatedly. The models of aging are most clearly defined in iteroparous species, which have a distinct soma separate from the germ line. Aging needs to be considerably qualified when applied to species with other kinds of life history. It is mistaken, for example, to regard the postreproductive end of life of semelparous species, which usually occurs in highly determinate fashion, as being comparable with the more protracted process of senescence in iteroparous species.

Aging of human beings is a physiological process corresponding to a progressive loss in homeostatic capacity of the body systems, ultimately increasing the vulnerability to environmental threats and to certain disease status. Nobody can escape from aging. However, it is evident that the process progresses differently among individuals of the same age. In any given subject, senescence is heterogenous among organs and also among their constitutive tissues, cells, and subcellular structures (2). Each and every organ of the human body develops and fails at its own rate, which is referred to as its age (3). This systematic aging occurs

Table 1 Core Age Markers of Each of the Body System

Aging type	Decline in	Average onset age (yr)
Electropause	Electrical activity of brain waves	45
Biopause	Neurotransmitters	Dopamine 30, acetylcholine 40, GABA 50, serotonin 60
Pineal pause	Melatonin	20
Pituitary pause	Hormone feedback loops	30
Sensory pause	Touch, hearing, vision, taste, and smell sensitivity	40
Psychopause	Personality health and mood	30
Thyropause	Calcitonin and thyroid hormone levels	50
Parathyropause	Parathyroid hormone	50
Thymopause	Glandular size and immune system	40
Cardiopause/ Vasculopause	Ejection fraction and blood flow	50
Pulmonopause	Lung elasticity and function with increase in blood pressure	50
Adrenopause	DHEA	55
Nephropause	Erythropoietin level and creatinine clearance	40
Somatopause	Growth hormone	30
Gastropause	Nutrient absorption	40
Pancropause	Blood sugar level	40
Insulopause	Glucose tolerance	40
Andropause	Testosterone in men	45
Menopause	Estrogen, progesterone, and testosterone in women	40
Osteopause	Bone density	30
Dermopause	Collagen, vitamin D synthesis	35
Onchopause	Nail growth	40
Uropause	Bladder control	45
Genopause	DNA	40

Source: From Ref. 3.

throughout the entire body from the time of about 30 to 45 years of age (Table 1). To further complicate the situation, there is regional variability of skin aging over the body. It is indeed quite evident that at any time in adult life, the face, scalp, forearms, trunk, and other body sites show different manifestations of aging. In addition, scrutinizing skin aging at the tissue level (epidermis, dermis, hypodermis, hair follicle), and further, at the cellular level (keratinocytes, melanocyte, fibroblast, dermal dendrocyte, etc.) shows a patchwork of aging severity.

Intracellular and extracellular molecules are involved differently by aging. Within each organ system, aging manifests as a progressive, approximately linear reduction in maximal function and reserve capacity at the molecular level. Some aspects of aging can be viewed as a predetermined programmed process. In addition, many of the age-associated physiological decrements are thought to result in part from environmental insults, either acute or chronic. However, in some instances, there are relatively few supportive data. To add to difficulties, physical growth and senescence are both characterized by cumulative progression of interlocking biological events. They are not always separated because at some time in the life of the organism they may proceed as if they were in tandem.

Cellular Senescence in Perspective

Granted that death is the ultimate failure of the organisms to withstand the onslaughts of an inimical environment, what is it in the aging process itself that brings about the termination of the replicative ability of cells as the individual becomes progressively older? What is it in cells and organisms that weakens their resistance to the hostile exogenous forces? How is it that some cells and organisms are programmed to die even without the assault from adverse environmental threats?

Many in vitro studies have demonstrated that the age of any tissue is strongly reflected in the behavior of cultured skin-derived cells (4). Replicative senescence of human cells is thus related to and perhaps caused by the exhaustion of their proliferative potential. According to the telomere hypothesis, somatic cells lack sufficient amounts of activity of the enzyme

telomerase to maintain the telomeric repeats in the face of the end replication problem. With each round of cell division, mortal cells lose some of their telomeric repeats (5). Since telomere length predicts the replicative capacity of cells, it may provide the best biomarker for cellular aging.

Stress-induced premature senescence (SIPS) occurs following many different sublethal stresses such as those induced by H_2O_2, other reactive oxygen species (ROS), and a variety of chemicals (6). Cells engaged in replicative senescence share common features with cells affected by SIPS, including morphology, senescence-associated β-galactosidase activity, cell cycle regulation, gene expression, and telomere shortening (6). The latter process is attributed to the accumulation of DNA single-strand breaks induced by oxidative stress. According to the thermodynamic theory of aging, the exposure of cells to sublethal stresses of various natures can trigger SIPS, with possible modulations of this process by bioenergetics. Thus, SIPS could be a mechanism of the in vivo accumulation of senescent-like cells in the skin (7).

Cellular senescence and cancer are closely related by several biological aspects, including p53 mutation (5,8,9), telomere shortening (10), vitamin A depletion (11), and defects in intercellular communications (12). The age-related mottled subclinical melanoderma, even at a subclinical stage, might be a predictive sign for a carcinoma-prone condition (13–15).

Skin Aging: 1, 2, or 7 Mechanisms?

Conceptually, human aging is one single chronological process of physiological decline progressing with age. This basic process exhibits multiple facets affecting differently the organs, tissues, and cells. This is particularly true in the skin. Over the past decades, the understanding of aging skin has considerably expanded, with a welcome emphasis on differentiating the intrinsic chronological aging changes from photoaging (Table 2) resulting from habitual chronic sun exposure (16). According to this concept, the changes observed in the skin appearance as a result of aging reflect two main processes. Firstly, the intrinsic changes in the skin are caused by the passage of time modulated by hereditary factors, along with modifications occurring inherently in the structure, physiology, and mechanobiology. Secondly, photodamage is a result of the cumulative exposure of the skin to ultraviolet (UV) exposure. Clinically, these two types of aging are manifested differently, with intrinsic aging giving rise to smooth, dry, pale, and finely wrinkled skin, and photoaging giving rise to coarse, roughened, and deeply wrinkled skin accompanied by pigmentary changes such as solar lentigines and mottled pigmentation. Differences between these two types of aging can be seen within one individual when comparing an area of skin commonly exposed to the sun, for example, the face, the neck, and the dorsal forearms, with an area commonly masked from the sun, for example, buttock skin.

This concept that is based on a duality in skin aging has been challenged because it may appear as an oversimplification in clinical practice (17). Thus, another classification of skin aging in seven distinct types was offered (Table 3). The important variables included the endocrine and overall metabolic status, the past and present life style, and several environmental threats, including cumulative UV and infrared exposures, and repeated

Table 2 Comparison of Intrinsic Aging and Photoaging

Feature	Intrinsic aging	Photoaging
Clinical appearance	Smooth texture, unblemished surface, fine wrinkles, some deepening of skin surface markings, some loss of elasticity, redundant skin	Nodular, leathery surface sallow complexion, yellowish mottled pigmentation, coarse wrinkles, severe loss of elasticity
Epidermis	Thin and viable	Marked acanthosis, cellular atypia
Elastic tissue	Increased, but almost normal	Tremendous increase, degenerates into amorphous mass
Collagen	Bundles thick, disoriented	Marked decrease of bundles and fibers
Glycosaminoglycans	Slightly decreased	Markedly increased
Reticular dermis	Thinner, fibroblasts decreased, inactive mast cells decreased, no inflammation	Thickened, elastosis, fibroblasts increased, hyperactive mast cells markedly increased, mixed inflammatory infiltrate
Papillary dermis	No grenz zone	Solar elastosis with grenz zone,
Microvasculature	Moderate loss	Great loss, abnormal and telangiectatic

Table 3 Cutaneous Aging Types

Aging type	Determinant factor
Genetic	Genetic (premature aging syndromes, phototype related, ethnic background)
Chronologic	Time
Actinic	Ultraviolet and infrared irradiations
Behavioral	Tobacco, alcoholic abuse, drug addiction, facial expressions
Endocrinological	Pregnancy, physiological, and hormonal influences (ovaries, testes, thyroid)
Catabolic	Chronic intercurrent debilitating disease (infections, cancers), nutritional deficiencies
Gravitational	Earth gravity

Source: From Ref. 17.

mechanical solicitations by muscles and external forces such as earth gravity. In this framework, the past history of the subject is emphasized. Accordingly, the global aging is considered to represent the cumulative or synergistic effects of specific features, each of them being independent from the others. Such a concept allows to individualize or integrate typical processes, including, among others, menopausal aging and smoking effects. Increased awareness of the distinct age-associated physiological changes in the skin may allow for more effective and specific skin care regimens, preventive measures, and dermatological treatment strategies in the elderly. As a consequence, the immutability of skin aging can be challenged (2,18). However, factors of skin aging share some common mechanisms (19). For instance, molecular mechanisms imply hyaluronate-CD44 pathways in the control and maintenance of epithelial growth and the viscoelastic properties of the extracellular matrix that offer new opportunities for preventive intervention (1).

Environmental Aging and Photoaging

Environmental influences produce obvious alterations to the texture and quality of the skin, the major extrinsic insults being chronic exposure to UV radiation. The action spectrum of photodamages is not fully characterized. The cumulative effects from repeated exposures to suberythemal doses of UVB and UVA in human skin are involved in these processes (20). The role of UVB in elastin promoter activation in photoaging is obvious. UVA significantly contributes to long-term actinic damage, and the spectral dependence for cumulative damages does not parallel the erythemal spectrum for acute UV injury in human beings.

Both UVA and UVB initiate a number of cellular responses, including ROS production within both dermal and epidermal cells. More specifically, cultures of human keratinocytes derived from donors of different ages and from paired sun-exposed and sun-protected sites of older donors demonstrate that both chronological aging and photoaging affect gene expression although in a quite distinct manner. Chronological aging alone, strikingly increases the baseline expression of the differentiation-associated gene small proline-rich protein (SPR2) and of the interleukin (IL)-1 receptor antagonist (RA) gene. By contrast, it has relatively little effect on the UV-inducibility of several other genes, including the proto-oncogenes c-myc and c-fos, the GADD 153 a gene inducible by growth arrest and DNA damage, and the IL-1α and 1L-β genes. Photoaging is different because it increases the UV-inducibility of c-fos, but decreases the baseline expression of the differentiation-associated genes IL-1 RA and SPR2 (21,22). The physiological impact of photodamages occurs at variable pace on the different skin structures. For instance, skin loosening and solar elastosis show clinical manifestations independently from the severity in mottled melanoderma (14).

Photoaging has profound effects on both the epidermis and dermis. The epidermis becomes atrophic compared to sun-protected areas on the same individual, often with disordered keratinocyte maturation. Histological features of photoaged skin are most apparent in the dermis where the extracellular matrix (ECM) shows marked alterations in composition (23). The collagen network of the dermal ECM is responsible for skin strength and resiliency and is intimately involved in the expression of photoaging. The major fibrillar collagen components of the dermis are of the types I and III. In photoaged human skin, precursors of both proteins are significantly reduced in the papillary dermis, and their reduction correlates with clinical severity of photoaging (24). This reduction results from a combination of reduced procollagen biosynthesis and increased enzymatic breakdown by matrix metalloproteinases

(MMPs) (25). Collagen breakdown products within photoaged dermis can negatively influence procollagen biosynthesis by fibroblasts (26). Fibrillar collagens are closely associated with the small chondroitin sulfate proteoglycan and decorin. Its distribution closely mirrors that of type I collagen in the dermis, regardless of level of extrinsic aging (27). Decorin allows interaction between the fibrillar collagens and the microfibril-forming type VI collagen that further interacts with type IV collagen, an important component of the basement membrane at the dermal–epidermis junction. Type VI collagen therefore is likely to play an important physiological role in the organization of the dermal ECM. Type VI collagen is concentrated in the papillary dermis and it seems little affected by photoaging (28). Type VII collagen was reported to be involved in the mechanism of wrinkle formation. This collagen is the major constituent of anchoring fibrils below the basement membrane providing cohesiveness between the epidermis and dermis. In photoaged skin, the number of anchoring fibrils along the basement membrane is significantly reduced, thus increasing the potential for fragility and blistering in photoaged skin (29).

The elastic fiber network supplies recoil and elasticity to the skin. The process of elastic fiber formation is under tight developmental control, with tropoelastin deposited on a preformed framework made of fibrillin-rich microfibrils. Mature elastic fibers are also encased in fibrillin and form a continuous network throughout the dermis. The elastic fiber network comprises thick elastin-rich fibers within the reticular dermis, a network of finer fibers with reduced elastin in the lower papillary dermis, and cascades of discrete fibrillin-rich microfibrillar bundles, with only discrete elastin, in the upper papillary dermis merging with the dermo-epidermal junction. Fibrillin is both a product of dermal fibroblasts and keratinocytes. The elastic fiber network shows considerable disruption in chronically photoaged skin. Firstly, photoaged skin contains abundant amounts of dystrophic elastotic material in the reticular dermis (30), which is immunopositive for tropoelastin, fibrillin, lysozyme, and immunoglobulins (31) Versican, a large chondroitin sulfate proteoglycan, appears to be regulated along with dystrophic elastin, resulting in a relative increase in photoaging. Although immunohistochemically identifiable fibrillin is present following actinic damage, the architecture and fibrillin-rich microfibrils are markedly altered. Minimally photoaged skin shows a similar marked loss of fibrillin-positive structures, implying that remodeling of the fibrillin-rich microfibrillar network is an early marker of photoaging (32).

All structural changes found in the dermis particularly affect the biomechanical properties of the skin. A cutaneous extrinsic aging score was derived from the difference between comparative photoexposed and photoprotected areas (33).

Phototype and Ethnic Aging

People of colored skin comprise the majority of the world population, and Asian subjects comprise more than half of the total earth population. The most obvious ethnic skin difference relates to skin color, which is dominated by the presence of melanin (34,35). The photoprotection derived from this polymer influences the rate of the skin-aging changes between the different racial groups. However, all racial groups are eventually subjected to the photoaging process. Generally, Caucasians have an earlier onset and greater skin wrinkling and sagging signs than other skin types, and in general, increased pigmentary problems are seen in colored skin, although East Asians, living in Europe and North America, have less pigment spots. Induction of a hyperpigmentary response is thought to be through signaling by the protease-activated receptor-2, which together with its activating protease, is increased in the epidermis of subjects with colored skin (36). Changes in skin biophysical properties with age demonstrate that the more darkly pigmented subjects retain younger skin properties compared with the more lightly pigmented groups.

Endocrine Aging

Irrespective of age, most of the skin components are under the physiological control of endocrine and neuroendocrine factors (Table 4). As such, skin is recognized as a hormone-dependent organ (37–39). Like any other system in the body, the aging process affecting the hormonal functions basically results in deteriorations expressed by hormone deficiencies, which in turn can influence the aging machinery operative in the skin (39). Quite distinct are the skin manifestations of some endocrinopathies, which may mimick or interfere with skin aging (37–39). All endocrine glands are affected by the global aging process. A few direct

Table 4 Neuroendocrine Receptors Active in the Skin

1. Adrenergic receptors
2. Androgen and estrogen receptors
3. Calcitonin gene-related peptide receptor
4. Cholinergic receptors
5. Corticotropin-releasing hormone and urocortin receptors
6. Glucocorticoid and mineralocorticoid receptors
7. Glutamate receptors
8. Growth hormone receptor
9. Histamine receptors
10. Melanocortin receptors
11. Miscellaneous neuropeptide receptors
12. Miscellaneous receptors
13. Neurokinin receptors
14. Neutrophin receptors
15. Opioid receptors
16. Parathormone and PTH-related protein receptors
17. PRL and LH-CG receptors
18. Serotonin receptors
19. Thyroid hormone receptors
20. Vasoactive intestinal peptide receptor
21. Vitamin D receptor

Abbreviations: CGRP-R, calcitonin gene-related peptide receptor; CRH-R, corticotropin-releasing hormone and urocortin receptors; GH-R, growth hormone receptor; MC-R, melanocortin receptors; NK-R, neurokinin receptors; NT-R, neutrophin receptors; PTH, parathormone; PTHrP, PTH-related protein receptors; LH/CG-R, PRL and LH-CG receptors; VIP-R, vasoactive intestinal peptide receptor; VDR, vitamin D receptor.
Source: From Ref. 39.

Table 5 Hormones and Neurotransmitters Produced by the Skin

1. Hypothalamic and pituitary hormones
2. Neuropeptides and neurotrophins
3. Neurotransmitters/neurohormones
4. Other steroid hormones
5. Parathormone-related protein
6. Sex steroid hormones
7. Thyroid hormones

Source: From Ref. 39.

consequences interfere with skin aging. They are mostly related to the declined activity of the pituitary gland, adrenal glands, ovaries, and testes.

Some hormones and neurotransmitters are synthesized by nerves, as well as by epithelial and dermal cells in the skin (Table 5). A number of environmental and intrinsic factors regulate the level of cutaneous neuroendocrine system activity. Solar radiation, temperature, humidity, as well as diverse chemicals and biological xenobiotics represent important environmental factors. Some internal mechanisms affecting the neuroendocrine system of the skin may be generated in reaction to some environmental signals or result from local biological rhythms or from local or general disease processes (37).

The paradigm of deleterious hormonal effects is presented by the influence of corticosteroids on skin atrophy. Cushing syndrome and iatrogenic effects of topical and systemic corticotherapy can equally be involved. Corticosteroids are known to regulate the expression of genes encoding collagens I,III,IV,V, decorin, elastin, MMPs 1,2,3, tenascin, and tissue inhibitors of MMPs 1 and 2 (40). However, the exact molecular mechanisms of skin atrophy induced by corticosteroids are not yet known. The corticosteroid-induced atrophy can be one of the most severe forms of skin aging corresponding to dermatoporosis (1).

The most important endocrine compound produced by the skin is vitamin D, which is a regulator of the calcium metabolism and exhibits other systemic effects as well. For example,

epidemiological evidence suggests that sunlight deprivation with associated reduction in the circulating level of vitamin D_3 may result in increased incidence of carcinomas of the breast, colon, and prostate (41). Vitamin D_3 and its analogues also modulate the biology of keratinocytes and melanocytes of the skin in vivo (42).

Growth hormone (GH) is secreted by the pituitary gland under the control of several hypothalamic and peripheral modulators that exert either positive or negative influences. The final balance among the modulating factors determines the pulsatile and circadian secretion of GH. Moreover, physiological changes occurring in particular conditions (i.e., puberty, pregnancy, aging, and severe acute illness) affect the GH secretion. The peripheral effects of GH are mainly exerted by insulin-like growth factor (IGF), produced by the liver upon GH stimulation. The circulating IGF-1 is bioavailable and functionally active depending upon its binding with the IGF-binding proteins (IGF-BPs).

Skin is a target of the GH-IGF system, which exerts a significant influence on the dermal and epidermal physiology (43). GH, IGF-1, IGF-2, and IGF-BPs are present in the skin and are involved in its physiological homeostasis, including the dermo-epidermal cross talking. Thus, not only systemic but also paracrine and/or autocrine cutaneous activity of the GH-IGF system contributes to skin homeostasis (43,44). GH supplementation induces skin changes, a part of which may correspond to some corrective effects on aging skin (45,46).

The progressive decline in dehydroepiandrosterone (DHEA) serum concentration with age, and conversely its supplementation have not demonstrated prominent effects on the skin except on sebum production.

Sex hormones manifest a variety of biological and immunological effects in the skin (47). Estrogen, alone or together with progesterone, prevents or reverses skin atrophy, dryness, and wrinkles associated with chronological aging or photoaging. Estrogen and progesterone stimulate proliferation of keratinocytes while estrogen suppresses apoptosis and thus prevents epidermal atrophy. Estrogen also enhances collagen synthesis, and estrogen and progesterone suppress collagenolysis by reducing MMP activity in fibroblasts, thereby maintaining skin thickness. Estrogen maintains skin moisture by increasing hyaluronic acid levels in the dermis; progesterone increases sebum excretion.

Both the climacteric period following menopause and the andropause decade may negatively affect the skin (47–49). Hormone replacement therapy (HRT), during the climacteric period, helps limitating these changes (50–53). However, there is a limitation because good and poor responders seem to exist (54). Smoking habit may also interfere with the treatment result (55).

Catabolic Aging

The elderly often exist on a substandard diet deficiency in many of the nutrients thought to be essential to maintain health. Protein-containing foods such as meat and fish tend to be too expensive or troublesome to prepare. Dietary faddism, confusional states, and forgetfulness are also responsible for an inadequate diet. These situations predispose skin changes that often amplify the alterations induced by age-related hormone deficiency.

Insufficient fresh fruit and/or vegetables give rise to vitamin C deficiency leading to scurvy. In this disorder, there is a defect in coagulation resulting in purpura, particularly in a punctate perifollicular pattern on the legs. In the elderly, iron deficiency is also common and may result in anemia, generalized pruritus, and some diffuse hair loss.

Essential fatty acid and vitamin A deficiencies because of dietary faddism or deprivation in the elderly cause xerosis (56). Many of the elderly are also deficient in zinc, and it has been suggested that this may be an important factor in preventing wound healing. Zinc supplementation, however, does not improve healing.

Chronic hemodialysis is another example of catabolic aging affecting the mechanical properties of skin (57,58).

Gravitational Aging

Skin of any part of the body is subjected to intrinsic and extrinsic mechanical forces. Among them, earth gravitation is important by influencing skin folding during aging. Any force generated by the skin or applied to it transduces information to cells that may in turn respond to it (59,60). The effects of mechanobiology may particularly be evidenced in the fibroblasts, dermal dendrocytes, keratinocytes, and melanocytes (61–63). Physical forces of gravity involve

mechanotransduction in the skin (64) and affect cell tensegrity and the cell mechanosensitive ion channels. As a result, the structure of the dermal extracellular matrix is affected.

The Case of Wrinkles

There is evidence that wrinkles are not related to the genuine microrelief (65,66). In addition, the microanatomical supports of wrinkles are varied (65–67). They depend on subtle changes in the structure of the superficial dermis elastotic deposits in the upper reticular dermis, loosening of the hypodermal connective tissue strands, or, universely, on hypertrophic binding of the dermis to the underlying facial muscles (66–68). The wrinkle severity rating (69) is influenced by the nature of the altered connective tissue. Similarly, the skin mechanical properties are under these influences (70,71).

Photoaged facial skin does not always present clinically with characteristic wrinkling. In some individuals, usually of light phototype, smooth unwrinkled skin and telangiectasia predominate. These people appear to be more at risk of developing basal cell carcinomas (BCC) on sun-exposed facial skin (72,73). Recent work has confirmed that there is an apparent inverse relationship between the degree of facial wrinkling and the occurrence of facial BCCs. Mechanistically, little is known regarding how these two clinical outcomes occur in response to the same environmental stimulus, namely sun exposure, but it appears that facial wrinkling may, to some extent, preclude the occurrence of BCCs in sun-exposed sites (73).

Smoking is an additional cause of wrinkling (74). Degeneration of elastic fibers by ROS and the repeated mechanical solicitations by some muscle contractions play a putative role in the formation of the smoker's wrinkles.

CONCLUSIONS

Aging is apparent at all levels of the physiology and anatomy of the body. Organs, tissues, cells, and molecules have their own aging processes that differ in their clinical relevance. The individual may perceive a global appearance of skin aging. By contrast, prevention and correction of skin aging may benefit from targeting some of the specific underlying biological processes.

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23 A Quantitative Approach to Age and Skin Structure and Function: Protein, Glycosaminoglycan, Water, and Lipid Content and Structure

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INTRODUCTION

The anatomic facets of skin are infinite, making a complete review of age-related changes in skin structure problematic. This overview, therefore, focuses on certain readily quantifiable aspects of the skin: protein, glycosaminoglycan (GAG), water, and lipid content and structure. Where possible, we address differences between intrinsic aging, physiologic aging, and extrinsic aging due to photoexposure, wind, relative humidity, and other environmental factors, although we acknowledge that this distinction is not always easily made. Ultimately, we hope to unify each topic and the general understanding of skin structure with respect to aging. This chapter reviews each category of structure and function in turn, beginning with a brief description of commonly used quantitative methods of study. Each section includes a table presenting research data, a discussion of the experimental outcomes and, wherever possible, an evidence-based summary of the effect of age on the parameter discussed.

TECHNIQUES

One quantitative method of protein study involves measurement of racemized aspartic acid in skin protein. This racemization represents a major type of nonenzymatic covalent modification of proteins initially synthesized using only L-amino acids. Aspartic acid racemization (AAR) leads to an age-dependent accumulation of D-aspartic acid in more long-lived human proteins (1). The study of AAR in total skin yields data mainly representative of collagen, which comprises the majority of dermal protein and has 10 to 20 times more aspartate residues per unit mass than elastin. However, racemization in collagen is slow because of conformational constraints of the triple helix (1). Racemization occurs more quickly in elastin. Skin samples can be purified such that, if indicated, the AAR due to elastin alone can be studied and its longevity measured (1). Elastin in various tissues including the aorta and lungs reveals high levels of AAR. This indicates a lack of turnover and accumulation of elastin damage in diverse aging tissues, possibly as part of programmed aging (1).

Raman spectroscopy is a nondestructive analytical method for determining the structure and conformation of molecular compounds. Although these results are not quantitative, per se, they are highly informative, as they do not require sample preparation or pretreatment and thus eliminate much potential interference. Recently, near-infrared Fourier transform (NIR-FT) Raman spectroscopy has emerged as being specially suited for the investigations of biologic material (2). NIR-FT Raman spectroscopy exploits an effect wherein a small amount of monochromatic light scattered by a substance has a frequency that differs from that of the incoming beam. This frequency difference represents the vibration frequency of the chemical bonds in the structure being analyzed. Frequency shifts can be analyzed and presented as spectra, with bands characteristic for chemical bonds in the examined molecules (2). This method gives highly reproducible results with only minor differences seen in spectra of different skin types (2).

PROTEINS

Collagen

Table 1 summarizes data pertaining to skin collagen. Collagen, which comprises approximately 70% to 80% of the dry weight of the dermis, is primarily responsible for the skin's tensile strength. Each collagen molecule consists of three polypeptide chains, each containing about 1000 amino acids in their primary sequence. In the collagen molecule, the α-chains are wrapped around each other to make a triple-helical conformation (3). In chronologically aged skin, the rate of collagen synthesis, activity of enzymes that act in the posttranslational modification, collagen solubility, and thickness of collagen fiber bundles in the skin all decrease (4,5). Also, the ratio of type III to type I collagen increases with increasing age (4,6). In photoaged skin, however, collagen fibers are fragmented, thickened, and more soluble (4). The increased fragmentation of collagen, especially in photoaged skin, is secondary to upregulation of collagen-degrading matrix metalloproteinases (MMPs) by UV radiation (7,8). In addition to the acute upregulation of these enzymes by UV exposure, the same MMP enzymes are also gradually increased during chronological aging (9). Aside from increased degradation and fragmentation, chronologically aged skin exhibits decreased fibroblast function and decreased mechanical stimulation, resulting in reduced collagen synthesis and replacement (10). It is plausible that both reduced collagen deposition in elderly skin and enhanced degradation of collagen in photoaged skin could explain the development of dermal atrophy and might relate to poor wound healing in the elderly.

Histological data, though not quantitative, reveal important information about orientation and arrangement of collagen fibers in skin. Lavker et al. compared skin from the upper inner arm of old (age 70–85) and young (age 19–25) individuals using light, transmission electron, and scanning electron microscopy (14). Interestingly, they suggested that the upper inner arm might be an optimal site for analyzing sun-protected skin, as it is not exposed to the pressure deformations and reformations occurring in the buttock. They found that in young adults, collagen in the papillary dermis forms a meshwork of randomly oriented thin fibers and small bundles. The reticular dermis consists of loosely interwoven, large, wavy, randomly oriented collagen bundles. However, the collagen within each bundle is packed together closely (14). In aged skin, the density of the collagen network appears to increase. This likely reflects a decrease in ground substance that would otherwise form spaces between the collagen fibers (14). Also, rather than appearing in discrete rope-like bundles of tightly packed fibers, collagen forms aggregates of loosely woven, mostly straight fibers. As fibers become straighter in aged skin, there is less room for the skin to be stretched, so tensile strength increases (14). Using immunoelectron microscopy, Vitellaro-Zuccarello et al. found similar age-related trends in skin collagen. They also noted greater intensity of collagen III staining in subjects older than 70 years (15). Hence histological and more recent methods are in agreement, revealing that increased age is associated with decreased collagen content and straightening of collagen fibers forming looser bundles, an increased type III-type I collagen ratio, and decreased ground substance.

From a biochemical standpoint, chronological aging induces increased markers of oxidation, glycoxidation, lipoxidation, and glycation in skin collagen (13). In particular, skin collagen's cross-linking lysine residues undergo significant oxidative changes with age. Lysine oxidase, a copper-dependent enzyme, converts lysine to allysine at all ages. Recently it has been shown that allysine is further oxidized to a stable end product, 2-aminoadipic acid. This oxidative change results in significant accumulation of 2-aminoadipic acid in collagen of aged skin; increased oxidative end product is also seen in diabetes, renal failure, and sepsis (13).

Elastin

Table 2 summarizes skin elastin data. The skin's intact elastic fiber network, which occupies approximately 2% to 4% of the dermis by volume, provides resilience and suppleness. This network shows definite changes associated with aging, especially between the ages of 30 and 70. In sun-exposed skin, an excessive accumulation of elastoic material occurs. Accumulation of new elastin in response to photoaging is also apparent from upregulation of the elastin promoter activity and increased abundance of elastin mRNA (1,16). Bernstein et al. compared photoaged skin with intrinsically aged skin, and found a 2.6-fold increase in elastin mRNA, a

(*text continued on page 250.*)

Table 1 Data: collagen

Source	Methods	Results	Notes
Shuster et al. 1975 (11).	Caucasian males: 74; Caucasian females: 80, aged 15–93 yr: biopsies were taken from the midpoint of extensor aspect of forearm using high-speed 5-mm punch. Some postmortem samples were included. Samples were defatted in acetone, dried to constant weight, and hydrolyzed; their hydroxyproline content was measured.	Linear decrease in absolute collagen content with age, 1% per yr. Collagen density decreases with age ($p < 0.001$). A significant relationship is noted between skin thickness and collagen content for all males ($p < 0.001$) and for females > 60 yr ($p < 0.001$).	Collagen decreases with age. This method may be subject to preparation artifacts.
Gniadecka et al. 1998. (12).	Study of Caucasians included 10 people aged 74–87 yr and 10 people aged 22–29 yr. Obtained Raman spectra from buttock skin and forearm skin, using NIR-Raman spectroscopy.	Photoaged skin: collagen fibers are fragmented, thickened, and more soluble, elastin fibers form conglomerates, and amount of GAG increases. Chronologically aged skin: changes are more subtle. Despite an overall increase in the number of collagen fibers, these are thinner and less soluble. Also there is a relative increase with age in the collagen III:collagen I ratio.	Raman spectroscopy allows a detailed analysis without preparation artifacts. The information here agrees with many of the other studies represented in this table.
Lovell et al. 1987 (6).	Strips of abdominal skin obtained at laparotomy or postmortem from 30 subjects aged 0–90 yr. Some samples were cut and acid hydrolyzed; hydroxyproline content was determined using an automated amino acid analyzer and the total collagen calculated from hydroxyproline estimations. Collagen content was calculated both per unit weight of freeze-dried skin and per unit surface area. Other skin samples were digested with CNBr, and type I:type III ratios were calculated using SDS polyacrylamide gel electrophoresis. Pepsin digestion and HPLC separation of denatured α-chains were also used to calculate type I:type III ratios. Indirect immunofluorescence enabled analysis of frozen skin samples antibody-labeled for types I, II, IV, and V collagens.	Hydroxyproline content and estimates of total collagen content did not vary significantly with age. SDS gel electrophoresis showed type III collagen content of skin samples from two young donors (age 5 yr) was 20–23%. In people aged 14–65 yr, measurements were relatively constant; showed content of type III collagen to be 18–21.5%. In people older than 65 yr, there was greater variation; levels of type III were increased and were as high as 31%. The HPLC method was less conclusive because pepsin digestion and separation of collagen component chains were incomplete, especially in skin samples from older individuals. Immunofluorescence data showed no gross changes in the distribution of various collagen types during aging.	Electron microscopy measurements represented a small study population and are therefore somewhat questionable ($n = 5$). Hydroxyproline approximations of collagen content appear more reliable, as they include 29 subjects, dispersed between age 3 mo and 82 yr; however, this still reflects a small number of subjects in any given age range. The shift in collagen type with aging prompts the question of whether this was due to increased type III synthesis or decreased type I synthesis. This study does not provide an answer.

(*Continued*)

Table 1 Data: collagen (*Continued*)

Source	Methods	Results	Notes
	Scanning electron microscopy was used to study the diameters of bundles of collagen fibers.	Scanning electron microscopy: decrease in number of collagen fiber bundles per unit area in the papillary area with increasing age. The oldest subject (82 yr) had reduced bundle width ($p < 0.0001$) compared with 4 other subjects aged 15–58 yr.	
Fisher et al. 1996 (7).	Adult buttock skin (number of subjects not clarified) was irradiated with 2 MED UVB in vivo (twice the dose required to cause barely perceptible reddening). Irradiated and adjacent nonirradiated sites were removed at various times following irradiation and snap frozen. RNA contents were then analyzed using northern blot. Band intensities were quantified using a PhosphorImager. Protein content in skin was quantified using Western blot. Nuclear extracts from irradiated and nonirradiated skin were also temporally analyzed for AP-1 and NF-κ B binding to double-stranded DNA probes by electrophoretic mobility shift assays.	Transcription factors NF-κ B and AP-1 showed 2.5–3-fold increased binding to DNA within 15 min of irradiation, lasting up to 4 hr after irradiation. Induction of interstitial collagenase, streptomelysin 1, and 92 kDa gelatinase mRNAs were maximal (6–60-fold, $p < 0.05$) at 16–24 hr, and returned to near baseline within 48–72 hr. Gelatinase mRNA of 72 kDa was detectable but was only elevated 1.6-fold at 24 hr post UVB exposure. Confirmation that mRNA rises corresponded with actual collagenase protein rises was done using Western blot.	Within minutes of UVB exposure, transcription factors AP-1 and NF-κ B showed increased binding to DNA, stimulating synthesis of various collagenase mRNAs, which resulted in increased synthesis of collagenase proteins. Authors provide direct, in vivo mechanistic evidence of UVB exposure resulting in increased collagenases in skin.
Fisher et al. 1997 (8).	Caucasian adults underwent irradiation of skin at 4 separate buttock sites, with each site exposed 1, 2, 3, or 4 times to radiation delivered at 48-hr intervals at ½ MED. Skin specimens were obtained from irradiated and adjacent nonirradiated sites at 24 hr after the last exposure.	After a single exposure to ultraviolet irradiation, collagenase and 92-kDa gelatinase activity were elevated 4.4 ± 0.2 times the value in nonirradiated skin and 2.3 ± 0.4 times, respectively. Collagenase and gelatinase activity remained maximally elevated after the 2nd, 3rd, and 4th exposures on days 3, 5, and 7, respectively	Repeated UV exposure leads to sustained induction of the MMPs.
Varani et al. 2000 (9).	Seventy-two subjects provided skin samples from sun-protected areas; age groups compared were 18–29 yr, 30–59 yr, 60–79 yr, and 80+. Levels of MMP-1 were assessed using Western blot, and levels of MMP-9 and MMP-2 were assessed using gelatin zymography with scanning laser densitometry for quantitation. Levels of type 1 and 3 pro-collagen were measured using Western blot.	In the 80+ yr group compared with the 18–29 yr group, there was a 40%, 52%, and 82% increase in MMPs 1, 9, and 2, respectively, ($p < 0.01$, 0.05, and 0.001). In the 60–79 yr group compared with the 18–29 yr group, there was a 23%, 20%, and 44% increase in MMPs 1, 9, and 2, respectively, ($p < 0.05$, NS, and $p < 0.05$, respectively). There was a 52% decrease in type 1 pro-collagen expression in 80+ yr-olds versus 18–29 yr-olds ($p = 0.022$).	Chronologic aging results in elevated expression of MMPs and decreased expression of types 1 and 3 pro-collagen.

Varani et al. 2006 (10).	Young (18–29 yr) versus old (80+ yr) subjects participated. Replicate 2- and/or 4-mm punch biopsies of sun-protected hip skin were obtained from each individual. For fluorescence microscopic and ultrastructural analysis and for the assessment of type 1 pro-collagen levels, 4-mm punches were used and 2-mm biopsies were used for routine light microscopy and for isolation of dermal fibroblasts in culture.	Fibroblasts from young skin produced greater type 1 pro-collagen than those from old skin (82 ± 16 versus 56 ± 8 ng/mL, $p < 0.05$) A reduction in mechanical stimulation in chronologically aged skin was inferred from greater percentage of cell surface attached to collagen fibers (78 ± 6 versus $56 \pm 8\%$, $p < 0.01$) and more extensive cell spreading (1.0 ± 0.3 versus 0.5 ± 0.3, $p < 0.05$) in young versus old skin.	Authors hypothesize that old fibroblasts have an age-dependent reduction in the capacity for collagen synthesis while simultaneously experiencing a loss in mechanical stimulation resulting from fewer intact collagen fibers.
Sell et al. 2007 (13).	Human skin samples from 117 people, aged 10–90 yr, were obtained at autopsy. Amounts of 2-aminoadipic acid and 6-hydroxynorleucine (a marker of allysine) in the collagen were determined in acid hydrosylates of processed samples using ion-monitoring gas chromatography. Quantitative contents of 2-aminoadipic acid and 6-hydroxynorleucine were compared in young and old subjects as well as in those with histories of diabetes, renal failure, and sepsis.	2-aminoadipic acid ($p < 0.0001$), but not 6-hydroxynorleucine ($p = 0.14$) significantly increased with age, reaching levels of 1 and 0.3 mmol/mol lysine at late age (mean 82 yr), respectively. Significant increases in 2-aminoadipic acid, but not 6-hydroxynorleucine, were also seen in patients with diabetes ($p < 0.0001$, levels of 2-aminoadipic acid up to < 3 mmol/mol), renal failure (levels of 2-aminoadipic acid up to 8 mmol/mol), and especially sepsis ($p = 0.0001$).	2-aminoadipic acid, a pan-marker for all forms of lysine oxidation, significantly increased in aging human skin. Levels of its precursor, allysine, are in steady state, suggesting ongoing oxidation of allysine to form the stable end product, 2-aminoadipic acid.

Abbreviations: CNBr, Cyanogen bromide; SDS, sodium dodecyl sulfate; HPLC, high performance liquid chromatography; MED, Minimal erythemogenic dose; AAR, aspartic acid racemization; GAGs, glycosaminoglycans; MMPs, matrix metalloproteinases.

Table 2 Data: Elastin

Robert et al. 1988 (19).	Analyzed 6-mm punch biopsies of skin from buttock and upper inner arm of 50 individuals (40 males, 10 females). Used a specific elastic staining procedure, then automated computerized image analysis. Calculated percent of surface area covered by elastic fibers, length, and number of elastic fibers per unit surface area in superficial (papillary) and deep (reticular) dermis as a function of age.	Percentage surface area coverage by elastic fibers increased with age in superficial and deep dermis: Males: superficial dermis, $r = 0.66$; $p < 0.001$. Deep dermis, $r = 0.56$, $p < 0.01$. Similar correlation in females did not reach significance because of small sample size. Mean fiber length also increased with age in the superficial and deep dermis: $r = 0.036$, $p < 0.02$. Number of elastic fibers per unit surface area showed no significant change with age in either the superficial or deep dermis.	Continuous increase with age in the length and relative surface area of elastic fibers. This appears to contradict these authors' rheological studies on the same patients that show a continuous decrease in skin elasticity with age (19). The authors attribute this to the possibility of continuous enrichment with age in polar amino acids, carbohydrates, lipids, and calcium of the skin elastic fibers. These structural changes in elastic fibers may interfere with their proper functioning.
Ritz-Timme et al. 2003 (1).	Skin samples measuring 2 × 2 cm were taken from ventral abdomen during autopsy. Paraffin sections of all samples were examined histologically. Then, elastin was purified and AAR was quantified. Specific ages are not given.	In total skin, specimens displayed slight age-dependent increases in D-aspartyl residues; in purified elastin the rate of increase was rapid and highly correlated with age ($r = 0.98$).	This paper goes into great detail, which is beyond the scope of this overview, but does provide interesting insight into elastin degradation.
Ritz-Timme et al. 2002 (21).	Review paper.	Accumulation of new elastin in response to photoaging can be seen from upregulation of the elastin promoter activity and increased abundance of elastin mRNA. However, de novo synthesis of elastin in adult tissues is ineffective.	This paper provides a review of AAR and its role in skin aging.

Bernstein et al. 1994 (16).	Sixteen males aged 49–66 yr. Punch biopsies measuring 4 mm taken from the sun-damaged neck and photoprotected buttock. Studied samples using northern blot analyses, transient transfections with a human elastin promoter/reporter gene, and immunohistochemical staining with elastin and fibrillin antibodies. Analyzed samples in pairs to determine effects of photoaging.	Northern analysis of frozen sections: up to a 2.6-fold increase in elastin mRNA in exposed versus nonexposed skin. Analysis of mRNA from fibroblast cultures: 5.3-fold increase in elastin expression and 2.5-fold increase in fibrillin expression in photodamaged skin. Transient transfection of cultured cells revealed 5-fold increase in elastin promoter activity. Score for elastin staining in superficial dermis, protected skin: 0.62 ± 0.52; exposed skin: 5.0 ± 0.76. ($n = 8$, $p < 0.000001$). Score for fibrillin staining in superficial dermis, protected skin: 0.75 ± 0.46; photoaged skin: 3.1 ± 0.64 ($p < 0.0001$).	This study does not include a very broad age range. Also, since it used several different methods to establish mechanistic details, each method had a very small sample size (3 in Northern blot analysis of total RNA from frozen sections, 3 in Northern blot analysis of fibroblast culture, 2 for transient transfection method, and 8 for immunohistostaining method). The increase in elastin promoter activity and mRNA do not account for the degree of accumulation of elastoic material seen histologically in superficial and middermis of photoaged skin. It is suggested that most of the material staining as elastin in photoaged skin is structurally abnormal. Authors propose that elastin degradation may be slower than production, with accumulation of partially degraded elastic fibers.
Seite et al. 2006 (17).	Ninety-one skin biopsies taken from unexposed (buttock area) skin, ages 21–80 yr, 30 specimens from semiexposed (forearm skin), ages 22–64 yr, and 24 specimens from severe exposure (facial skin), ages 45–65 yr. UV exposure's (280–400 nm) influence on lysozyme deposition measured using 122 samples from buttock skin, ages 20–40 yr. Measurement of elastin and lysozmye via direct immunofluorescence with computer-aided quantitation.	Reduced elastin content with age in buttock skin (groups aged 61–80 yr had significantly less elastin than groups aged 21–50 yr). Relative amounts of elastin in the face of subjects aged 51–70 yr were abnormally high compared with buttock and forearm skin of those age groups. UVA (320–400 nm), especially long-wave UVA (340–400 nm), induces lysozyme deposition in elastin fibers to a significantly greater extent than simulated solar radiation (280–400 nm). 25% of elastin fibers in buttock skin were covered with lysozyme, compared to 66% of elastin fibers in facial skin, supporting the association of lysozyme with solar elastosis. Lysozyme was shown to inhibit degradation of elastin fibers by human leukocyte esterase.	Authors use an ample sample size and explain their methodology thoroughly to reveal mechanistic explanation for solar elastosis based on increased deposition of lysozyme in sun-exposed skin; lysozyme inhibits human leukocyte esterase to prevent proper degradation of elastin and allow accumulation of partially degraded fibers. The lower elastin content in sun-protected, older skin compared with sun-protected younger skin implies that human leukocyte esterase naturally works uninhibited to reduce elastin content with age; however, the UV-induced accumulation of lysozyme inhibits elastin's degradation with age in sun-exposed skin.

Abbreviations: AAR, aspartic acid racemization; UV, ultraviolet.

5.3-fold increase in elastin expression, and a 5-fold increase in elastin promoter activity in photodamaged skin (16). However, these apparent increases in elastin synthesis do not account for the massive accumulation of elastoic material seen histologically in photoaged skin (16). Some attribute this to elastin degradation being slower than synthesis, leading to an accumulation of partially degraded fibers. Recent work has revealed that proteins such as elafin and lysozyme, expression of which is induced by UVA radiation, prevent elastin degradation by human leukocyte (neutrophil) elastase (17,18). In purified skin elastin, the amount of racemized aspartic acid increases rapidly and is highly correlated with age ($r = 98$) (1). This indicates that skin's elastin, like elastin in the aorta and lung, is long-lived and accumulates damage over time (19,20).

In innate aging, fragmentation of elastic fibers results in decreased number and diameter. Computerized image analysis of elastin-stained skin biopsies from the buttock and upper inner arm reveals an age-related increase in mean elastin fiber length and percentage surface area coverage in the dermis, but these fibers are thought to be abnormally enriched in polar amino acids, carbohydrates, lipids, and calcium (19). Through different mechanisms, photoaging and intrinsic aging ultimately result in a deficiency of functional, structurally intact elastic fibers (5). The finer oxytalan fibers in the papillary dermis are depleted or lost altogether; eluanic and elastic fibers become progressively abnormal. These alterations largely account for the widely recognized decrease in the skin's physiological elasticity with increased age (19).

Examination of intrinsically aged skin elastin and fibrillin with immunohistochemical staining revealed that elastin was located in the papillary dermis just below the basement membrane, as small fibers mostly oriented perpendicular to the epidermis. In the deeper dermis, fibers were thicker and oriented differently. Areas surrounding adnexal structures and larger vessels in the deep dermis were also intensely stained (16). Photoaged skin demonstrated similar small-diameter fibers just below the basement membrane within a zone lacking excessive staining, which was of variable thickness (16). This may correspond to the SLEB (subepidermal low echogenic band) seen in ultrasound imaging. Beneath this area of relatively sparse staining was a region of poorly formed, clumped, thick fibers. This staining pattern occupied the superficial to middermis, below which staining again resumed its well-defined pattern as seen in sun-protected skin (16).

Elastin therefore exhibits numerous age-related changes, including slow degradation and accumulation of damage in existing elastin with intrinsic aging, increased synthesis of apparently abnormal elastin in photoexposed areas, and abnormal localization of elastin in the upper dermis of photodamaged skin. These factors lead to the histologically evident elastoic accumulation and contribute to characteristic changes in ultrasound images of aged skin.

General Protein Structure

Table 3 summarizes data pertaining to other facets of skin protein structure. Through Raman spectroscopy, little difference is seen between photoexposed and protected areas in young individuals; the majority of proteins in young skin are in helical conformation. Intrinsically aged skin shows slightly altered protein structure, and photoaged skin reveals markedly altered protein conformation, with increased folding and less exposure of aliphatic residues to water (2,12). Amino acid composition of proteins and free amino acids in aged skin also differ significantly from those of young skin, including an increase in overall hydrophobicity of amino acid fractions from the elderly (22). Since free amino acids are believed to play a key role in stratum corneum (SC) water binding, this shift in their composition, combined with the evidence of altered tertiary protein structure, provides insight into the increased incidence of xerosis in aged individuals.

Aside from protein structure, the level of expression as well as the spatial distribution of certain proteins in the skin appear to change with intrinsic aging and photoaging. For example, in normal skin, the extracellular matrix protein 1 (ECM1) is mainly expressed in the basal cell layers of the epidermis and in dermal vessels. The protein's expression is increased throughout the epidermis of photoaged skin but significantly reduced in the basal and upper epidermal layers of intrinsically aged, UV-protected skin (23). Acute exposure to UV radiation also induces increased expression of the protein throughout the epidermis in healthy young skin (23). Hence, UV-related stress may also influence distribution and expression of various proteins involved in maintaining skin structure.

Table 3 Data: Generalized Protein Structure

Source	Method	Results	Notes
Gniadecka et al. 1998 (12).	Study of Caucasians included 10 people aged 74–87 yr and 10 people aged 22–29 yr. Obtained Raman spectra from buttock skin and forearm skin, using NIR-Raman spectroscopy.	Young group: Little difference in spectra of photoexposed versus photoprotected sites. Most skin proteins were in a helical conformation. Older group: Intrinsically aged (buttock) skin: Resembled young dorsal forearm or buttock, except for a significant ($p = 0.008$) shift of amide I peak position toward lower frequencies in older skin, suggesting minor conformational changes of protein structure. Photoaged (dorsal forearm) skin: In addition to the amide I band, the amide III band was also significantly shifted to lower frequency compared with aged photoprotected and younger skin spectra. Also, decreased intensity of the amide III band indicated severe conformational changes in protein in structure, with an increase in protein folding and less exposure of aliphatic amino acids to surrounding water.	In young skin, most proteins are in helical structure and we do not see much difference between sun-exposed and sun-protected regions. Chronologically aged skin has proteins in slightly altered conformation. Photoaged skin has proteins in markedly altered conformation, with increased folding and less exposure of aliphatic residues to water. This enables the proteins in photoaged skin to bind less water.
Jacobson et al. 1990 (22).	Amino acid composition was quantified in 3 fractions isolated from scales of SC from the lower leg. The three fractions studied were free amino acids (FAA), soluble hydrolysate (SH) and whole-cell hydrolysate (WCH). "Old" subjects ($n = 20$) were 60 yr or older; "young" subjects ($n = 20$) were 30 yr or younger.	In normal subjects, each of the 3 fractions showed significant difference ($p < 0.03$) in amino acid composition as a function of age. The FAA and SH fractions revealed an increase in hydrophobic amino acids.	This is an interesting study that goes into great detail regarding specific amino acid composition, which is beyond the scope of this overview. Nonetheless, the general shift toward increased hydrophobicity is an important trend that should be noted.
Gniadecka et al. 1998 (2).	Used NIR-FT Raman spectroscopy to examine 3-mm punch biopsies from buttock, lower leg, back, and arm in 44 individuals aged 18–35 yr.	Most proteins in the whole skin and SC were in α-helix conformation. This was supported by the frequencies of amide I and III maxima and by a strong C-C stretch band at 935 cm^{-1}.	These data further support that in young skin, proteins are mostly in α-helical conformation.

(*Continued*)

Table 3 Data: Generalized Protein Structure (*Continued*)

Source	Method	Results	Notes
Sander et al. 2006 (23).	Evaluated buttock skin and photoexposed skin of 12 young (<30 yrs) and 12 older (66–73 yrs) subjects. ECM1 expression was investigated using immunohistochemistry with densitometric image analysis for semiquantitative results. Acute UV exposure was created by irradiating buttock skin over 10 days with a solar simulator.	In normal human skin, ECM1 is expressed mainly in basal cell layers of epidermal keratinocyes and dermal vessels. Intrinsically aged, UV-protected skin showed a significantly reduced expression in basal (~10% decreased staining intensity) and upper (~8% decreased staining intensity) epidermal cell layers compared with young skin ($p < 0.05$). In photoaged skin, expression is significantly increased in the lower (~15% increased staining) and upper (~18% increased staining) epidermis compared with age-matched UV-protected sites ($p < 0.01$). Acute photoexposure also results in marked increased epidermal ECM1 expression (~8–10%, $p < 0.05$).	Semiquantitative data reveal acute and chronic UV-related stress, which appears to influence expression and distribution of ECM1. Future studies may reveal similar impacts of intrinsic and photoaging on other skin proteins.

Tables 1 to 3 present age-related data on collagen, elastin, and generalized protein structure, respectively. See text for discussion.
Abbreviations: AAR, aspartic acid racemization; FAA, free amino acids; SH, soluble hydrolysate; WCH, whole-cell hydrolysate; ECM, extracellular matrix protein; ECM1, extracellular matrix protein 1; SC, stratum corneum; UV, ultraviolet.

GLYCOSAMINOGLYCANS

GAGs are composed of specific repeating disaccharide units. Those attached to a core protein are referred to as proteoglycans and are found widely distributed throughout the skin. GAGs most often present in human skin are hyaluronic acid (not attached to a protein core) and the proteoglycan family of chondroitin sulfates, including dermatan sulfate (24). GAGs are especially important in skin because they bind up to 1000 times their volume in water. Therefore, skin hydration is highly related to the content and distribution of dermal GAGs, especially hyaluronic acid (24).

Table 4 summarizes data regarding GAGs. GAGs increase in photoaged skin compared with young or intrinsically aged skin (12,24). This seems paradoxical, as photoaged skin appears leathery and dry, unlike newborn skin, which also contains high levels of GAGs. Confocal laser scanning microscopy reveals that GAGs in photodamaged skin are abnormally deposited on elastoic material, rather than diffusely scattered as in young skin (24). This aberrant localization may interfere with normal water binding by GAGs, despite their increased number.

A recent study that used laser capture microdissection and quantitative real-time PCR (polymerase chain reaction) on punch biopsy specimens from human buttock skin found that decorin, a proteoglycan, may have a significant role in collagen fiber diameter (26). Decorin mRNA is expressed in the reticular, but not the papillary dermis, and like other GAGs, levels of decorin are increased with chronological age. It is suggested that a decreased collagen-decorin ratio may contribute to the changes in ECM structure in aging skin (26).

WATER

In young skin, most of the water is bound to proteins and, appropriately, is called bound water (2). This is important for the structure and mechanical properties of many proteins and their mutual interactions. Water molecules not bound to proteins bind to each other, and are called tetrahedron or bulk water (2).

Data pertaining to water structure and aging are summarized in Table 5. Intrinsic aging does not appear to alter water structure significantly (12). However, in photoaged skin, Raman spectroscopy reveals an increase in total water content. Again, this seems paradoxical, as aged skin is often dry and weathered. However, structurally, significantly more of the water in aged skin is in tetrahedron form. Thus, as proteins are more hydrophobic and folded, and GAGs are clumped on elastoic material, they interact less with water, and water in aged skin binds to itself instead. This lack of interaction between water and surrounding molecules in photoaged skin likely contributes to its characteristically dry and wrinkled appearance.

LIPIDS

The "brick and mortar" model is often employed to describe the stratum corneum's protein-rich corneocytes embedded in a matrix of ceramides, cholesterol, and fatty acids, and smaller amounts of cholesterol sulfate, glucosylceramides, and phospholipids. These lipids form multilamellar sheets amid the intercellular spaces of the stratum corneum, and are critical to the SC's mechanical and cohesive properties, enabling it to function as an effective water barrier (28). Changes in SC lipid content have been linked to skin conditions such as xerosis and possibly atopic dermatitis (28).

Table 6 summarizes data pertaining to skin lipids and age. Many authors agree that overall lipid content of human skin decreases with age (28–30). Using high performance thin-layer chromatography (HPTLC), Rogers et al. found a 30% decrease in the face, hand, and leg of older subjects, but the older group only extended to age 50. No significant change was seen in proportional composition of lipid classes or ceramide species (28). Schreiner et al. used small-angle X-ray diffraction to compare lipid composition in subjects aged 23 to 27 years with subjects aged 63 to 69 years (31). They did not see any overall difference in lipid quantity or composition between the groups. However, the aged group consisted of only four subjects, and again, included a narrow age range. Saint-Leger et al. studied the lower legs of 50 subjects and

Table 4 Data: Glycosaminoglycans

Source	Method	Results	Notes
Bernstein et al. 1996 (24).	Included 6 males, aged 52–60 yr, with significant photodamage. Punch biopsies measuring 4 mm were taken from the sun-damaged posterior neck and sun-protected buttock. Histometrically studied GAG content of papillary dermis using immunoperoxidase stains specific for hyaluronic acid and chondroitin sulfate. Expressed measurements as percent of fields stained positively for these GAGs. Studied location of GAGs using confocal laser scanning microscopy, staining specifically for GAGs and elastin.	Significant increase in GAG staining in sun-damaged versus sun-protected skin from the same individuals: Hyaluronic acid: Sun-protected: 13.7 ± 1% Sun-exposed: 24.4 ± 0.5% ($p < 0.05$). Chondroitin sulfate: Sun-protected: 6.77 ± 0.25% Sun-damaged: 23.37 ± 0.6% ($p < 0.0001$). Superficial dermal GAGs in sun-damaged skin are clumped and deposited almost exclusively on the solar elastoic material, rather than diffusely between the fine network of collagen and elastic fibers as in normal (photoprotected) skin, wherein concentration of dermal GAGs is greatest just beneath the epidermis and decreases gradually with increasing depth.	One would expect that increased GAG content would give skin a youthful appearance, as it does in newborn skin. These authors state that the abnormal location of GAGs in photodamaged skin may explain the apparently paradoxical weathered appearance of photodamaged skin despite increased GAGs. This study does not consider possible anatomical variation between neck and buttock, separate from the factor of photodamage. Furthermore, the narrow 52–60-age range limits the study to one of photoaging and does not consider intrinsic aging.
Takahashi et al. 1996 (25).	To quantify main disaccharide units of skin GAGs, high-performance lipid chromatography was used after labeling with 1-phenyl-3-methyl-5-pyrazolone. After comparing 6 “sun-exposed people” with 6 other “sun-protected people,” the authors compared sun-exposed and sun-protected skin within 6 individuals.	Total amount of disaccharide units in sun-exposed skin was significantly greater than that in sun-protected skin ($p < 0.05$). Also saw a decrease in the ratio (δ)Di-HA (disaccharide-hyaluronic acid)/δDi-4s (disaccharide-dermatan sulfate) in photoaged skin.	This article addresses photoaging but not intrinsic aging. The increase in GAGs in photodamaged skin agrees with results of Bernstein et al. (above). The significance of the increased hyaluronic acid-dermatan sulfate ratio is unclear.

Lochner et al. 2007 (26).	Full-thickness punch biopsies isolated from human buttock skin of 5 young (21–35 yr) and 5 older (61–68 yr) subjects. Distribution and expression of collagens 1 and 3 and decorin mRNA were measured using laser capture microdissection and quantitative real-time PCR in young versus old subjects. Decorin and collagen expression were also measured before and after single exposure with two minimal erythemal doses of simulated solar irradiation after 24 hr.	Decorin mRNA is expressed in the reticular but not in the papillary dermis. Expression is 105% higher in older than in younger subjects. Simulated solar exposure resulted in downregulation of decorin mRNA in both groups [−35% in young; −35% in older). Collagens I and III expressions were downregulated with increasing age (29% and 60% lower levels in collagens I and III mRNA, respectively, in older subjects compared with young) and after single UV irradiation (21% decrease seen with collagen I and 60% seen with collagen III).	Small sample size, but otherwise convincing evidence of decreased expression of decorin and collagens I and III with age and also with UV radiation. The exact mechanistic significance is unclear, but authors concluded that decreasing collagen to decorin ratio inflected by both age and UV irradiation may affect collagen bundle diameter in aging skin.

Table 4 summarizes data on age-related changes in GAG structure and localization. See text for discussion.
Abbreviations: GAGs, glycosaminoglycans; Di-HA, disaccharide-hyaluronic acid; δDi-4s, disaccharide-dermatan sulfate; UV, ultraviolet; PCR, polymerase chain reaction.

Table 5 Data: Water Structure

Source	Method	Results	Notes
Gniadecka et al. 1998 (12).	Study of Caucasians included 10 people aged 74–87 yr and 10 people aged 22–29 yr. Obtained Raman spectra from buttock skin and dorsal forearm skin, using NIR-Raman spectroscopy.	Younger group: Most water molecules in young skin were bound to other macromolecules (the 180 cm-1 band was absent). Saw no significant difference in water content or structure in sun-exposed versus sun-protected skin. Older group: Intrinsically aged (buttock) skin: no significant difference in water content or structure compared with young skin. Photoaged (dorsal forearm) skin: increased content of nonbounded water (180 cm-1 band present). Total hydrogen-bonded water is significantly decreased in photoaged skin ($p = 0.03$). Saw an overall (30%) increase in water content of photoaged skin.	In young skin, water is primarily present in bound form. This does not appear to change with intrinsic aging. However, in photoaged skin, overall water content increases and proportionally shifts such that less of it is in bound form.
Gniadecka et al.1998 (2).	Used NIR-FT Raman spectroscopy to examine 3-mm punch biopsies from the buttock, lower leg, back, and arm in 44 people ages 18–35 yr.	Over 90% of water in whole skin is present in the bound form.	These data support that in young people, water is mainly present bound to macromolecules.
Wright et al. 1998 (27).	MRI chemical shift imaging was used to noninvasively study nine volunteers of both sexes. Obtained localized (1) H spectra of the skin, quantified free water content, normalized to skin thickness.	Relative concentration of free water in the skin, normalized to skin thickness, was slightly greater in older subjects and intanned subjects.	These data are consistent with other studies showing overall increase in the free, unbound water content of aged and sun-exposed skin.

Table 5 presents data pertaining to age and skin water structure. See text for discussion.

Table 6 Data: SC and Skin Surface Lipids

Source	Methods	Results	Notes
Schreiner et al. 2000 (31).	Included 10 normal subjects, aged 25.5 ± 2.5 yr, 10 subjects with dry skin, aged 30 ± 6 yr, and 4 subjects aged 66 ± 3 yr. Performed small-angle X-ray diffraction and lipid analysis on whole SC samples from the lower legs. Measured the percent and quantity of different barrier lipid classes.	No significant difference in the number of total ceramides, free sterols, and FFAs between younger and older groups. While not statistically significant, there was an apparent increase in percentage of FFA and compensatory decrease in percentage of ceramides.	According to this study, lipid compositions of different skin types do not differ significantly; however, the aged group only consists of four subjects.
Rogers et al. 1996 (28).	Included 28 female Caucasians, aged 21–50 yr. Studied lipid composition from 8 sequential SC tape strippings of face, hand, and leg. Corneocytes were removed from the tape strippings by sonication in methanol, and lipid extracts were treated and separated using HPTLC on 20 × 10 cm plates. HPTLC plates were developed, dried, and stained, then quantitated using a scanning densitometer at 420 nm. Samples of ceramide fractions from the leg site were also used to analyze ceramide 1 esterified fatty acids in relation to age in the following groups: 26–29 yr ($n = 9$), 41–43 yr ($n = 9$), 57–60 yr ($n = 10$).	All lipid classes decreased with increasing age [Overall, saw a 30% decrease in lipid content in aged subjects. Decrease was most marked for all ceramide species (1–6) in the face and hand, and for cholesterol in the face ($p < 0.05$)]. Percentage ratios of each of the major lipid classes and of the individual ceramide species remained constant. Esterified FA analysis: Levels of ceramide 1 linoleate decreased with increasing age. There were no significant age-related changes in other ceramide 1 esterified FAs or in FFA species.	This study included a fairly narrow age range and does not consider changes in elderly skin. Also, the authors only took 8 strippings from each site; this does not necessarily include the whole SC. This study did account for differences in the amount of SC removed by measuring protein content in strippings and normalizing the mass of each lipid fraction removed.
Saint-Leger et al. 1988 (30).	SC lipids were collected from the right and left legs of 50 subjects of varying ages.	The SC lipid profile was generally constant from age 50 yr upward. Aging was associated with a decrease in sterol esters and triglycerides. Changes in lipids did not seem to account for the increasing xerosis in aged populations.	This is again a very site-specific study. While the sample size is probably adequate, the findings in SC taken from individuals' legs may or may not be generalizable to other body surfaces. The question of how or whether lipid content affects xerosis may also require further study.
Cua et al. 1995 (32).	Included 7 females aged 24.9 ± 1.1 yr, 7 males aged 28.7 ± 0.5 yr, 7 females aged 75.3 ± 2.4 yr, and 8 males aged 73.8 ± 1.2 yr. Measured 11 anatomical regions' SSL contents.	Skin surface lipid content was not statistically different between age groups on all regions except for the ankle, where lipid content was lower in the elderly ($p < 0.05$).	This study accounts for the intraindividual variation one might expect of the skin covering different body surfaces and nicely controls for gender and age. A similarly designed study with a greater number of subjects could be very useful for further confirmation.

Table 6 presents data for age-related changes in lipid content and composition. See text for discussion.
Abbreviations: SSL, skin surface lipid; SC, stratum corneum; FFA, free fatty acids; FAs, fatty acids.

found that the lipid profile was constant from age 50 upward; overall, aging was associated with a slight decrease in sterol esters and triglycerides (30). Cua et al. noted significant regional variation within individuals as they studied 11 sites on 29 people, comparing individuals in their third decade of life with those in their eighth decade. Interestingly, they, too, found little relationship between skin surface lipid content and age, except on the ankle, where the elderly demonstrated decreased lipid content (32). From these contradictory studies, it is difficult to conclude with certainty whether lipid content decreases with age. Many confounding factors may hinder such studies, including seasonal and diurnal variation, general interindividual variation, and the use of several different methodologies by different researchers.

CONCLUSIONS

Collagen becomes less soluble, thinner, and sparser in intrinsically aged skin, but is thickened, fragmented, and more soluble with photoaging (12). UV exposure leads to activation of transcription factors that stimulate increased production of collagenases and MMPs, which lead to breakdown of skin collagen and other proteins (7–9). Also, the ratio of type III to type I

Table 7 Recommendations for Future Studies

Problem	Recommendation
Interindividual variation	**A.** Larger sample sizes to justify significance. Given the tremendous range of variation in skin bioengineering parameters, researchers should consider designing studies using power calculations, wherein before the study, they determine what would be a clinically relevant difference in measurement between young skin and old skin (δ), a significance level (α, usually 0.05) and power level (1-β, usually 8). For a test comparing means of measurements, sample size needed for significance at the specified level is calculated as follows: $n = 2^*[z_{(1-\alpha/2)} + z_{(1-\beta)}]^2/(\delta/s)^2$ Values for $z_{(1-\alpha/2)}$ and $z_{(1-\beta)}$ for 5% significance and 80% power, respectively, are 1.96 and 0.8416. The use of power calculations assumes a normal distribution of the data and equal numerical allocation of participants to the groups being compared (33).
	B. Given the likely significant effects of age-associated hormonal changes on the skin (34), studies should attempt to normalize for or at least regularly report differences in hormonal status among groups tested.
Intraindividual variation	**A**. The average of three measurements should be recorded at any test site; i.e., statistics should analyze the mean of three skin thickness measurements on a certain region of the skin to help minimize "noise" of individual variation.).
	B. Standardization of skin sites tested; e.g., we could agree to focus research on a limited number of specific areas that ideally address issues of lifelong environmental exposure; we suggest, somewhat arbitrarily: 1) the midvolar forearm, exactly halfway between the centers of the wrist and elbow joints 2) the middorsal forearm, exactly halfway between the centers of wrist and elbow joints 3) the mid upper inner arm, exactly halfway between the mid-axilla and elbow joint 3) the exact center of the forehead 4) the upper-mid buttock, 3 cm below the iliac crest and halfway between the midline and left or right border, so as to minimize effects of lifelong compression and decompression that occurs on the lower buttock 5) the midline of the back, over spinous process of T2.
Instrument and other measurement-related variation	**A.** Standardization of, or at the very least accurate and consistent reporting of settings used on bioengineering tools, including frequency settings and gain swept curves on ultrasonography machines.
	B. Since diurnal variation has been demonstrated in skin thickness and echogenicity studies (4, 35, 36), and may also be a factor in blood flow and water content, studies should be designed to minimize this variation; all individuals should be tested at the same or comparable time of day, and timing of the measurements should be reported, if not standardized.

Table 7 presents several recommendations that may help to decrease variation among future studies.

collagen is reported to increase with age (6,12,15). Histologically, young collagen is randomly organized into a meshwork of loosely interwoven bundles. Age leads to a loosening within these bundles and straightening of collagen fibers, increasing skin's tensile strength (14). Biochemically, the aging process leads to progressive oxidation of collagen's lysine residues resulting in accumulation of 2-aminoadipic acid (13).

Elastin is a long-lived protein in human skin; it appears to accumulate damage with age and sun exposure. New elastin is synthesized in greater quantities in aged skin, but it is thought that this synthesis results in abnormally structured elastin (16,21). Also, elastin degradation does not appear to keep pace with new synthesis in aged skin. This results in massive accumulation of elastoic material, especially in photoaged skin; degradation is further impaired by UV-induced expression of proteins elafin and lysozyme (17,18). The abnormal structure of this excessive elastin prevents it from functioning as it does in young skin.

Studies of primary and tertiary skin protein structure in aged skin reveal an environment unfriendly to water, with an overall increase in hydrophobic amino acids and greater folding such that aliphatic residues are more hidden from water (12,22). Also, although total amounts of GAGs appear to be increased in aged skin, these are abnormally localized on the elastoic material in the superficial dermis; thus, they are unable to bind water as well as if they were scattered appropriately throughout the whole dermis (24). Hence it is not surprising that, although aged skin contains increased amounts of water, most of this water is bound to itself in tetrahedral form, rather than being bound to proteins and GAGs as it is in young skin (12). These factors together likely contribute to increased xerosis and withered appearance of aged skin.

While it tends to be an accepted assumption that lipid content decreases with age, quantitative studies are contradictory. Some indicate a marked age-related decrease in skin lipids, at least up to age 50 (28), while others indicate little or no relationship (31,32). Future studies may be benefited by increased sample size and standardization of method, body site, and season of study. Table 7 presents suggestions for greater standardization, and hopefully greater consensus, among future studies.

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24 Glycation End Products

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INTRODUCTION: THE GLYCOXIDATIVE AGING PROCESS

Numerous studies have been carried out in an attempt to elucidate the biochemical and molecular mechanisms of the aging process. The basic biochemical process underlying the aging process was first introduced in 1956 with the free radical theory of aging (1). This theory states that oxidative damage to DNA and other cellular components is the main driving force behind aging. More recent versions of this theory predict that mitochondria are a major source of reactive oxygen species (ROS) that cause oxidative damage. The idea that genetically damaged mitochondria accumulate with time and are causally responsible for the aging phenotype via a disturbed energy production and excessive ROS production is at the core of the so-called mitochondrial theory of aging (2). In 1989, Monnier proposed the Maillard theory of aging, stating that the fundamental aging process might be mediated by the Maillard reaction (the nonenzymatic reaction between reducing sugars and proteins, also known as the glycation process) (3). The free radical-glycation/Maillard reaction theory of aging brings those two views together. It suggests that free radicals (ROS) and reactive carbonyl species (RCS) from Maillard reactions may represent interactive elements of a more complex biochemical pathway. The age-related deterioration then results from the cumulative damage induced by ROS, by RCS, and by their interactions (4). Glycation of mitochondrial proteins results in the excessive formation of intracellular superoxide (5). It was recently shown that senescent human fibroblasts are characterized by a partial uncoupling of the respiratory chain, resulting in increased proton leakage and enhanced electron transport activity (6). Stöckl et al. (7) even suggested a cause-effect relationship between impaired mitochondrial coupling and premature senescence. Others have proposed a key role for high-level ROS-generating enzymes of the NOX family NADPH oxidases in causing age-related diseases (8,9). Oxidative damage to DNA has been found to be an important determinant of life span at least in lower organisms such as *Drosophila melanogaster*. Studies in higher organisms argue for a role of oxidative stress in age-related disease, especially cancer; however, the data remain inconclusive on whether oxidative stress determines life span (10). The general consensus appears to be that the aging process is multifactorial and that it results from an accumulation of damage with an underlying glycoxidative mechanism. Our interpretation of the glycoxidative model of aging is presented schematically in Figure 1.

We will now focus on how glycation contributes to the aging process in skin.

FORMATION OF GLYCATION END PRODUCTS

The nonenzymatic protein glycation reaction was originally described by Maillard to explain the browning reactions taking place during food preparation (11). The initial phase of the Maillard reaction results in the reversible formation of early glycation products. During this process, several reactive intermediates are generated, including ROS and/or RCS like (methyl)glyoxal and glycolaldehyde. The reaction can progress beyond that stage, leading to the formation of a variety of products collectively termed “advanced glycation end products (AGEs)”. Heat-generated AGEs that are formed in common foods represent “exogenous” forms of Maillard reaction products, which are ingested on a daily basis. Cigarette smoke was found to contain RCS that are able to react with proteins to form AGEs (12). The “endogenously” formed Maillard products are formed in vivo when reducing sugars or reactive intermediates react with body proteins. Carbohydrates (sugars) are part of a healthy

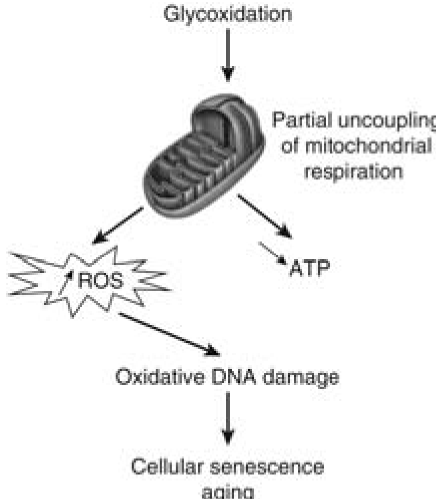


Figure 1 Glycoxidative model of aging. Glycoxidative damage, induced by ROS and RCS, causes impairment of the oxidative phosphorylation process in mitochondria. This leads to mitochondrial leakage of free radicals and less-efficient ATP production, resulting in oxidative DNA damage, cellular senescence, and aging. *Abbreviations*: ROS, reactive oxygen species; RCS, reactive carbonyl species. *Source*: From Refs. 4, 7, 10.

diet. They supply the body with energy, mainly in the form of glucose. When excess (reducing) sugars are available, they can react with other molecules such as proteins. Both exogenous and endogenous forms of AGEs are believed to play a role in aging and disease through what has been generally termed "carbonyl stress" (13).

Some AGEs are characterized by intra- and intermolecular cross-linking with typical fluorescent properties (14). Their formation can be induced in model proteins by incubation with reducing sugars (e.g., fructose) or RCS (e.g., methylglyoxal) in vitro. The progressive formation of AGEs can be followed with fluorimetric detection at wavelengths associated with AGEs (excitation/emission wavelengths of 370/440 nm). The speed of formation depends on the nature of the glycating species. As shown in Figure 2, the in vitro formation of fluorescent AGEs in a model protein (bovine serum albumin, BSA) occurs faster with glycolaldehyde than with 3-deoxyglucosone or methylglyoxal. The reaction can take up to several weeks when less-reactive sugars such as glucose or fructose are used to initiate the reaction.

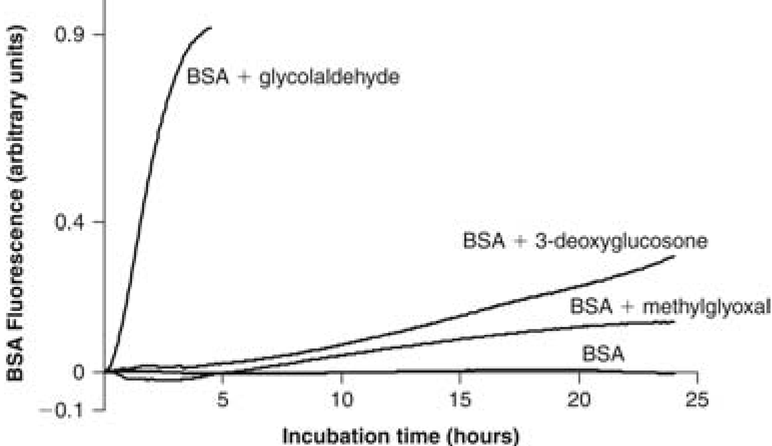


Figure 2 Kinetics of the formation of fluorescent AGEs in the model protein BSA upon incubation with glycation inducers glycolaldehyde, 3-deoxyglucosone, and methylglyoxal. Fluorimetric detection at wavelengths associated with AGEs (excitation/emission wavelengths of 370/440 nm). *Abbreviations*: AGE, advanced glycation end product; BSA, bovine serum albumin.

ACCUMULATION OF GLYCATION END PRODUCTS IN SKIN

Measuring Glycation End Products in Skin

Since the endogenous formation of AGEs is a slow process, the body's long-lived proteins such as collagen are mostly susceptible to AGE accumulation. Therefore, the formation and accumulation of AGEs have been extensively studied in the skin. The presence of glycation end products in the skin has even been proposed as a marker and predictor for the progression of certain systemic diseases (15). The initial method for quantifying AGEs was based on the measurement of collagen-linked fluorescence in tissue biopsies (16). Later, more specialized techniques were developed to analyze specific AGEs such as N^{ε}-(carboxymethyl)lysine and pentosidine (17). More recently, the in vivo measurement of skin fluorescence at wavelengths associated with AGEs (including excitation/emission wavelengths of 370/440 nm) has been introduced as a noninvasive clinical tool to study age-related phenomena in human skin (18). Skin autofluorescence has recently been shown to be correlated with the presence of specific AGEs (19). Skin autofluorescence measurement was then proposed by these authors as an independent predictor for the development of microvascular complications in type 2 diabetes mellitus (20). We have been using a similar method to measure in vivo skin autofluorescence at wavelengths associated with AGEs (including excitation/emission wavelengths of 370/440 nm) in a healthy population. The data were collected with an LS 50B fluorescence spectrometer (PerkinElmer, Waltham, MA, U.S.A.) equipped with a fiber-optic cable (Fig. 3).

Accumulation of Glycation End Products as a Function of Age and Body Weight

In humans, AGEs have been demonstrated to accumulate in the body as a function of age (21) and in age-related diseases (22). Obesity and overweight are risk factors for various disorders, including diabetes (23). Even in nondiabetic individuals, glycated hemoglobin values were shown to increase progressively with age and obesity (24). The increase in overweight and obesity prevalence is evident in the western societies over the past few decades. More recently, this trend of weight gain was observed in other countries, e.g., in Asian countries (25,26), and it is expected to develop worldwide. Although excessive weight gain is a complex, multifactorial chronic condition involving genetic, physiological, metabolic, and psychological components, a dramatic increase in energy-rich food intake accompanied by a significant reduction in physical activity are major contributors to obesity. It is therefore of interest to investigate whether weight gain and obesity have an impact on the accumulation of AGEs in skin.

Long-term consumption of the reducing sugar fructose was shown to accelerate the endogenous induction of collagen-linked fluorescence and skin collagen cross-linking in rats (27). Increasing evidence suggests that reduction of sugar intake by caloric restriction in various species leads to decreased carbonyl stress and reduced accumulation of AGE

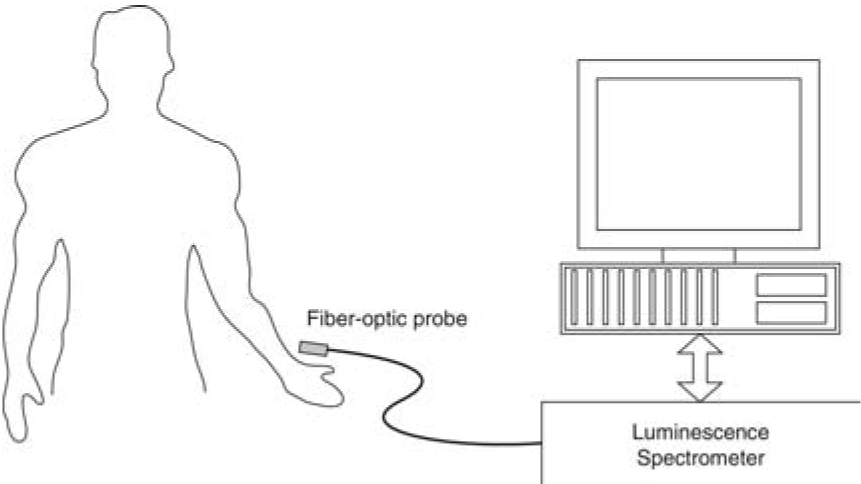


Figure 3 Experimental setup for in vivo measurement of skin autofluorescence at wavelengths associated with AGEs (excitation/emission wavelengths of 370/440 nm) using an LS 50B fluorescence spectrometer (PerkinElmer, Waltham, MA, U.S.A.) with a fiber-optic cable. *Abbreviation*: AGE, advanced glycation end product.

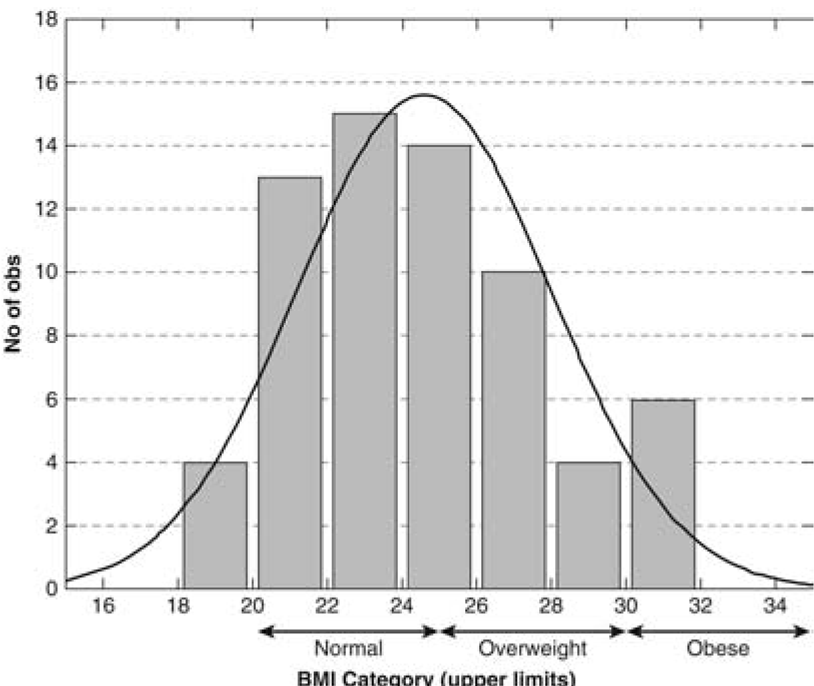


Figure 4 The distribution of BMI for test panel described in the text. The distribution was close to normal with a median of 24.2. A total of 37 volunteers had a normal BMI, 23 were overweight, and 6 were obese. *Abbreviation*: BMI, body mass index.

products in skin collagen (28,29). In calorie-restricted mice, the rate of glycated collagen and AGE accumulation in skin were also shown to be slowed down, which was inversely related to maximal life span (30).

We used in vivo skin fluorescence to evaluate the association between body fat levels (on the basis of impedance measurement) of healthy but slightly overweight individuals and their AGE-related skin fluorescence. A panel of 66 female Caucasian nonsmoking healthy volunteers living in Belgium, aged between 21 and 59 years (median 42 years), was enrolled in this study. As indicated in Figure 4, the body mass index (BMI, calculated as weight in kg divided by the square of height in m) of the volunteers was close to normal and ranged from 18.3 to 32.0 (median 24.2). Skin color was measured with a chromameter (Minolta, Osaka, Japan) and expressed as individual typology angle (ITA°). The ITA-value decreases with increasing pigmentation of the skin. Multiple linear regression techniques were used to take into account the impact of skin color on the in vivo AGE-related fluorescence (excitation/emission wavelengths of 370/440 nm). As shown in Table 1, the fluorescence intensity attributed to AGEs increased as a function of panelist age ($p = 0.008$) and with increasing percentage of body fat ($p = 0.04$) (independent of the age effect). These data suggest that within a population of healthy women with weight from normal to slightly overweight, an increasing level of fluorescent AGEs accumulates in the skin as a function of age and body weight. In a separate study, we have found that within a test panel of 448 female Caucasian volunteers

Table 1 Statistical Outcome of the Multiple Linear Regression Analysis on the Association Between In Vivo Skin Fluorescence and Percentage Body Fat, Chronological Age, and Skin Color (ITA)

	Fat (%)		Chronological age		Skin color (ITA°)	
Fluorophore	Partial regression coefficient	*p*-value	Partial regression coefficient	*p*-value	Partial regression coefficient	*p*-value
AGEs (370/440)	**0.67**	**0.04**	**0.58**	**0.008**	**2.48**	$<\mathbf{10^{-6}}$

Statistically significant parameters are indicated in bold.
Abbreviation: AGE, advanced glycation end product.

Table 2 Statistical Outcome of the Multiple Linear Regression Analysis on the Association Between In Vivo Skin Fluorescence and Smoking Behavior, Chronological Age, and Skin Color (ITA)

Fluorophore	Smoking behavior		Chronological age		Skin color (ITA°)	
	Partial regression coefficient	*p*-value	Partial regression coefficient	*p*-value	Partial regression coefficient	*p*-value
AGEs (370/440)	**9.43**	**0.03**	**0.32**	**0.02**	**2.22**	**$<10^{-6}$**

Statistically significant parameters are indicated in bold.
Abbreviation: AGE, advanced glycation end product.

living in the New York area, the independent contribution from body weight and chronological age on the skin AGE-related autofluorescence was confirmed in the group of panelists younger than 40 years ($n = 147$). In the older panelists ($n = 301$), the effect of age on the AGE-related fluorescence was much stronger and became dominant over the effect of body weight (31).

Accumulation of Glycation End Products as a Function of Age and Smoking Behavior

On the basis of a systematic review of 25 prospective cohort studies, it was recently concluded that active smoking is associated with an increased risk of type 2 diabetes (32). Literature data suggest that smoking causes mitochondrial dysfunction via inhibition of complex IV activity (cytochrome c oxidase) (33). This mitochondrial dysfunction could contribute to increased endogenous production of ROS (34). Cigarette smoke is also considered to be an inducer of glycoxidative reactions (12). Nornicotine, a constituent of tobacco and metabolite of nicotine, can catalyze Maillard-like reactions under aqueous conditions. The plasma of smokers as compared with nonsmokers contains higher concentrations of nornicotine-modified proteins (35). A significant rise in the mean levels of the glycation product fructosamine and total plasma glycated proteins were found in smokers when compared with nonsmoking controls (36). An increase in dermal collagen cross-linking can be induced in rats by exposure to cigarette smoke (37). We used in vivo skin fluorescence to evaluate the association between smoking behavior and AGE-related skin fluorescence in normal healthy individuals.

A panel of 94 female Caucasian healthy volunteers living in Belgium, aged between 18 and 81 years (median 43 years), was enrolled in this study. There were 16 active smokers and 78 nonsmokers. Multiple linear regression techniques were used to take into account the impact of skin color on the in vivo AGE-related fluorescence (excitation/emission wavelengths of 370/440 nm). As shown in Table 2, the fluorescence intensity attributed to AGEs increased as a function of panelist age ($p = 0.02$) and with smoking ($p = 0.03$) (independent of the age effect). These data suggest that within a population of healthy women an increasing level of fluorescent AGEs accumulates in the skin as a function of age and smoking behavior.

CONSEQUENCES OF GLYCATION END PRODUCT FORMATION AND ACCUMULATION IN SKIN

Structural Consequences

The glycation process is characterized by the formation of AGEs with intra- and intermolecular cross-links and with typical fluorescent properties. It is well known that the accumulation of cross-linked AGEs alters the structural properties of tissue proteins. The cross-linking also reduces their susceptibility to being removed by catabolic processes, thereby contributing further to the accumulation (38). The structural changes resulting from a slow accumulation of AGEs in the skin contribute to the modification of the skin's biomechanical properties. At the clinical level, they have been associated with an increase in stiffness or brittleness of the skin (39,40). This is in agreement with data showing that in vitro glycated skin samples are characterized by a loss of biomechanical properties (41). The structural changes are not solely restricted to the dermal compartment of the skin. In a diabetic population, it was shown that glycation of plantar epidermal proteins could play an important role in the stiffening of plantar skin (42). The glycation process can therefore play an important role in explaining the changes in mechanical properties of the skin that occur over several decades of life (43,44).

Functional Consequences

The accumulation of AGEs in long-lived proteins and the resulting structural changes occur rather slowly. The glycation process can have more important short-term consequences as well. The presence of AGEs can increase cellular oxidative stress and promote inflammatory reactions (45). The in vivo formation of reactive intermediates such as methylglyoxal can cause immediate cellular damage by causing the deactivation of enzymes with an important role in cellular defence or survival (46).

Deactivation of enzymes involved in maintaining energy homeostasis such as creatine kinase is expected to result in a generalized decline in biological performance (47). Creatine kinase is responsible for transferring the energetic phosphor group between creatine/creatine phosphate and adenosine triphosphate (ATP). Clinical studies with ^{31}P nuclear magnetic resonance spectroscopy have indicated that the capacity to regenerate energy levels after a mild stress is compromised in older skin (48). As illustrated in Figure 5, creatine kinase is susceptible to in vitro deactivation upon incubation with methylglyoxal as well as with a cigarette smoke extract. Methylglyoxal decreases mitochondrial membrane potential and intracellular ATP levels, suggesting that carbonyl stress-induced loss of mitochondrial integrity could contribute to the cytotoxicity of the intermediate glycation products (49). As previously shown in Figure 1, the consequence of mitochondrial damage is a partial uncoupling of the mitochondrial respiration, mitochondrial leakage of free radicals, and less-efficient ATP production, resulting in oxidative DNA damage, cellular senescence, and aging (4,7,10).

Deactivation of protective enzymes such as catalase, superoxide dismutase, glutathione reductase, and peroxidase will result in a reduced antioxidant defense capacity of the cells. The importance of the activity of the superoxide dismutase (SOD)/catalase duo has been clearly established. SOD catalyzes the dismutation of the superoxide anion into oxygen and hydrogen peroxide. Then catalase will neutralize hydrogen peroxide to oxygen and water. Sun exposure results in a disturbed catalase to SOD ratio, at least in the upper layers of the skin (50). Low catalase activity levels have been measured in the skin of patients with vitiligo (51), polymorphic light eruption (52), and *Xeroderma Pigmentosum* (53). In vitro studies emphasize the importance of maintaining the optimal ratio between the different antioxidant enzymes and its relation to cellular senescence and sensitivity to oxidative stress (54). The observation that the life span of *Caenorhabitis elegans* could be extended after supplementation with SOD/catalase mimics supports the hypothesis that an increased efficiency at which ROS can be neutralized by antioxidant enzymes may counteract the aging process (55).

Finally, deactivation of enzymes involved in the DNA repair process can have major consequences for cellular viability and tissue functioning. Defects in cellular DNA repair

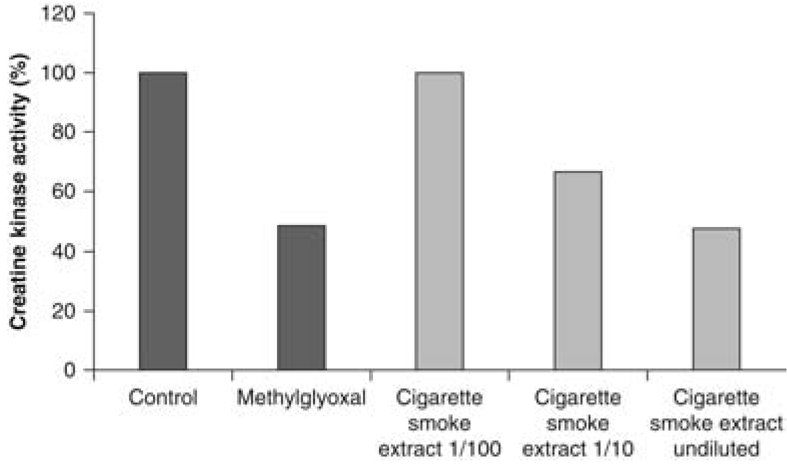


Figure 5 A cigarette smoke extract prepared by smoking six research cigarettes from Tobacco Health Research, University of Kentucky (ref.: 2R4F, 11/2001) into 7 mL of HPLC grade water, containing 7 μL of Triton X−100. In vitro incubation of creatine kinase with cigarette smoke condensate caused a dose-dependent decrease of creatine kinase enzyme activity. The undiluted cigarette smoke condensate caused 50% loss of enzyme activity, which was similar to the effect caused by the glycation intermediate methylglyoxal at 4.5 mM.

processes have been linked to genome instability. This then potentially leads to an accumulation of mutations, cellular dysfunction, and aberrant phenotypes at the tissue and organism levels. Depending on which part of the DNA repair process is affected, the clinical manifestation of those genetic defects occurs in the form of heritable cancers and/or premature-aging syndromes (56,57).

TECHNOLOGIES TO CONTROL GLYCATION IN SKIN

Protecting Against Glycation End Product Formation

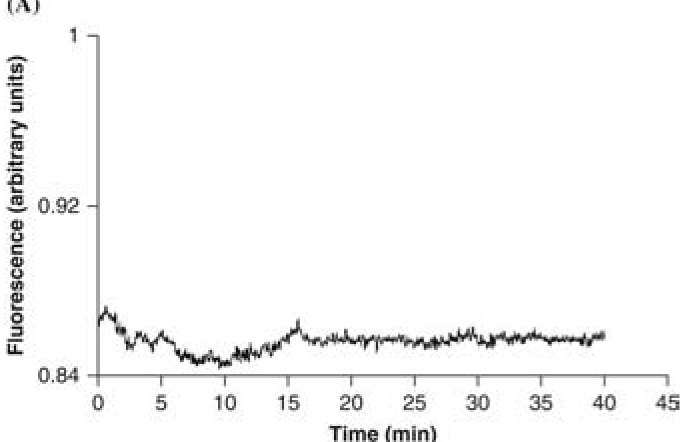
The overwhelming evidence linking the consequences of the formation of glycation end products in the skin with aging has strongly stimulated the development of technologies aimed at slowing down AGE formation. Technologies for either the prevention of AGE formation in the skin or the reduction of existing AGEs have been proposed by various laboratories around the world. We intend to limit this review to the description of technologies aimed at preventing the glycation process and its consequences. In our hands, none of the ingredients that claimed to reduce the amount of preexisting AGEs (so-called AGE breakers) have shown to reduce the extent of AGE cross-linking in our in vitro model systems. This does not exclude the fact that other mechanisms may lead to their removal in a more complex in vivo system.

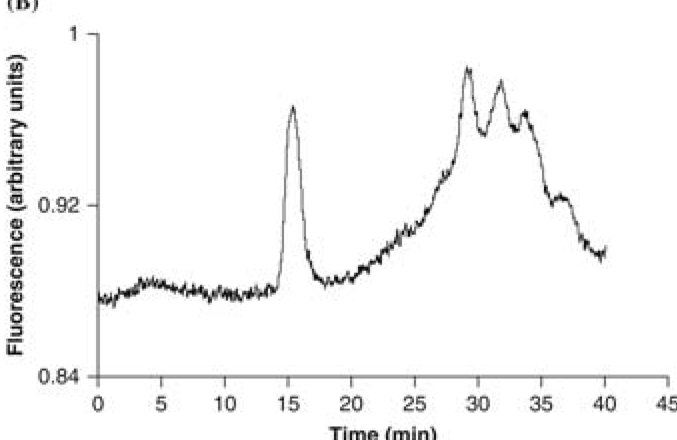
To characterize AGE-modified proteins used in studies of formation and structural effects of glycation, an in vitro assay was developed that surveys the content of cross-linked proteins with characteristic fluorescent properties. The assay procedure involves the high performance liquid chromatography (HPLC) separation by gel filtration of adducts resulting from the reaction between a model protein and a glycation inducer at 37°C. The quantification is based on fluorimetric detection at wavelengths associated with AGEs (excitation/emission wavelengths of 370/440 nm). An example is shown in Figure 6, with creatine kinase as the model protein before (A) and after (B) incubation with the glycation inducer glycolaldehyde. The higher molecular weight adducts elute earlier and have the characteristic fluorescent properties.

This procedure can then be used to evaluate the efficiency of glycation inhibitors in preventing the formation of AGEs in vitro. Aminoguanidine can be used as a positive control with well-known anti-glycation activity (58). As shown in Figure 6 (C), this compound significantly prevented the development of higher molecular weight AGEs in our in vitro testing model and can be proposed for topical use in products aimed at protecting the skin from the damages induced by the glycation process.

In any preventative technology, it is essential to control the initial steps of the reaction process. As described before, the glycation and oxidation reactions are intimately linked at an early stage of the process. For this reason, it is advisable to evaluate the activity of known antioxidants such as flavonoids for their ability to control the initial stage of the glycation process. Experiments conducted in our laboratories demonstrated that more than 75% of the flavonoid-like materials we tested had an antioxidant activity (based on the luminescent ABEL R antioxidant test kit for vitamin C type antioxidants) similar to their anti-glycation effect. In line with published data, the overall outcome suggested that the presence of a hydroxyl function at the C-3′ and C-4′ position of the B-ring is a requisite for both antioxidant (59) and anti-glycation activity (60) (see Fig. 7). An additional series of experiments using an ex vivo skin model confirmed the activity of a blend of eight antioxidants in preventing the damage caused by UV exposure in glycated skin (unpublished results). Finally, we demonstrated in a clinical study that the topical application of a product containing a mixture of antioxidants as well as aminoguanidine and another scavenger of RCS on a group of human volunteers for a period of two months resulted in a significant reduction of the skin fluorescence intensity at the wavelengths attributed to AGEs (unpublished results).

These data suggest that the control of the glycation process requires a multifactorial approach rather than the simple blockage of the Maillard reaction to inhibit the formation of a covalent bond between a reducing sugar and a protein. The many different steps occurring during the initial stage of the glycoxidation process, leading to the formation of the early glycation products, followed by the development of AGEs, cannot be controlled by one single molecule. On the contrary, a mixture of antioxidants with proven efficacy in protecting cellular





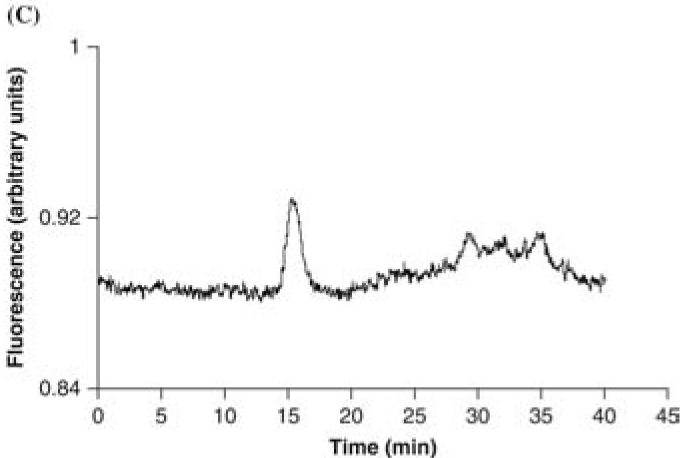


Figure 6 The formation of fluorescent AGEs in the model protein creatine kinase upon incubation with glycolaldehyde. Creatine kinase was glycated in vitro and subjected to gel filtration HPLC analysis with fluorimetric detection at wavelengths associated with AGEs (excitation/emission wavelengths of 370/440 nm). The enzyme migrates as multiple peaks in the retention window between 15 and 40 minutes. The chromatogram of untreated creatine kinase is depicted in the top panel (**A**) and shows no fluorescent signal. Incubation of creatine kinase with the glycation inducer glycolaldehyde (panel **B**) resulted in the appearance of fluorescent peaks with higher molecular weight that are attributed to the glycation of creatine kinase. Coincubation with the glycation inhibitor aminoguanidine partially prevented the formation of the fluorescent protein modifications (panel **C**). *Abbreviation*: AGE, advanced glycation end product.

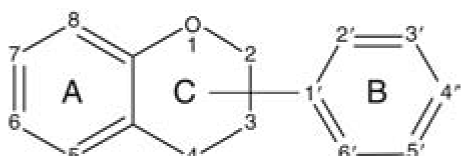


Figure 7 Basic structure of flavonoids

lipids and proteins as well as the mitochondrial and nuclear DNA together with ingredients that directly inhibit the glycation process (e.g., aminoguanidine) should be used in the same preparation to provide the skin with a more complete protection and thereby slow down the aging process.

Protecting Against Consequences of Glycation

Previously in this chapter, we reviewed the structural consequences of glycation, which result in the loss of skin resilience and elasticity because of the cross-linking of elastin and collagen in the dermis. As this cross-linking reaction is nonenzymatic and occurs slowly, it could be prevented by the regular topical application of products containing the anti-glycation ingredients we described above. The indirect consequences of the glycation process can have, in our opinion, far more impact on the skin, as they potentially undermine all the essential functions of the skin cells. As described above, the early stage of the glycation process involves the formation of RCS such as methylglyoxal, glyoxal, glycolaldehyde, and 3-deoxyglucosone. This can increase cellular oxidative stress, promote inflammatory reactions, and deactivate some of the key enzymes responsible for the protective (e.g., catalase, superoxide dismutase), reparative, as well as the metabolic function (e.g., creatine kinase) of the cells.

In an in vitro system, it is possible to prevent the loss of enzymatic activity using the technologies described above. An example is shown in Figure 8, whereby aminoguanidine prevents the loss of creatine kinase activity induced by incubation with methylglyoxal (47). In a complex in vivo system, it is advisable to use this in combination with ingredients that will compensate for the deficient metabolic activity, the lower protective enzyme capacity, and the reduced DNA repair enzyme activity.

Treatment with a blend with creatine, the reduced form of β-nicotinamide adenine dinucleotide (NADH), and *N*-acetyl-L-carnitine helps to replenish the energy reserve pool while reducing intracellular ROS generation in older skin cells (61).

The UV stable synthetic SOD/catalase mimic manganese complex EUK-134 can be used topically to compensate for a loss of antioxidant enzyme activity (62,63) and as a result help restore the protective capacity of the skin against oxidative damage. The indirect consequence is a 50% reduction of the UV-induced cellular apoptosis (sunburn cells) after treatment with nanomolar concentrations of EUK 134.

Similarly, the glycation-induced loss of DNA repair activity can be boosted with DNA repair enzymes, e.g., UV endonuclease from micrococcus luteus and 8-oxoguanine DNA glycosilase (OGG1). They are encapsulated in a liposomal preparation and marketed by AGI dermatics under the trade name of liposomal UV endonuclease and liposomal OGG1, respectively. A significant amount of research has been done (64,65) to prove that the topical application of a preparation containing a liposomal suspension of these two enzymes is

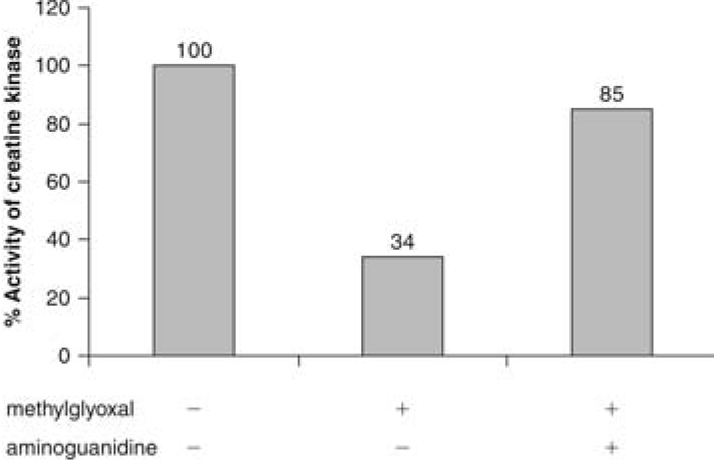


Figure 8 In vitro incubation of creatine kinase with the glycation inducer methylglyoxal decreased the creatine kinase enzyme activity by 65%. The loss of enzyme activity could partially be prevented by the glycation inhibitor aminoguanidine.

reducing the amount of DNA damage, and as a result reduces significantly the amount of cellular apoptosis.

Used together, these technologies provide a broad-spectrum protection from the direct as well as indirect damages caused by the interaction between sugars and our skin's proteins.

By interacting at every step of the process described in Figure 1, it is possible to minimize the impact of the glycation reactions on cellular viability, not only by preventing the Maillard reaction from occurring but also by restoring the enzymatic activity, which is essential for the natural protection mechanisms of the cells. Technologies that optimize metabolic activity restore the protective capacity and boost DNA repair will thereby enhance the cellular resistance to stress induced by exposure to the environment and slow down the aging process.

CONCLUSIONS

There has been an interesting evolution over the last 10 years in understanding the causes of the premature skin-aging process. From the very general free radical theory of aging, to the more specific Maillard theory of aging, the scientific research seems to seek deeper into the real causes of the premature dysfunction of cellular protection and repair mechanisms. It has been postulated that the defense mechanism expressed by mammalian cells is the result of an evolutionary process that has allowed cells to adapt to an oxidative, DNA damaging environment. Assuming this is true, it becomes difficult to understand why exposure to the environment can still result in premature skin aging.

Two important factors could explain this condition:

1. A significant boost in the level of exposure to oxidative stress due to an increase in environmental pollution and higher levels of UV exposure (more frequent travels and depletion of the ozone layer).
2. A malfunctioning of the cellular defense mechanisms caused by deactivation of the protection and repair mechanisms, leading to the accumulation of deficient lipids, proteins, and DNA.

The gradual increase in the level of oxidative stress caused by the increased pollution of the world's atmosphere is a well-accepted fact that we do not intend to discuss within the scope of this chapter. The deactivation of the cellular defense mechanism however is of far more interest, especially in the light of the gradual increase in prevalence of obesity and cigarette smoke exposure over the past few decades. Both these factors cause AGEs to accumulate faster in the skin.

In this chapter, we have reviewed how the glycoxidation process has an impact not only on the structural proteins of the skin such as keratins, collagen, and elastin but more importantly on the functional enzymes with an essential role in providing the cells with the capacity to energize, repair, and protect skin cells.

In complete agreement with Monnier (3), we believe that the glycoxidation process plays a major role in the premature-aging process, as it is involved in three major processes involved in the accumulation of damage in the cells (Fig. 1).

1. The glycoxidation process affects the cell's metabolic function by causing damage to mitochondrial proteins, resulting in a heightened release of intracellular ROS.
2. The glycoxidation process causes a deactivation of the essential enzymatic antioxidant activity, thereby further enhancing the oxidative damage to cellular components.
3. The glycoxidation process is linked to the deactivation of the enzymatic DNA repair mechanism, thereby contributing to the accumulation of DNA damage.

As a result, we see an increase in apoptosis or cellular senescence, possibly even the development of cells with erroneous genomic structure. The combination of these three factors can therefore explain why the glycation process contributes to skin aging.

The faster accumulation of AGEs in skin as a function of body weight and smoking behavior is believed to be accompanied by an enhanced level of damage induced by the glycoxidation process and a concomitant reduction of the cellular defense mechanisms.

The technologies we have described in this chapter can intervene at multiple steps in this sequence of events. The topical application of products containing a well-balanced mix of technologies to control glycoxidation and its consequences can help to restore a functional level of cellular defense. These products should therefore be of great help in maintaining cellular homeostasis and preventing premature skin aging.

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25 Spectrophotometric Intracutaneous Analysis (SIAscopy)

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WHAT IS SIASCOPY?

Spectrophotometric intracutaneous analysis (SIA)scopy is a skin-imaging technique that allows the rapid, noninvasive in vivo quantification and assessment of (eu) melanin, (oxy) hemoglobin, and dermal collagen within human skin. A powerful feature of SIAscopy is that it produces independent linear measurements of each of these endpoints, which can also be mapped over the skin, producing images called SIAscans. SIAscopy was originally developed for the assessment of malignant melanoma (1) where the accurate assessment of melanin, blood, and collagen has been shown to increase diagnostic accuracy (2) for the disease.

SIAscopy measures underlying histological parameters through the use of a model of tissue coloration, providing a cross-reference between spectral measurements and histology. The model is constructed by computing the spectral composition of light remitted from the skin, given parameters specifying its structure and optical properties, providing a unique mapping between the spectral measurements and the histological parameters (3). For each histological component, a parametric image is then created, providing the magnitude of each at all pixel locations. This approach requires two inputs: the first is a set of parameters that characterize a given tissue by specifying its components, their optical properties, their quantities, and their geometry; the second is a method for computing the remitted spectra from the given parameters.

CONSTRUCTION OF THE MATHEMATICAL OPTICAL MODEL OF HUMAN SKIN

The skin consists of a number of layers with distinct functions and optical properties as shown in Figure 1. Light incident to the skin penetrates the superficial layers, and while some of it is absorbed, much is remitted back and can be measured.

The stratum corneum is a protective layer consisting of keratinized squamous cells (corneocytes), and it varies in thickness across the body. Apart from forward scattering of incident light, it is optically neutral (4). The epidermis is composed of several layers of differentiating keratinocytes and also contains pigment-producing cells, melanocytes, and their product, the melanins. The melanins are complex heteropolymers that strongly absorb short-wavelength radiation, i.e., light in the blue part of the visible spectrum and radiation in the ultraviolet (UV) waveband (in the latter case, therefore, acting as a filter to protect the deeper layers of the skin from the well-documented harmful effects of UV radiation). Within the epidermal layer, there is very little scattering and that which does occur is forward directed. This means that all light not absorbed by melanin can be considered to pass into the dermis. The dermis is composed largely of collagen fibers, and in contrast to the epidermis, it contains sensors, receptors, blood vessels, and nerve endings. Hemoglobin, present in blood vessels within the dermis, acts as a selective absorber of light. The dermis consists of two structurally different layers, papillary and reticular, which differ principally in the size of collagen fibers. The scale of the collagen fibers in the papillary dermis (diameter of an order of magnitude less than the incident visible light) makes this layer highly scattering, i.e., any incoming light is scattered with a proportion directed back toward the skin surface. The scatter is greatest at the blue end of the spectrum, decreasing with increasing wavelength

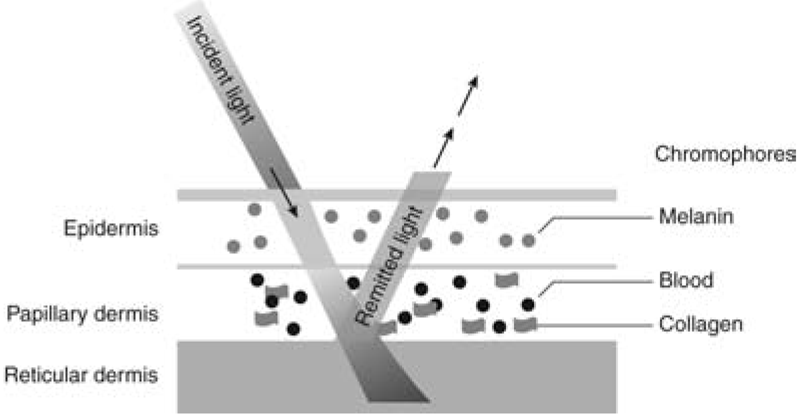

Figure 1 A schematic representation of skin layers (*labels on the left*) and chromophores (*labels on the right*).

(through green and red and into the infrared). Within the reticular dermis, in contrast, the larger scale of collagen fiber bundles causes highly forward-directed scattering (1). Thus, any light reaching this layer passes deeper into the skin and does not contribute to the remitted spectrum.

From these first principles, therefore, the mathematical optical model for normal skin has three layers corresponding to epidermis, upper papillary dermis (with prevalence of blood), and lower papillary dermis. The range of wavelengths used by the SIAscope technique, from 400 to 1000 nm, covers the entire visible spectrum and a small range of near infrared. Recently, the model has been verified by comparing its output to that generated by a stochastic Monte Carlo method using a public domain implementation (Figs. 2 and 3) (5).

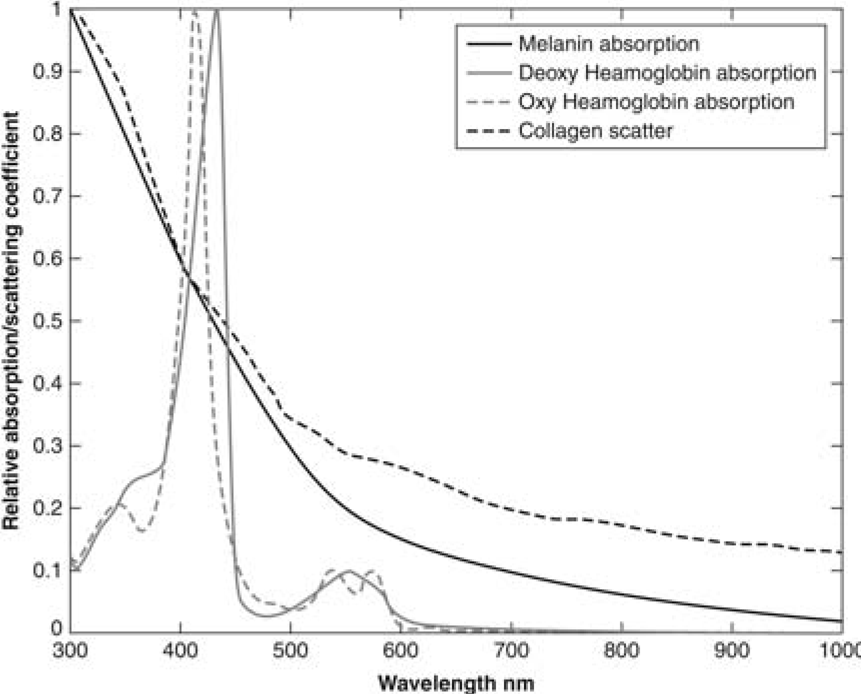

Figure 2 Absorption coefficients of principal components of human skin.

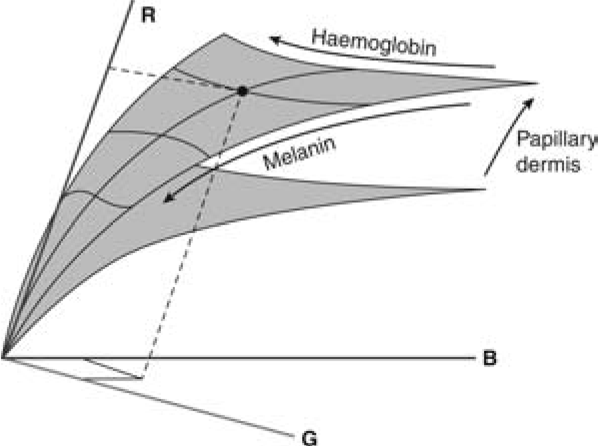

Figure 3 Schematic relationship between two reference systems: color system, with axes R, G, and B; and the histological components hemoglobin, melanin, and collagen.

CONTACT SIASCOPY

Contact SIAscopy uses a small handheld scanner (Fig. 4), which is placed in contact with the skin. This contact ensures that the distance of the skin from the lens is known and fixed, which allows exact calibration of the spectral imaging used. This control of imaging geometry allows the synthesis of accurate gray scale concentration maps each of (oxy) hemoglobin, (eu) melanin, collagen, and the position of melanin relative to the dermo-epidermal junction (Fig. 5). A small

Figure 4 SIAscope.

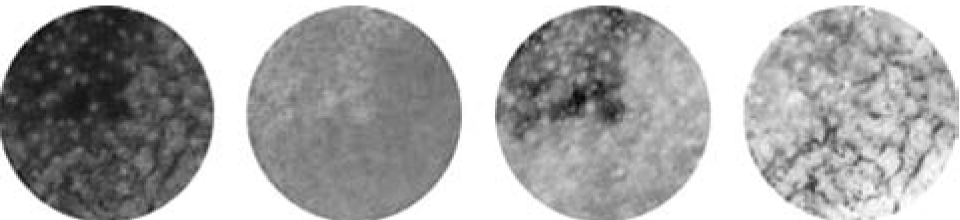

Figure 5 SIAscans showing, from left: color, collagen, melanin, and hemoglobin.

amount of matching fluid is used to ensure that optical aberration due to the refractive index of air is removed effectively.

The contact SIAscope provides a rapid and convenient method for assessing and characterizing intrinsic and extrinsic skin aging and also assessing the effects of cosmetic products, for example, the reduction of solar lentigines (6,7).

CHROMOPHORE MAPPING

We have given the term "chromophore mapping" to the synthesis and subsequent analysis of the gray scale molecular concentration maps produced by SIAscopy. These maps are readily accessible by a wide range of image analysis techniques, allowing sophisticated analysis of the arrangements of chromophores within them. We used these techniques in a study performed on 400 female Caucasian subjects aged 10 to 70 years, recruited in equal five-year cohorts (7), and demonstrated remarkable relationships for the melanin, hemoglobin, and collagen endpoints (obtained using a contact SIAscope) with age, consistent with ingoing hypotheses relating to the extent and timetabling of expression for each of these optical skin components. Moreover, sufficient dynamic range was present within the data to allow the use of this technique to track changes in these chromophores because of treatment.

SURFACE ANALYSIS

Further development of the contact SIAscope method has yielded an analysis of fine surface topograhical features ("microtexture" and fine lines and wrinkles) (8). This technique uses the fact that light returning from the skin is a blend of deeply scattered light and direct surface reflection resulting from the stacked nature of the stratum corneum. If the deeply scattered light is isolated and removed, the remaining directly reflected light carries information pertaining to the skin surface. Other workers have used this phenomenon by acquiring separate images taken in different polarization states (9). This is a useful technique, but requires specialist hardware and can suffer from registration problems between image sets.

Highly detailed information regarding the surface of the skin can be obtained using contact SIAscopy because the instrument polarizers operate only within the visible region of the spectrum and do not interact with infrared light (Fig. 6). This results in suppression of surface reflection in the visible spectrum only (which is why a matching fluid is required to obtain accurate collagen measurements). By using a technique described in (8), a prediction of

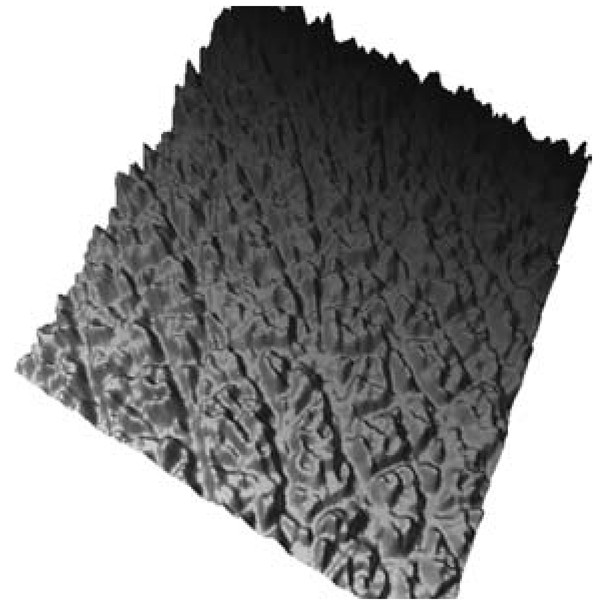

Figure 6 Skin surface micro architecture measured with a SIAscope.

what the polarized infrared signal should be is made from the polarized-visible spectra and then compared with the actual unpolarized infrared measurement. The difference is then converted into a measurement of direct surface scatter. The result is a detailed map of skin topology, which is both rapid and simple, lending itself naturally to large scale cosmetic testing and development.

HYDRATION

An adaptation of the surface analysis technique can also be used to assess skin hydration levels in vivo, allowing investigation into the effects of skin moisturizers and also diseased skin conditions such as eczema. This technique operates on the principle that direct surface reflection measured by the surface analysis technique is lower in hydrated stratum corneum. An example of skin moisturization changing over time following the application of a topical moisturizer is shown in Figure 7, with the false color images showing the spatial changes in hydration over a 10-minute period.

NONCONTACT SIASCOPY

Noncontact SIAscopy (NCS) uses a digital camera as a broad-band spectrometer to recover chromophore information over a wide area. The same mathematical model underpinning contact SIAscopy is used to create a mathematical model of the camera response to varying amounts of hemoglobin and melanin (10). The mathematical model is based on the Bayer filter response curves, the light-sensitive array that sits at the focal point of a digital camera, and the spectral power distribution of the light source (usually a flash) used with the camera. To measure the response curves of the Bayer filter, a double-monochromator is used to illuminate it with specific and highly calibrated narrow (<10 nm) wavebands of light.

A problem still exists, however, because the geometry of the scene being imaged is not known and, therefore, calibration of the measured information is difficult. To overcome this problem, a ratio of different Bayer filter wavebands forms the input to the mathematical model. The use of ratios removes the artifacts of geometry, as they are present equally in all wavebands. From first principles, this approach reduces the number of chromophores that can be measured, such that NCS is able to measure only hemoglobin and melanin. NCS is, however, extremely flexible allowing imaging of full faces, or, indeed, any body part (if the

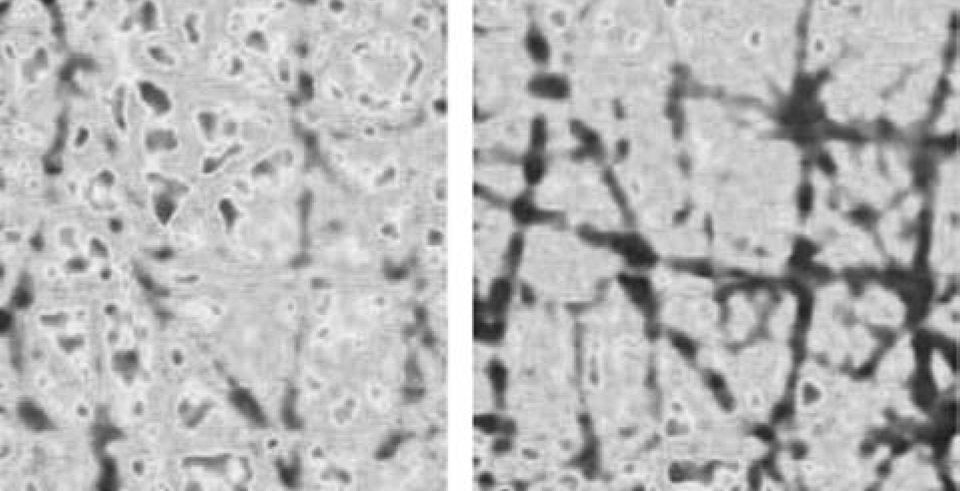

Figure 7 Changes in hydration levels in human skin (same area) following application of a topical moisturizer (before and 10 minutes after application). "Dry" skin (*left*) and "moisturized" skin (*right*) with pseudocolor scale indicating hydration state.

Figure 8 Noncontact SIAscopy in use.

Figure 9 NCS images with (*left to right*) cross-polarized color, melanin, and hemoglobin maps.

camera is used apart from the stand). Apart from the ability to measure large fields, NCS also has the advantage of eliminating the potential artifacts of pressure "blanching" that can potentially occur with any skin contact measure (Fig. 8).

Figure 9 shows NCS melanin and hemoglobin SIAscans. Localized subsurface hyperpigmentation can clearly be seen in the melanin SIAscan and telangiectasia in the hemoglobin SIAscan.

Because the NCS technique now allows routine acquisition of full-face melanin and hemoglobin chromophore maps, the method has proven an ideal clinical partner in assessing the effects of cosmetic treatments. In a recent double-blinded study, NCS was used to provide a quantitative means of measuring the effect of a vehicle containing 2% *N*-acetyl glucosamine (NAG) and 4% niacinamide (N) versus a vehicle control, applied topically, full-face, twice-daily for eight weeks, to two groups of 100 females aged 40 to 60 years, respectively, on melanized hyperpigmented spots (11). Analysis of the NCS melanin maps demonstrated clear treatment effects for the NAG + N combination versus vehicle control, resulting in a significant ($p < 0.05$) reduction in melanin spot area fraction and a significant ($p < 0.05$) increase in melanin evenness.

Finally, it should be noted that a separate study has shown an excellent correlation between NCS-derived melanin concentrations and eumelanin concentrations in human skin biopsies, spanning Fitzpatrick skin types I-VI (12). It must be concluded, therefore, that large-field chromophore mapping by NCS brings a new level of sensitivity and specificity to measurement of human skin color.

CONCLUSION

Whereas even modern high-resolution imaging still only describes skin appearance, SIAscopy explains it by separating the molecular components responsible for that appearance in the first place. In this way, SIAscopy provides the clinician and researcher alike with a powerful new tool to both measure and characterize human skin.

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26 The Visioscan-Driven ULEV and SELS Methods

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INTRODUCTION

Melanocytes and melanins are largely responsible for the phototype-related color of the skin. The varied colors of skin depend on the nature, amount, size, and distribution of melanin pigment produced by melanocytes and transferred to keratinocytes. The system is governed by the epidermal melanin unit corresponding to a functional entity composed of one melanocyte and its neighboring keratinocytes into which the melanosomes are transferred. Chronic ultraviolet (UV) radiation provides a positive signal to the exposed epidermal melanin unit. In this instance, the number of active melanocytes increases, and individual melanocytes are stimulated to produce more melanins. In addition, melanosome transfer from melanocytes to the surrounding keratinocytes is enhanced through the intervention of the protease-activated receptor 2 (1).

According to the individual melanin phenotype, age, and cumulative UV exposure, the skin commonly develops a discrete-to-severe mottled appearance. Freckles in youths and solar lentigines in older individuals are the clinical representatives of these influences. Such aspects are due to an increase in the keratinocyte melanin content (melanotic hypermelanosis), which may or may not be associated with melanocytic hyperplasia. The resulting mottled pigmentation is an early key feature of photoaging.

Photography under UVA light, which is highly absorbed by melanin, is a convenient way to highlight any discrete variation in skin pigmentation. Provided that the lighting is kept constant and the camera calibrated beforehand, this technique highlights any change in pigmentation. By using a UVA source, any skin blemish can thus be conveniently assessed by a regular photography system (2–7). As such, UV photography is used as a diagnostic tool, but it has rarely been employed for measuring pigmentation. Indeed, the latter application is not satisfactory because casual equipments generate shadows superposed to the skin pigmentation. To respond to this drawback, a charge couple device (CCD) camera equipped with an internal UV-emitting unit (VisioScan® VC98, C+K electronic, Cologne, Germany) was developed (Fig. 1). The video sensor chip must be closely applied to the skin surface to avoid shadows. The uniform illumination of the skin brings out a sharp picture of a 6 mm × 8 mm area of the skin surface. The high resolution of the system allows close assessments of the skin. The connection of the VisioScan VC98 to the computer is made through an image digitalization unit, which configures the image in pixels of 256 gray levels, where 0 is black and 256 is white. This leads to the so-called UV-light enhanced visualization (ULEV) method.

SUBCLINICAL MELANODERMA

The mosaic subclinical melanoderma (MSM) is a mottled pattern enhanced under ULEV examination (8,9). This feature is particularly prominent in photosensitive individuals with a phaeomelanin-enriched phenotype. Using this method, the increased contrast between the faint or almost invisible hyperpigmentation and the surrounding skin is the combined result of a greater reflection of UVA than visible light by collagen, and its greater absorption by melanin inside the epidermis. The boundary between the subclinical melanoderma and the surrounding skin is sharply marked. Since UV rays penetrate less deeply into the skin than visible light, it is considered that UV photography and the ULEV method catch mainly the pigmentation inside the epidermis. Thus, the pigments localized in the dermis are not detectable using these methods. Therefore, a clear distinction can be made using ULEV between an enhanced melanotic hypermelanosis and a dermal melanoderma. This distinction is useful because the former type is accessible to treatment and the latter type is unresponsive and remains as a melanin tattoo. These two conditions coexist, for instance, in melasma.

Figure 1 VisioScan camera.

Several MSM patterns have been identified (8–11). They are listed in Table 1. Spotty perifollicular dots (Fig. 2) are commonly seen on the scalp and face, occasionally extending to other seborrheic regions (8,10–12). This physiological pattern is recognized as early as during adolescence, and it does not seem altered by aging. By contrast, the interfollicular region exhibits a combination of pinpoint lesions, small macules, and globular macules (Fig. 3). This pattern appears later in life and is subject to variations with cumulative photoexposure and aging. These hypermelanotic blemishes may become confluent, and they appear more prominent on chronically sun-exposed skin. This aspect is regarded as an early sign of

Table 1 Patterns of Subclinical Skin Mottling

- Pinpoint: minute irregularly distributed darker spots
- Follicular dots: speckled perifollicular darker rings
- Small macules: small interfollicular darker areas
- Globular macules: accretive and circinate confluence of smaller macules
- Streaky macules: elongated darker areas along wrinkles
- Confluent macules: massive darker areas

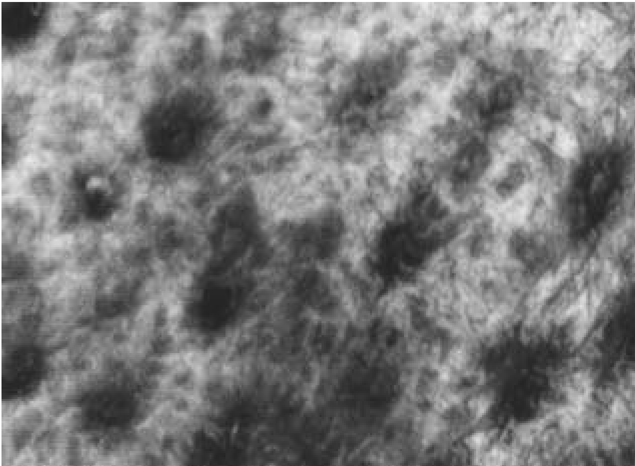

Figure 2 Perifollicular dots of subclinical melanoderma.

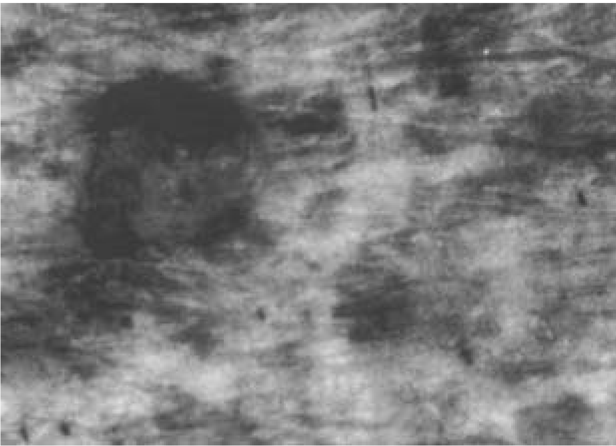

Figure 3 Macular interfollicular subclinical melanoderma.

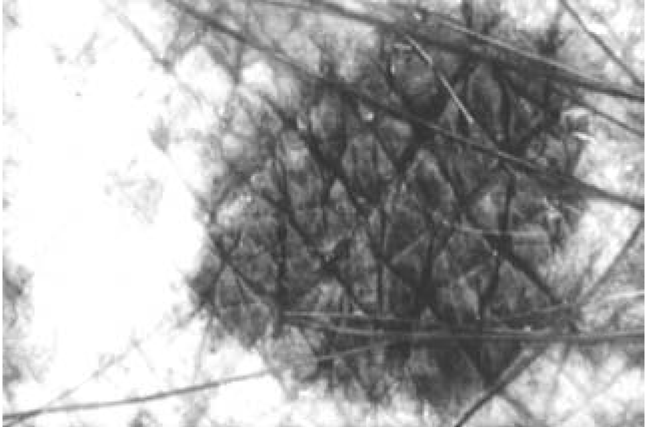

Figure 4 "Brown spot" on photoaged skin.

photoaging (9,13,14). Still another aspect is given by the streaky pattern elongated along wrinkles. This aspect is typically present on the sunny side slope of facial frown lines (8).

ASSESSMENT OF WHITENING AGENTS

The ULEV method can be employed for assessing the efficacy of cosmetic whitening products (8,15–18). Image analysis of the pictures offers objective quantification of the depigmenting effect on the epidermal melanin unit. The method has been applied to the assessment of "brown spots" (Fig. 4) corresponding to solar lentigines or incipient pigmented seborrheic keratoses (15,16,18). Such evaluations are more easily handled than the bleaching effect on melasma (17). Using the ULEV method, the observed effects of whitening agents cannot be distinguished according to the putative biological effects on tyrosinase, protease-activated receptor 2 or any other step of melanization.

Beyond conventional cosmetic whitening agents, some drugs can alter the activity of the epidermal melanin unit. Both topical corticosteroids and vitamin D analogues decrease the MSM severity as assessed by the ULEV method (14).

SKIN SURFACE MICRORELIEF AND SCALINESS

The quantitative evaluation of the skin surface microrelief is of interest in assessing some of the therapeutic and cosmetic interventions as well as for the determination of the severity in irritation damages to the skin. A measurement where the skin can be directly monitored

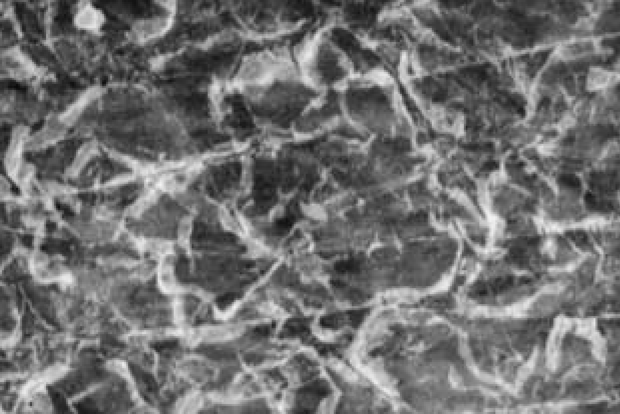

Figure 5 Scaly aspect of the skin.

optically using an image digitalization process without any sampling or replica collection should represent a great advance in technology (19–21).

The regular observation of ULEV pictures reveals any hyperkeratotic and scaly aspect of the skin surface. The lesions look whitish in contrast with the gray aspect of the normal-looking skin (19–21). The SELS (surface evaluation of living skin) method is an additional way, allowing direct and noninvasive measurement of the skin topography using the VisioScan camera (22). The actual picture of the skin can be presented with false colors and viewed either two dimensionally or tridimensionally. Four different parameters have been selected to be displayed and analyzed in the evaluation of the skin surface. Skin smoothness (SE_{sm}) is calculated from the average width and depth of the wrinkles. Skin roughness (SE_r) represents the opposite parameter to the first. Scaliness parameter (SE_{sc}) aims at representing the level of dryness of the stratum corneum (SC). The wrinkle parameter (SE_w) is calculated from the proportion of horizontal and vertical wrinkles.

In the SELS assessment, swelling the stratum corneum with water or a moisturizer does not appear to affect the roughness of the skin surface, but the value of the scaliness parameter and the number of wrinkles decrease, while smoothness increases.

The UVA of the VisioScan VC98 highlights desquamation (Fig. 5). A semiquantitative assessment is possible, but the interpretation of data is not always straightforward. For improving this assessment using the VisioScan, the transparent, sticky Corneofix® F20 tape (C+K electronic, Cologne, Germany) is slightly pressed onto the skin surface. The superficial corneocytes stick to the tape and are harvested for the assessment of their numbers and sizes. As the light is absorbed differently because of the different thickness of corneocyte clumps, they appear as dark pixels in the image. Looking at the gray level distribution in the histogram, a quantitative assessment can be performed. The regular VisioScan software distinguishes five scaliness levels. The drawback of this automatic cutting is that the shutter does not properly recognize the background in some images. In these instances, the observer can set the shutter manually by comparing the original and the segmented image with an accuracy of $\pm 0.1\%$.

COMEDOGENESIS

Microcomedones and keratin-filled funnel-like acroinfundibula can be easily identified using ULEV examination (Figs. 6 and 7). The method can thus be useful in the assessment of comedogenic and comedolytic compounds. Acne physiopathology is also highlighted by this method.

SEBUM EXCRETION

Sebum flow dynamics can be assessed using lipid-sensitive films (23). The assessment benefits from image analysis of the sebum-enriched spots. The VisioScan VC98 camera can be used for that purpose (24). The head of the camera is covered by the opaque microporous lipid sensitive Sebufix® F16 tape (C+K electronic, Cologne, Germany) before being applied to

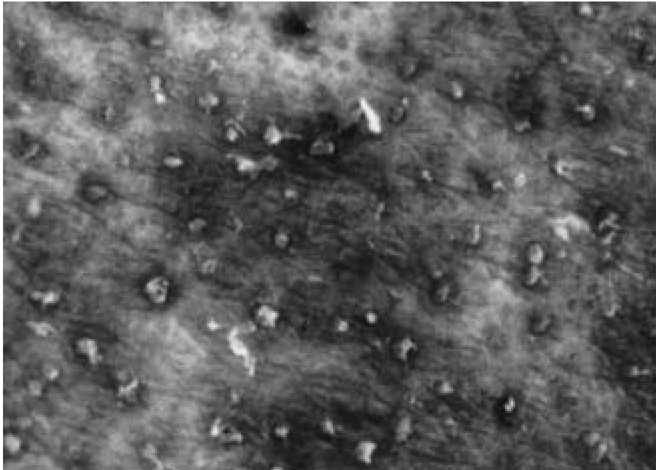

Figure 6 Microcomedones.

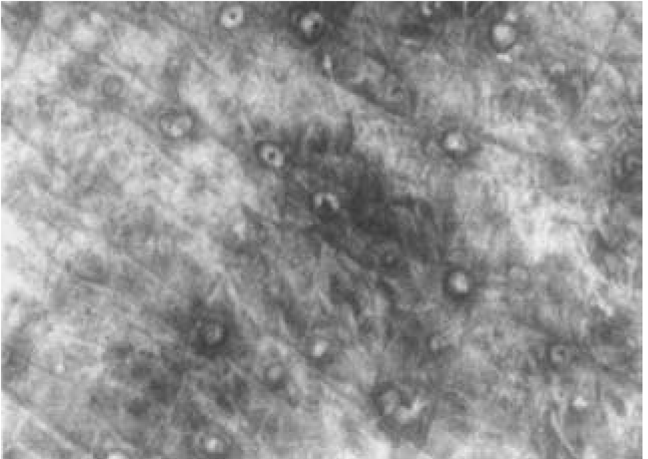

Figure 7 Open comedones.

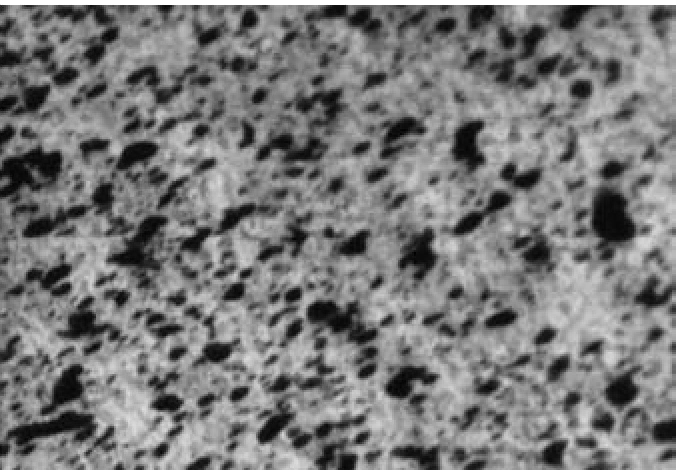

Figure 8 Sebum droplets in a Sebufix foil.

the skin. After about 30 seconds, the mean instant sebum follicular output (SFO) corresponding to the area of the transparent spots of lipid droplets is assessed using computerized image analysis (Fig. 8). The face of the Sebufix F16 in contact with the skin is glue free. Thus, the sebum can enter the micropores of the sebum-sensitive foil without any restriction. This leads to a short

Figure 9 Gray hairs dispersed in normally pigmented hairs.

measuring time after which the foil is ready to be evaluated. This requirement is important to avoid any occlusion effect. Indeed, occlusion leads to stratum corneum swelling and to increasing skin temperature, thus influencing the sebum measurements.

The more oily the skin, the shorter the collecting time is necessary. Studies performed any time after cleansing the skin is thus possible. The supplied holder presses the lipid-sensitive film lightly but constantly on the skin and leads to reproducible measurements. The special foil specifically measures the sebum output from the follicular reservoir. With this procedure, the sebum output at the skin surface can be monitored live in real time.

HAIR GRAYING

The loss of melanin content in the hair shaft is a natural manifestation of aging leading to apparent hair whitening. Hair graying can be observed as early as 20 years of age in Caucasians and 30 years in Africans; it has been reported that on average, in a cohort of Caucasians, 50% of people had at least 50% gray hair by age 50 (25). The biological processes underlying hair graying remain unclear (26). In addition, the process of hair graying is difficult to assess and quantify in vivo. The ULEV method highlights this physiological phenomenon by enhancing the contrast between graying hair, which appear bright white, and the other hair shafts (Fig. 9).

TRICHOBACTERIOSIS

Some bacterial species tend to clump along hair shafts. They are embedded in a biofilm. This condition corresponds to trichobacteriosis formerly called trichomycosis. The ULEV method is a convenient way to observe these structures, which appear as bright white sheaths encasing the base of the hair shafts (Fig. 10). This aspect is probably related to the fluorescence exhibited

Figure 10 Trichobacteriosis characterized by bacterial sheaths encasing hairs.

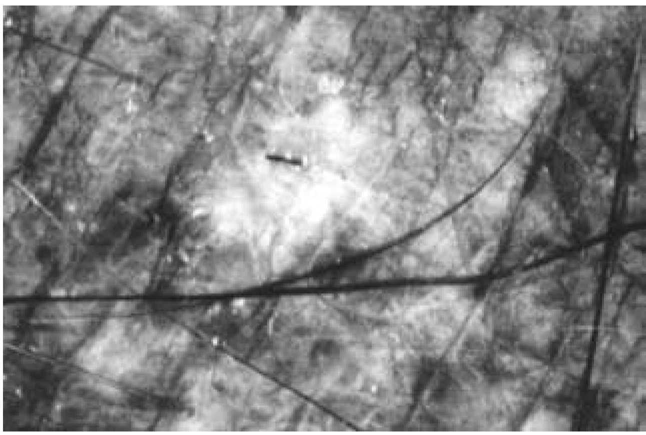

Figure 11 Area of depigmented skin in the background of mosaic subclinical melanoderma.

by trichobacteriosis under Wood's light. A similar phenomenon can occur at the site of other fluorescent lesions such as erythrasma and pityriasis (tinea) versicolor.

DANDRUFF ASSESSMENTS

Under ULEV examination, dandruffs appear as small white objects dispersed along hair shafts (27,28). Because of the high contrast with hair, a quantitative assessment is made possible without any specific sampling procedure.

RISK ASSESSMENT OF SKIN CANCERS

A correlation was found between MSM severity and the risk for developing actinic keratoses and basal cell carcinomas on facial skin and the scalp (29–34). A peculiar aspect corresponds to focal depigmentation suggesting the destruction of some epidermal melanin units (Fig. 11). Such finding could help in identifying early adult life subjects at risk of skin cancers.

CONCLUSION

UVA photography and its more recent development, using a CCD camera equipped with an internal UVA-emitting unit, are useful by different aspects. The MSM revealed by the ULEV method is one of the earliest clinical signs of photoaging. The same method also highlights scaliness and desquamation as well as a series of other potential specific conditions mentioned in this chapter.

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27 New Trends in Antiaging Cosmetic Ingredients and Treatments: An Overview

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INTRODUCTION

Aging is a natural process in which both intrinsic aging, also described as biological aging, and photoaging lead progressively to a loss of structural integrity and physiological function of the skin (1–4).

With intrinsic aging, structural changes occur in the skin as a natural consequence of the biological changes over time and produce a certain number of histological, physiological, and biochemical modifications. Intrinsic aging is determined genetically (influence of gender and ethnic group), variable in function of skin site, and also influenced by hormonal changes. Visually it is characterized by fine wrinkles. By comparison, "photoaging" is the term used to describe the changes occurring in the skin, resulting from repetitive exposure to sunlight. The histological, physiological, and biochemical changes in the different layers of the skin are much more drastic. As a result, the photodamaged skin may present various symptoms such as deep wrinkles, actinic keratoses, solar elastosis, yellowing, pigmentation disorders and premalignant lesions, skin atrophy, senile purpura, telangiectasia, laxity, and roughness.

The demographic changes in the industrialized populations (United States, Europe, and Japan) are the consequence of the rise in the number of elderly persons: the life expectancy for men (about 80 years of age) and women (far above 80 years). Women with a longer average life expectancy will spend a long lifetime in the menopausal status. They will enjoy their leisure time and are very much aware of the concept of preservation of the youthful appearance (face and whole body) and good health (4). These categories of seniors (3 or 4 $\times$ 20 years) have more financial means to spend on cosmetic products than do the very young population. Consumers are focused on their health and appearance, creating a great demand for antiaging cosmetic and oral treatments. We observe the following trends in these demands: an appeal for "Green and Natural," more natural and less invasive cosmetic substitutes instead of cosmetic surgery and skin injections without the side effects. Modern topical antiaging cosmetic products can be characterized as cosmeceutical preparations: formulated with "natural," functional active ingredients and with innovative delivery systems (penetration enhancers, nanoparticles, and porous delivery systems) (5). The entrance of a large number of men older than 60 years, who like women want to preserve their appearance and good health, has provoked the development of a specific range of skin care and antiaging products for men. The aims of this chapter are (*i*) to describe the histological, physiological, biophysical, and biochemical characteristics of biological aging and photoaging, respectively, (*ii*) to describe the clinical visual and tactile examinations and bioengineering methods to qualify and/or quantify these symptoms, and (*iii*) to give a critical overview of the different topical antiaging cosmetic ingredients and treatments. Also, oral antiaging food supplement will be mentioned. Excellent review articles on skin aging have been published (1–4,6).

INTRINSIC SKIN AGING

Intrinsic aging or chronologic or biological aging is, by definition, unavoidable, since it represents the biological effect of time on the skin, which is not influenced by repetitive sun exposure. The skin that normally ages is smooth with some deepening of the surface (small wrinkles) and loss of isotropy in the microrelief. Histologically, there is an epidermal and dermal atrophy, gradual reduction in the amount of collagen, hyperkeratosis, and some atrophy—a reduced number of melanocytes, Langerhans cells, and fibroblasts. A shortening in telomere DNA and metabolic oxidative damage are believed to play a major role in the

intrinsic aging process. Also, the glycation reaction on skin proteins occurs with time and contributes to the intrinsic aging (see the chapter "Glycation End Products" by Declercq et al.) (7). The synergy of intrinsic aging and photoaging during the life span produces a deterioration of the cutaneous barrier. Aged skin is more susceptible to extreme dryness, itching, cutaneous infections, autoimmune disorders, vascular complications, lack of elasticity, and an increased risk for premalignant and malignant lesions. The major intrinsic skin aging factors are anatomical variations, gender, ethnicity, and hormonal changes (4).

Ethnicity

It is obvious that differences in pigmentation have the greatest effect on aging. High levels of melanin pigmentation are protective with regard to aging. Black skin shows smaller differences between exposed and unexposed sites. Skin cancer incidence rates between white Caucasian and Africans indicate that pigmentation provides a significant level of protection from UV radiation. It appears that wrinkling in Asian skin occurs later and with less severity than in Caucasian skin, probably due to ethnicity but also due to differences in lifestyle and diet. For a complete recent review of the properties of skin related to race, sex, and site, the reader is referred to the chapter "Biophysical Characteristics of the Skin: Relation to Race, Sex, Age, and Site" by Couturaud (8).

Anatomical Skin Sites

Large variations in some skin properties (hydration, transepidermal water loss, epidermal lipids, sebum secretion, and mechanical properties) have been observed with respect to the studied body site. There are also large differences in skin thickness in function of the body site, ranging from very thin on the eyelids to more than 5 mm on the sole of the feet. A regional variation is clearly observed when considering the quantity and composition of lipids in the stratum corneum. Because of thickness and sebum secretion, the viscoelastic properties of the skin are very different at the forehead, nose, and cheeks compared with the forearm.

Gender and Hormonal Changes in Cutaneous Tissues

The influence of gender on the physicochemical properties of the skin is often reported in the literature (8); however, no clear-cut conclusive information on the influence of sex on intrinsic aging is reported. Nevertheless, there are significant morphological differences according to sex: total skin thickness is greater for men on most skin sites.

The changes of estrogen levels in the female skin when comparing pre- with postmenopausal women have well been described in the literature. Intrinsic aging in men is a progressive phenomenon: one observes a smooth decline in skin properties in function of time. The same curve shows clearly a sharp acceleration of decline around 50 to 60 years, which corresponds to the passage from pre- to postmenopause.

EXTRINSIC SKIN AGING

Chronic repetitive exposure of human skin to solar UV rays causes marked morphological, histological, biochemical, and biophysical changes that are described as photoaging. As already mentioned here above, the clinical signs of photoaging are fine and coarse wrinkles, actinic keratoses, solar elastosis, yellowing, pigmentation disorders and premalignant lesions, skin atrophy, senile purpura, freckles, solar comedones, telangiectasia, laxity, roughness, and extreme dryness. Most of the alterations are apparent in the dermis of photoaged skin and include massive accumulation of abnormal elastic fibers, loss of collagen, and increase of GlycoAminoGlycans (GAGs). The fibrous network degenerates into amorphous masses modifying the structure of the matrix. UV-mediated damage to elastic fibers and fibroblasts provokes the accumulation of this elastotic material. Alteration in the dermal extracellular matrix in photoaged skin could involve the production of abnormal matrix molecules by the fibroblasts. Also is noted the increase of activity of metal-binding matrix-degrading enzymes (9).

Exposure to UV Light

Research in the field of photaging has resulted in a better understanding of the molecular mechanisms of the aging process. UV light penetrates skin, and depending on its wavelength, it interacts with different skin layers located at different depths. Shorter wavelengths (UVB,

280–320 nm) are mostly absorbed in the epidermis, affecting predominantly keratinocytes. Longer-wavelength UV light (UVA, 320–340 nm) penetrates deeper and can interact with keratinocytes and dermal fibroblasts. The melanin-pigmented cells absorb UV light and thus protect skin cells from exposure to UV radiation. UVA light mostly acts indirectly through generation of reactive oxygen species (ROS). "ROS" is a general term for oxygen-derived species (superoxide anion, hydroxyl radical, and certain peroxides). ROS exert a multitude of effects such as lipid peroxidation, activation of matrix metalloproteinases and generation of DNA, and mitochondrial DNA damage (10,11). UVB light can also generate ROS, its main mechanism of action is the interaction with DNA, provoking DNA damage. The skin presents an antioxidant defense mechanism, including an enzymatic and nonenzymatic system. As an enzymatic system, we have superoxide dismutases, catalase and a selenium glutathione peroxidase, converting superoxide anion in hydrogen peroxide and hydrogen peroxide in water, respectively. As endogenous antioxidants we find glutathione, α-lipoic acid, coenzyme Q, etc. Other antioxidants, such as vitamins and polyphenolic compounds, can be obtained from diet. An increase of ROS (depletion of the antioxidant system) will cause oxidative stress, leading to potential tissue damage.

Sun damage also creates a state of chronic inflammation, with the release of proteolytic enzymes by the inflammatory system, disrupting the dermal matrix (1). Sunburn is a well-known acute effect of sun exposure and is clinically visible as an erythema triggered by inflammation. After a certain threshold of UV exposure is reached, a delayed and prolonged vasodilatation allows the passage of lymphocytes and macrophages in the tissues, which induces inflammation, leading to a histological appearance of chronic inflammation (9). The intake of antioxidants or anti-inflammatory compounds was suggested to reduce erythema provoked by UV irradiation (11).

Effect of Smoking

It is now well established that smoking has an aggravating effect on skin aging. Cigarette smoke represents one of the greatest exogenous sources of free radicals. The skin is diectly and intensively exposed to cigarette smoke and to smoke from exhalation. Even external exposure to cigarette smoke (secondhand cigarette smoke) prematurely ages the skin. Smoking provokes elastosis, telangiectasia, skin roughness, and premature wrinkles on facial skin due to the vascular constriction of nicotine (12). A clear dose-response relationship has been observed between smoking and wrinkling (4). Smoking also increases ROS formation and is an important risk for cutaneous squamous cell carcinoma.

DESCRIPTION OF THE DIFFERENT OBJECTIVE EVALUATIONS OF PHOTOAGING

Evaluation of Microrelief and Wrinkles

Wrinkles in the Facial Skin

Facial wrinkles are the most characteristic morphological changes observed in photoaged skin. Therefore, many quantificative methods to analyze wrinkles have been developed (13). First, direct visual evaluation of the face (front and lateral views) and rating of different well-selected aspects of photoaging (fine and coarse wrinkle, roughness, yellowing solar lentigines) by experienced examinators using a numerical scale are very popular in use and rather simple to carry out. The subjects themselves can judge their degree of photoaging and the efficacy of cosmetic products on the severity of wrinkles. Different clinical studies have shown that there is more or less a good correlation between the rating of the subjects and the evaluation of experienced investigators. Although visual evaluation is rather subjective and evaluator dependent, both methods are widely used in clinical testing of wrinkle-smoothing products. The same visual evaluation of the face can be carried out on macro digital photographic pictures obtained using a standard setup for positioning the face of the subject and lateral illumination. Evaluation of the wrinkles using silicone skin replicas has been employed complementary to visual examinations. Skin microrelief and coarse wrinkles (crow's feet) can be taken using these replicas. Many techniques using different principles are now available to analyze the replicas (mechanical and laser profilometry, two-dimensional image analysis, confocal micoscopy, etc.).

In vivo instruments have been developed to study directly the morphological structure of the skin. The PRIMOS® system is based on the image analysis with image triangulation using a micro-mirror device projection on the skin (14). It is possible to measure the skin topography by using a UVA-light video camera Visioscan VC98® with a SELS® software (Surface Evaluation of the Living Skin) technique providing the standard roughness parameters (Rt, Rm, Rz, and Rp) (15). The microrelief of the skin surface and particularly the isotropy/anisotropy of this microrelief can be evaluated from the images obtained using a charge coupled device (CCD) visible video camera (Intuiskin IntuiPro Pack®, Crolles, France). Image analysis gives the classical roughness parameters and the degree of anisotropy of the skin surface (16).

Reviscometer® (17)

Aged skin is characterized, among other symptoms, by profound changes in the orientation of the collagen fibers in the dermis. In young skin, these fibers are very small and oriented almost in all directions (isotropy). When the skin gets older, thickening of the fibers occurs with a preferential orientation of the thick collagen fibers (anisotropy). The degree of isotropy/anisotropy in the dermis can be evaluated by measuring the propagation speed of an acoustic shock wave applied on the skin surfaces following different orientations with a Reviscometer® (18). The propagation speed of a shock wave applied on the skin surface at different angles gives a degree of isotropy/anisotropy clearly related to skin aging.

Viscoelastic Properties of the Skin Using the Cutometer®

Photoaging will provoke profound changes in the structure of the dermis and consequently changes in the mechanical properties of this layer. The Cutometer® (19) is a suction device that measures the vertical displacement of the skin when a small vacuum (suction) is applied on the surface. The vertical displacement of the skin can be measured in function of vacuum (stree-strain curve) or in function of time when suddenly vacuum is applied and released (strain-time curve). From the strain versus time curve, the pure elastic, pure elastic recovery, and viscoelastic properties can be quantified and are clearly related to the degree of aging and skin laxity.

Ultrasound Measurements at 20 MHz

Querleux et al. (20) described on echographic pictures of photoaged skin, between the epidermis and reticular dermis, a dark echogenic band, which has been called subepidermal low echogenic band (SLEB). Several studies have proposed that the evaluation of the SLEB (thickness, number of low echogenic pixels, and the ratio of the number of pixels in the SLEB to the number of pixels in the deeper dermis) to be markers of photoaging (21–23). Also, the mean total thickness of the dermis and its density can be computed.

Hydration Measurements

Typical photoaged skin shows symptoms of roughness and dryness, although extreme dryness (ichtyosis) can be present on nonexposed skin areas (lower part of the legs in very old persons). Typical hydrating and smoothing products are used in the treatment of dryness. In addition, most antiaging cosmetic preparations contain hydrating ingredients and skin-smoothing ingredients. Quantitative hydration evaluation of the upper layers of the skin, e.g., horny layer and upper epidermis, are numerous: electrical impedance and capacitance measurements (24), Fourier transformed infrared spectroscopy with an attenuated total reflection unit, FTATR (25), and confocal Raman microscopy (26). The last two instruments, although they give quantitative data directly related to the amount of water present in the horny layer, are less used in routine clinical research because of the high price of purchase. Most routine hydration measurements are carried out using the electrical impedance/conductance properties [Dermalab®, Hadsund, Denmark (27), Skicon®, Hamamatsu, Japan (28), DPM Nova®, Portsmouth, New Hampshire (29)] or capacitance properties [Corneometer®, Cologne: Germany (30) and MoistureMeter®, Kuopio, Finland (31)] of an alternating electric current applied on the skin surface. It must be pointed out that the data of these instruments (electrical units or arbitrary units) are related to hydration but not linearly proportional to the percentage of water present in the horny layer.

CRITICAL OVERVIEW OF THE TOPICAL ANTIAGING INGREDIENTS AND TREATMENTS

Before starting to give an overview of the different categories of antiaging ingredients, it is important to make the following remarks.

As mentioned above, the clinical symptoms of photoaging are multiple: fine and coarse wrinkles, actinic keratoses, solar elastosis, yellowing, pigmentation disorders, premalignant lesions, skin atrophy, senile purpura, freckles, solar comedones, telangiectasia, laxity, roughness, and extreme dryness. Obviously, it is impossible with one antiaging cream/gel to treat all these symptoms simultaneously. Some symptoms such as actinic keratoses, purpura, pigmentation disorder, or telangiectasia can be reduced only with focused treatments containing specific ingredients. For example, a skin-whitening product, which reduces skin pigmentation problems on the face, hands, and arms.

As a consequence, we will discuss in this chapter the most cited clinical symptoms of photoaging, such as fine and coarse wrinkles, skin laxity, roughness, and dryness. With the exception of dryness (horny layer), these clinical symptoms are related to major alteration of the dermis.

Cosmetic or Cosmeceutical Antiaging Products?

The antiaging "active" ingredients, which will be described hereafter, are cosmetic products with properties very similar to a pharmaceutical product (drug-like benefits)—cosmeceuticals (32,33). However, the EEC legislation and particularly the FDA do not recognize such category as "cosmeceuticals" and consider these formulations as cosmetic products. The term cosmeceutical or its synonym terminology is often misused in cosmetic advertising and may be misleading to the consumer. He interprets a cosmeceutical to be similar to a pharmaceutical product and that these formulations have passed with success the tests for efficacy, safety, and quality control as required for a medical preparation (33). Indeed, product testing may also be warranted by the companies to document claimed efficacy and to support marketing (34). Generally speaking, the quality control testing on ingredients and safety testing are of good quality, and the used ingredients are mostly safe. However, these ingredients may not be as efficient as claimed, and the concentrations used in these formulations will not necessarily correspond to an "effective" concentration. This can be the case with many plant extracts with antioxidant properties. There are no proven effective topical antiaging ingredients/or treatment that completely eliminates the symptoms of skin photoaging, but there are products and treatments that can visibly reduce or slow down these symptoms: it is more correct to consider reduction of the appearance of aged skin. Many cosmetic products claim to reduce the clinical signs of photoaged skin; however, there are very few scientific, randomized, double-blind, placebo-controlled, clinical studies to support these claims (35). Finally, many antiaging claims are based on in vivo testing on cells or simple skin models but not in vivo on a sufficient number of human subjects.

Moisturizers

The classical moisturizers are used for treating dryness in the photoaged skin: polyols (glycerin, propylene glycol, butylene glycol and sorbitol), urea, lactic acid and salts, hyaluronic and salts, pyrrolodone-5-carboxylic acid and salts (PCA), panthenol, amino acids and proteins (collagen and proteins from wheat, rice, silk, soybean, and oat). See the chapter "Hydrating Substances" by Lodén (36). More sophisticated peptides and proteins are presently used as moisturizers. It concerns generally more lipophylic quaternary *N*-alkyl derivatives of proteins or small polypeptides with long side chains (ester binding) to increase the lipophilic character: binding to the horny layer and a better percutaneous absorption. Recently, the use of small peptides, which mimic the amino acid sequence of collagen or enzymes (biomimetic peptides), has been proposed as mosturizers (37). For a better percutaneous penetration, small fragments of hyaluronic acid were also suggested (38).

Surface-Smoothing Agents

Surface-smoothing silicone derivatives (39) or filmogen proteins such as quaternized proteins or silk, rice and oat (36), and skin feel agents (40) are used in antiaging products. The high adsorption to the skin surface provokes a smoothing of the skin surface and is at the same time humectant.

Retinoids

The use of retinoic acid as a topical antiaging ingredient has been extensively investigated. The efficacy of this topical drug has been scientifically proved: reversing fine wrinkles, non-homogenous skin pigmentation and rough skin surface (41). However, the typical side effects of topical application of retinoic acid, known as retinoid dermatitis, occur in most patients: erythema, scaling, and pruritis limit the use of this topical drug. Cosmetic research on similar molecules with the same antiaging properties but without the irritant side effects are going on. Retinol, retinal, and different retinol derivatives (retinyl palmitate = lipophilic derivate and retinyl propionate = hydrophilic derivative) are frequently proposed in topical antiaging treatments (42–44). Retinol and retinal must be metabolized in the skin to the active trans-retinoic acid. The incoporation of retinol and probably also retinal in cosmetic preparations poses the problem of stability (slow oxidation of retinol in function of time). New delivery systems and derivatives of retinol may solve the problem of stability of these preparations.

α- and β-Hydroxy Acids

Hydroxy acids such as glycolic acid and lactic acid at lower concentrations than those used in dermatological peelings are designed to remove the superficial epidermal layers of the skin and give a younger appearance (exfoliation and rejuvenation) (45,46). There is an effect on the synthesis of ceramides by the keratinocytes and on the lipidic structure of the stratum corneum (modulation of the barrier function). Stimulation of the epidermis and dermis cells. Sort of soft chemical peeling without the side effects of higher concentrations (redness, burning, and swelling). The use of more lipophilic derivatives of β-hydroxy acid (C8-lipohydroxy acid) has recently been proposed as a treatment of photoaged skin. The slower rate of penetration of this soft lipophilic exfoliating agent (reservoir effect in the horny layer) assures an effect at lower concentration with less side effects (47).

Antioxidants

In the field of cosmetic treatments, antioxidants are widely innovative ingredients in topical antiaging applications. The chapter "Antioxidants" by Weber et al. gives an overview of the current state of research on the use of antioxidants in topical cosmeceutical applications (48). The chapter on "Oral Cosmetics" by Demeester et al. (11) explains the benefits of antioxidants in antiaging oral cosmetics. The target of these active ingredients is to counter the ROS molecules produced in the skin. The most important antioxidants are vitamin C (49,50), vitamin E (51,52), coenzyme Q (53), and α-lipoic acid (54). The combined topical use of retinoids, niacinamide, *N*-acetyl glucosamine, and moisturizing peptides has recently been reported (55).

Some double-blind, vehicle-controlled antiaging treatments over long periods have been reported in the literature.

The topical application of a cream containing a mixture of folic acid and creatine showed improvements in the mechanical properties of the skin (56). Also, an improvement of the photoaged dermal matrix by topical application of a cosmetic "antiaging" product containing a lipoentapeptide, white lupin, and retinyl palmitate was reported by Watson et al. (44).

Plant antioxidants have recently been investigated with promising results: polyphenols in green tea, soya isoflavones genistein and daidzein, tannins in pomegranate, and resveratrol in seeds of grape.

The topical use of resveratol, a polyphenol from red grapes with great antioxidant activity in skin care formulation, has been reported by Baxter (57). The efficacy of soybean extract against photoaging was investigated in a double-blind, vehicle-controlled clinical study by Wallo et al. (58), indicating the promise of the soya isoflavones. Finally, the effects of green tea extracts in the clinical and histological appearance of photoaging skin were examined by Chiu et al. (59).

UV Filters

Protection of the skin against photoaging is ensured by the use of broad-spectrum UV filters either by radiation absorption or by light reflection. As a consequence, one reduces the total lifetime UV dose. Most cosmetic antiaging creams and lotions contain a mixture of UVA and UVB filters with SPF around 15 to 20.

Table 1 List of the Plant Extracts Mostly Used in Commercial Antiaging Cosmetics

Sesamum indicum, Prunus Amygdalis dulcis, Phyllanthus umblica, Siegesbeckia orientalis, Theobroma cacao, Bytospermum parkii, Mangifera indica, Mentha piperada, Aleurits moluccana, Glycurrhiza glabra, Arcostaphylos uva, Imperata cylindrica, Centella asiatica, Echinacea purpurea, Camelia sinensis, Thea sinensis, Hordeum vulgare, Crithium maritimum, Plantago lanceolata, Phellodendron amurense, Spirea ulmaria, Artemisia vulgaris, Santalum album, Rosmarinus officinalis, Centella asiatica, Curcuma longa, Aloe vera, Arnica calendula, Ginkgo biloba, various algae such as Fucus vesiculosus, Laminaria flexicaulis, Ascophyllum nodosum.

Small Lipopolypeptides

Is palmitoyl pentapeptide a new skin rejuvenation compound? It is a relatively small molecule: an ester between palmitic acid and a pentapeptide chain to enhance oil solubility for the sake of better skin penetration. The typical sequence of one of these pentapeptides is Lys-Thr-Thr-Lys-Ser, a sequence, which mimics the specific sequence of a domain in procollagen type I (Pal-KTTKS) or Matrixyl® (60–62). In vitro studies have shown that the addition of Pal-KTTKS stimulates the synthesis of key constituents of the skin matrix: collagen, elastin, and GAGs. In vivo studies with the topical use of the lipo-mimetic pentapeptides are currently under way and show an improvement of skin appearance (63,64).

Miscellanous Plant Extracts

When looking at the composition of most commercial antiaging formulations, one always notices the use of plant ingredients. Table 1 gives an overview of the plant extracts mostly used. The rationale of antiaging efficacy of these plant extracts is mainly based on folk medicine, general anecdotic or subjective information, and much less on sound clinical published studies concerning double-blind, vehicle-controlled antiaging treatments over long periods.

A Stretch-Mark Cream Which Was Used as an Antiwrinkle Cream

The last marketing furor in Europe and United States was regarding an antiaging product, a commercial product named Strivectin-SD® (65), a rather surprising story. As claimed by the company in their adverisement, originally the product was developed as a cream for treating stretch marks on abdomen and legs. This body cream contains many ingredients, including a patented Striadil Complex® (plant extracts such as Phyllantus emblica, Siegesbeckia orientalis, and others) and the patented antiaging lipo-pentapeptide. Preliminary clinical trials with Strivectin-SD showed that the product was able to reduce the appearance of existing stretch marks. Then it appeared that the product could also be used on the face for treating photoaging. So far, the scant clinical data are encouraging (61). The major question remains: Is there a sound scientific basis under the HYPE? How does this new antiaging cream exactly work? Besides the lipo-pentapeptide derivative, which are the other possible "active plant ingredients"? The formulation contains 24 different plant ingredients. Further clinical antiaging studies with these peptide-containing formulations and plant extracts are necessary in the future before these efficacy claims can be confirmed. At this stage, only preliminary antiaging testing results of this new cream were presented (6).

ORAL ANTIAGING COSMETICS

The term "oral cosmetics" is used with respect to dietary supplements, which claim to have beneficial physiological effect on skin, hair, or nails; these are preparations for oral use only such as capsules, tablets, liquids, or granulates. Oral cosmetics are mainly focused on antiaging and slimming/anticellulite preparations. Clearly, the oral route of admnistration requires special product characterizations such as toxicology, bioavailability, and the metabolization of its components. Furthermore, these clinical trials should be randomized, placebo controlled and double blind to minimize the bias of the results of the studies. In addition, any change in dietary habits or lifestyle should be avoided, and the intake of other food supplements should be kept under control during the complete study. An overview of the most recent antiaging oral cosmetics available and their results is given in the chapter "Oral Cosmetics" by Demeester et al. (11).

CONCLUSIONS

The importance of antiaging cosmetics is actually well established. Many molecules are promising "active" ingredients to slow down the different symptoms of photoaging. Even though a growing amount of scientific literature deals with clinical antiaging studies, there is still a need of further investigation to demonstrate the efficacy of these various ingredients in topical formulations.

However, a sound approach of antiaging strategy is a lifestyle, which does not have any marketing appeal but is probably effective. It consists of a healthy balanced diet, regular physical exercise, avoidance of smoking and excessive alcohol, a good basic day and night skin care treatment, use of sunscreens with high SPF during the day, maximal sun avoidance, particularly for those with a light complexion (phototypes I and II) and those with an outdoor lifestyle (35). The synergy of combining the topical use of antiaging creams with oral food supplements appears to be an interesting approach in the future.

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28 Antioxidants

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INTRODUCTION

In the field of dermatology, antioxidants are a widely used and innovative ingredient in topical applications. This chapter is intended to provide an overview of the current state of research on the use of antioxidants in cosmeceutical applications as well as safety aspects. The most important antioxidants, vitamin E, vitamin C, thiols, and flavonoids, will be introduced and their intriguing cooperation, as well as their role in signal transduction events, will be discussed.

The body is continuously exposed to oxidants. Endogenous sources arise as a consequence of normal metabolic pathways. For example, mitochondrial respiration produces superoxide and hydrogen peroxide, while enzymes such as lipoxygenase, xanthine oxidase, and NADPH oxidase produce hydroperoxides and superoxide. Exogenous oxidants arise from environmental pollutants such as smoke, smog, UV radiation, and diet. In response to these oxidants, a number of systemic antioxidants are available whose function is to scavenge reactive oxygen species, preventing damage to macromolecules such as lipids, DNA, and proteins. Antioxidant protection arises from molecules synthesized as part of metabolism, e.g., glutathione (GSH) and uric acid; essential vitamins, which must be taken in from the diet, e.g., vitamin E and C; and enzymes, which decompose reactive oxygen species, e.g., superoxide dismutase, catalase, and GSH peroxidases. These systems provide protection in various intra- and intercellular compartments. Usually there is a tight balance between oxidants produced and antioxidant scavenging; however, under certain conditions, the balance can be tipped in favor of the oxidants, a condition called "oxidative stress." Potentially oxidative stress can be caused by an increase in the number of oxidants, for example, as a result of cigarette smoking or UV irradiation, or by a deficiency of any one important antioxidant. This is of major concern since oxidative stress has been implicated in a number of conditions including atherosclerosis, skin cancer, and photoaging.

VITAMIN E

Vitamin E is the major lipophilic antioxidant in skin, and it is the most commonly used natural antioxidant in topical formulations. It is found in all parts of the skin, the dermis and epidermis as well as in the stratum corneum (SC), and is believed to play an essential role in the protection of biomolecules from oxidative stress.

Vitamin E is a family of eight naturally occurring isoforms: four tocopherols (α-,β-,γ-,δ-form) and four tocotrienols (α-,β-,γ-,δ-form) (1) (Fig. 1). All forms consist of a chromanol nucleus that carries the redox-active phenolic hydroxyl group and a lipophilic tail. While tocopherols contain a phytyl side chain, the isoprenoid tail of the tocotrienols is polyunsaturated, making the chain more rigid. The side chain is anchored in lipid membranes while the nucleus is located at the lipid/aqueous interface. Even though the radical scavenging activity

Figure 1 Naturally occurring forms of vitamin E. (**A**) Tocopherols contain a saturated side chain, (**B**) while the isoprenoid side chain of tocotrienols is polyunsaturated. The α-forms contain both methyl groups on the chromanol nucleus (1,2), while the β-forms contain only methyl group (1), the γ-forms only (2), and the δ-forms none.

of the different isoforms is essentially identical, their biological activity after oral administration differs dramatically (1). This phenomenon can be explained by the existence of an α-tocopherol transfer protein in the liver that positively selects RRR-α-tocopherol and incorporates it into VLDL, which leads to recirculation of the α-tocopherol pool, while this transfer protein does not recognize the other forms, which are therefore excreted more rapidly (2).

In skin, as in the other human organs, α-tocopherol is the predominant form of vitamin E with 5 to 10 higher concentrations than γ-tocopherol. Delivery of vitamin E to the SC occurs in two different modes. On the one hand, it is stored into differentiating keratinocytes and moves up into the newly formed SC, which leads to a gradient-type distribution of α-tocopherol with decreasing concentrations toward the skin surface (3). On the other hand, vitamin E is secreted by sebaceous glands and reaches the SC from the outside. In sebaceous gland-rich regions such as the face, this delivery mechanism is responsible for the enrichment of the outer SC with vitamin E (4). Sebaceous gland activity is low in children and starts to increase during puberty to reach a plateau at the age of 19 and remains relatively constant until it starts to decline in women starting in the cohort of 50 to 59 years of age and in men starting from 70 years of age. (5). Thus, children as well as women older than 50 years and men older than 70 years may have a compromised mechanism of vitamin E delivery to the skin surface. These collectives may potentially benefit from topical vitamin E supplementation.

After oral supplementation, vitamin E reaches the skin via sebaceous glands after a period of at least two to three weeks (6). These findings demonstrate that the sebaceous gland route is a significant delivery mechanism for vitamin E.

Various oxidative stressors have been shown to deplete vitamin E, among other antioxidants. In the epidermis, at least four minimal erythemal doses (MEDs) of solar-simulated ultraviolet (ssUV) radiation are needed to deplete vitamin E (7), while doses as low as 0.75 MED are capable of destroying vitamin E in the human SC (3). Mouse experiments have shown that a dose of 1 ppm × 2 hr of ozone (O_3) depletes SC vitamin E (8). Since this concentration of O_3 is higher than the naturally occurring levels of tropospheric O_3, the biological relevance of these findings for human skin is not yet clear. Benzoyl peroxide is used for the treatment of acne. A single application of a 10% w/vol formulation of benzoyl peroxide almost completely depletes SC vitamin E in vivo (9).

α-Tocopherol is widely used as an active ingredient in topical formulations. After topical application, it penetrates readily into the skin (10). Since the free form of vitamin E is quite unstable and light sensitive (it absorbs in the UVB range), the active hydroxyl group is usually protected by esterification with acetate. This increases the stability but renders the compound redox inactive. When administered orally, vitamin E acetate is hydrolyzed quantitatively in the intestines. There is some controversy, however, as to whether α-tocopherol acetate can be hydrolyzed in human skin. Chronic application of α-tocopherol acetate leads to an increase in free vitamin E in both the rat (11) and the mouse (12), where it was recently shown that UVB increases the hydrolysis of α-tocopherol acetate by induction of nonspecific esterases up to 10- to 30-fold (13). While one study suggested that bioconversion of α-tocopherol acetate does not occur in human skin (14), significant hydrolysis was demonstrated in recent studies using a human epidermis-tissue culture model and in vivo (15). A 0.15% formulation of vitamin E acetate increased the SC far more than oral supplementation with 400-IU α-tocopherol (16). A rinse-off application of vitamin E was also able to increase the vitamin E.

The availability of the free form of vitamin E needs to be considered when analyzing possible health benefits. The majority of studies have been carried out in animal models, while

only limited data exist for human studies. Lipid peroxidation is inhibited after topical application of α-tocopherol (17). Several studies indicate that topically applied α-tocopherol inhibits UVB-induced photodamage of DNA in a mouse model (18) and keratinocyte cultures (Trolox®, water-soluble derivative of vitamin E) (19). Protection against Langerhans cell depletion by UV light was observed after topical application of α-tocopherol in a mouse model (20). α-Tocopherol and its sorbate ester were studied in a mouse model of skin aging. Both antioxidants were found to be effective, sorbate even more so than α-tocopherol (21). Systemic administration of vitamin E in humans (only in combination with vitamin C) increased the MED and reduced changes in skin blood flow after UV irradiation (22,23). A rinse-off application of vitamin E was able to increase the vitamin E content in the barrier lipids. It was also able to decrease the formation of squalene monohydroperoxide from squalene by low-dose UVA (8 J/cm^2 (24).

Yet, several studies indicate that α-tocopherol acetate is not as effective as free vitamin E when applied topically. Inhibition of DNA mutation in mice was 5 to 10 times less effective (19). Also, in a mouse model, unlike free vitamin E, the acetate form seemed to be ineffective (25). In summary, even though some health benefits of vitamin E supplementation have been shown, there is still a need for controlled studies in humans under physiological conditions. So far vitamin E was found to have anticarcinogenic, photoprotective, skin-stabilizing properties. This topic is reviewed in detail by Thiele et al. (26).

The safety of vitamin E supplementation is a topic of current discourse. For years, oral application of vitamin E has been regarded harmless. Recent meta-analyses discuss an increase in overall mortality after oral vitamin E supplementation. However, another large meta-analysis found no overall increase in cancer mortality by vitamin E (27). In the framework of the SUVIMAX study, a recent publication suggests increased risk of skin cancer in women taking a mixture of vitamin E, vitamin C, β-carotene, selenium, and zinc, but not in men (28). A panel of experts concluded from clinical trial evidence that vitamin E supplements appear safe for most adults in amounts $\leq$ 1600 IU (1073 mg RRR-α-tocopherol or the molar equivalent of its esters (29). Safety data were obtained for oral supplementation. No large controlled studies exist for topical supplementation of vitamin E.

Recently, the tocotrienol forms of vitamin E have become a focus of interest, since they have been found to be more efficient antioxidants in some model systems than tocopherols (30). Even if they are not bioavailable after oral supplementation, topical application circumvents the exclusion by α-TTP in the liver. In fact, free tocotrienols readily penetrate into mouse skin (10), and tocotrienol acetate is hydrolyzed in skin homogenates and in murine skin in vivo (24). Topical application of a tocotrienol-rich fraction has been demonstrated to protect mouse skin from UV- and O_3-induced oxidative stress (31,32).

Benzoyl peroxide (BPO) is used for the treatment of acne. During seven daily applications of a 10% w/vol formulation of benzoyl peroxide, endogenous SC vitamin E was progressively depleted. Vitamin E in the SC was significantly retained after α-tocotrienol (5% w/vol) supplementation for seven days. α-Tocotrienol supplementation significantly mitigated the BPO-induced lipid peroxidation. The transepidermal water loss was increased 1.9-fold by seven BPO applications, while there was no difference between α-tocotrienol treatment and controls (9). In conclusion, tocotrienols bear a potential that yet remains to be explored.

VITAMIN C

Ascorbic acid or vitamin C is one of the most important water-soluble antioxidants, which is present in high amounts in the skin. While most species are able to produce ascorbic acid, humans lack the enzymes necessary for its synthesis. Deficiency in ascorbic acid causes scurvy, a disease already described in the ancient writings of the Greeks (33). Apart from the pure antioxidant function ascorbic acid is an essential cofactor for different enzymes. The antioxidant capacity of vitamin C is related to its unique structure (Fig. 2). Due to its pKa1 of 4.25, it is

Figure 2 Structural formula of vitamin C as the mono-anion ascorbate.

present as a monoanion at physiological pH, which can undergo a one-electron donation to form the ascorbyl radical with a delocalized electron and can be further oxidized to result in dehydroascorbic acid. Dehydroascorbic acid is relatively unstable and breaks down if it is not regenerated (see antioxidant network). In vitro ascorbic acid can scavenge many types of radicals, including the hydroxyl ($OH^{\bullet}$), the superoxide ($O_2^{\bullet-}$), and water-soluble peroxyl ($ROO^{\bullet}$) radicals as well as other reactive oxygen species such as O_3, and quenches singlet O_2. Because of its relative reduction potentials, ascorbate can reduce Fe(III) to Fe(II), which in turn can decompose hydrogen peroxide (H_2O_2) to the dangerous hydroxyl radical. Therefore, vitamin C can exert pro-oxidant effects in the presence of unbound iron (Fenton's reagent).

In the skin, vitamin C is found in all layers. In SC, it forms a similar gradient as vitamin E with decreasing concentrations toward the outside. Vitamin C is depleted by O_3, UV radiation, and BPO. One of the earliest discoveries of vitamin C benefits in the skin was the observation that it stimulates collagen synthesis in dermal fibroblasts (34). Recently, a pretranscriptional role of vitamin C has been described (35). Also, vitamin C is essential in the formation of competent barrier lipids in reconstructed human epidermis (36).

Several studies have investigated protective effects of vitamin C against oxidative stress. UVB-induced immunotolerance as a marker of damage to the immune system could be abrogated by topical application of vitamin C to murine skin (37). UVB-induced sunburn cell formation was mitigated by vitamin C in porcine skin (38). While one study reported a postadministrative protective effect of vitamin C-phosphate against UV-induced damage in mice (39), another study found no such effect in humans (40). Systemic application of vitamin C in combination with vitamin E protected against UV-induced erythema in humans (22). Another study described protection against erythema, sunburn cell formation, and thymidine dimer formation in pigs by a combination of vitamin E (1%) and vitamin C (15%) (41). In a keratinocyte cell culture system, vitamin C reduced UVB-induced DNA damage (19). In mice, an anticarcinogenic effect of vitamin C was described (42). However, no data regarding such benefits exist in humans.

Since vitamin C is not very stable, it is difficult to incorporate it into topical formulations. Esterification with phosphate is used to circumvent this limitation. In vitro experiments demonstrated that Mg-ascorbyl-2-phosphate penetrates the murine skin barrier and is bioconverted into free ascorbate (43).

THIOL ANTIOXIDANTS

Thiols share an oxidizable sulfydryl (SH) group. Glutathione (GSH) is a tripeptide (Fig. 3) whose SH group at the cysteine can be oxidized, forming a disulfide (GSSG) with another GSH. Physiologically, more than 90% of the GSH is in the reduced form. GSH peroxidases use GSH oxidation to reduce H_2O_2 and other water-soluble peroxides. The synthesis of GSH by the human cell is stimulated by *N*-acetyl-cysteine (NAC), which is hydrolyzed to cysteine intracellularly. Moreover, NAC acts as an antioxidant itself. Lipoic acid (1,2-dithiolane-3-pentanoic acid or thioctic acid, LA) is a cofactor of multienzyme complexes in the decarboxylation of α-keto acids. Applied as the oxidized dithiol dihydrolipoic acid (DHLA) it is taken up by cells and reduced by mitochondrial and cytosolic enzymes [NAD(P)H dependent]. It thereby forms an efficient cycle, since it can in turn regenerate GSSG to GSH and stimulate the GSH synthesis by improving cysteine utilization (44).

Figure 3 Chemical structures of thiols: (**A**) GSH consisting of glycine, cysteine, and glutamic acid. (**B**) Lipoic acid in its oxidized form as a disulfide.

General provisos in the use of thiols in skin applications are the typical smell and the poor solubility of LA in aqueous solutions below pH 7. Yet, several thiol agents have been tested for protective effects in the skin. For oral as well as topical application in mouse models, GSH-ethyl esters and GSH-isopropyl esters proved to be more efficient than free GSH. Oral supplementation decreased the formation of UV-induced tumors (43) and the formation of sunburn cells (45). Topical treatment partially inhibited UV-induced immunosuppression (46). NAC was able to reduce UVA-induced DNA damage in fibroblasts (47) and protected mice against UVB-induced immunosuppression after topical application (46) in a mode that did not involve de novo GSH synthesis (48). Lipoic acid was demonstrated to penetrate into mouse skin (49), while oral supplementation of lipoic acid has actually been shown to have an anti-inflammatory effect in mice (50) and to prevent symptoms of vitamin E deficiency in vitamin E-deficient mice (51).

POLYPHENOLS

Flavonoids are widely distributed plant pigments and tannins occurring in barks, roots, leaves, flowers, and fruits. Their roles in plants include photoprotection and contributing to the plant color. Consequently, our diet contains flavonoids, which can be found in a variety of foods from green vegetables to red wine.

In spite of the fact flavonoids have been used in traditional medicine for several centuries, it was not until 1936 that their first biological activity, the vitamin C-sparing action, was described by Rusznyak and Szent–Györgyi. As a result, they received the name of "Vitamin P." Flavonoids, also referred to as "plant polyphenols," have been recognized as potent antioxidants. Their free radical-scavenging and metal-chelating activities have been extensively studied. Nonetheless, given their polyphenolic structure (Fig. 4), the electron- and hydrogen-donating abilities constitute the major feature of their antioxidant properties (52). By opposition to the antioxidants previously described, flavonoids are not part of the endogenous antioxidant system but still interact with it through the antioxidant network (see below).

Among the applications found in traditional medicine, flavonoids account for anti-inflammatory, antiphlogistic, and wound-healing functions. Their effect on skin inflammation has been thought for a long time to be limited to the inhibition of the activity of 5-lipoxygenase and cyclooxygenase. However, recent studies suggest a more subtle mode of regulation of the inflammatory reaction by flavonoids. In fact, flavonoids such as silymarin, quercetin, genistein, and apigenin are effective inhibitors of NF-κB, a proinflammatory transcription factor, thereby reducing the transcription of proinflammatory genes and preventing inflammation (53–55).

Oral supplementation and topical application of green and black tea polyphenols show beneficial effects against UV radiation (UVR)-induced skin carcinogenesis in mice (56–58). In addition, these flavonoids as well as silymarin were found to prevent UVR-induced inflammation, ornithine decarboxylase expression, and activity (59), all of these events being potential contributors to carcinogenesis (60).

Procyanidins, also named "condensed tannins" are flavonoids found in pine bark (Pycnogenol®), grape seeds, and fruits, for instance. By direct protein interaction, they were shown to protect collagen and elastin, two dermal matrix proteins, against their degradation (61). Furthermore, some of these procyanidins exhibit a remarkable effect on follicle hair proliferation (62), thus extending the therapeutic applications of flavonoids to alopecia. Although the flavonoids are not part of our endogenous antioxidant defenses, they display a broad spectrum of properties particularly helpful in preventing UVR-caused deleterious effects in human skin.

Figure 4 Chemical structure of catechin, a flavane, as an example of a flavonoid. Flavanes share a common base structure (rings A, B, C, one), which is hydroxylated in different patterns.

THE ANTIOXIDANT NETWORK

When antioxidants react with an oxidant, they are converted to a form that no longer functions as an antioxidant and is said to be consumed. In order for the oxidized product to function again, it needs to be recycled to its native form. The antioxidant network describes the ability of the antioxidants to recycle and regenerate oxidized forms of each other, thereby providing extra levels of protection (Fig. 5). Thus, the process is synergistic, and the net antioxidant protection is always greater than the sum of the individual effects.

The major systemic antioxidants vitamin E, vitamin C, and GSH are present in different cellular compartments, and all have the ability to interact with one another. Typically the radicals formed on the antioxidants are more stable and longer lived than the damaging radicals produced in vivo, which is mostly due to a delocalization of the unpaired electron. Thus, they have more chance to interact with each other and be reduced than to react with macromolecules. Vitamin E is the major chain-breaking antioxidant, protecting biological membranes from lipid peroxidation, which is a difficult task considering the ratio of phospholipids molecules to vitamin E is ~1500:1. However, vitamin E is never depleted because it is constantly being recycled. When vitamin E becomes oxidized, a radical on vitamin E is formed (chromanoxyl radical). In the absence of networking antioxidants, this radical can either become pro-oxidant by abstracting hydrogen from lipids or react to form nonradical products (consumed). However, a number of antioxidants are known to be able to reduce the chromanoxyl radical and regenerate vitamin E (63). These include vitamin C (64), ubiquinol, and GSH (65). Vitamin C, the most abundant plasma antioxidant and first line of defense, can reduce the tocopheroxyl radical, forming the ascorbyl radical. Interactions between vitamins E and C have been demonstrated in various systems both in vivo [reviewed in (66)] and in vitro [reviewed in (67)]. The ascorbyl radical is practically inert and oxidizes further to form dehydroascorbic acid. This can be reduced back to native vitamin C by GSH. This process is known to occur both chemically (68) and enzymatically (69) in both erythrocytes (70) and neutrophils induced by bacteria (71); the latter may relate to a host of defense mechanisms. Glutathione is the major intracellular antioxidant. Oxidized GSSG is constantly recycled to GSH enzymatically by GSH reductase, thus providing a constant pool of GSH. Glutathione recycling relies on NAD(P)H as the electron donor. Thus, metabolic pathways involved in energy production provide the ultimate electron donors for the antioxidant network. It is also known that GSH can directly recycle vitamin E (65,72), as well as ubiquinol (73), another lipophilic antioxidant which itself is recycled in mitochondria as part of the electron transport chain.

Certain supplements are also known to contribute to the network by recycling antioxidants. Lipoic acid is a prime example since this potent antioxidant can recycle ascorbate, GSH, and ubiquinol in vitro [reviewed in (74)]. Recently, it has been demonstrated that flavonoids may also play a networking role since they are also able to recycle the ascorbyl radical (75). Thus, there exists a very organized defense system against free radical attack, which ultimately serves to protect and recycle antioxidants in various cellular compartments.

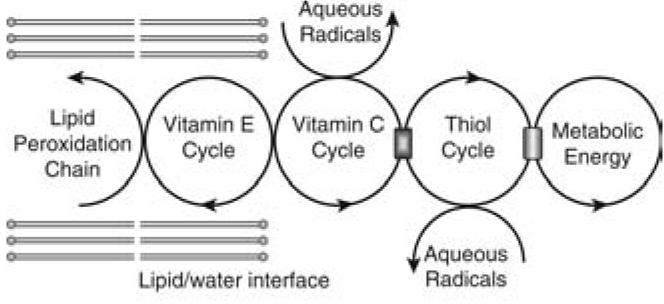

Figure 5 Schematics of the intertwined action of the antioxidant network. An ascorbate molecule can either recycle the vitamin E radical arising from breaking the lipid peroxidation chain or scavenge an aqueous radical. Glutathione can either regenerate ascorbate or scavenge a radical enzymatically. Glutathione itself then can be regenerated by the cellular metabolism.

REGULATION OF GENE TRANSCRIPTION

The skin is the largest human organ permanently exposed to a variety of stresses, among which oxidative insults such as UVR and ozone exposure account for the etiology of many skin disorders. However, oxidative damage is not responsible for all biological effects engendered by these stressors in the skin. Indeed, UVR causes changes in the expression of genes encoding proinflammatory cytokines, growth factors, stress response proteins, oncoproteins, matrix metalloproteinases (MMPs), etc. (76). Although the immediate target(s) of UVR is (are) still unknown, certain kinases and transcription factors can be activated by UVR, thereby increasing gene transcription (77). One transcription factor, NF-κB, appears of particular interest for the skin, since the lack of its inhibitory protein, IκBα, is associated with the development of a widespread dermatitis in knockout mice (78,79). Furthermore, reactive oxygen species, such as the ones produced after UVR, are suspected to play an important role in the activation of NF-κB (80). Consequently, antioxidants have been found to be among the most potent NF-κB inhibitors.

Vitamin E has specific effects on signaling events: Tocotrienols downregulate the 3-hydroxyl-3-methylglutaryl-coenzyme A (HMG CoA) reductase, a key enzyme of the mevalonate pathway that produces cholesterol (81). Inhibition of side-pathways of HMG-CoA-reductase is known to have shown anticarcinogenic effects. α-Tocopherol is able to inhibit protein kinase C, an important factor of atherosclerosis (82). Protein kinase C also regulates the activity of collagenase (MMP-1), an enzyme that degrades skin collagen. In cell culture models, α-tocopherol inhibits MMP-1 via inhibition of protein kinase C(83). Recently, it was demonstrated that vitamin E inhibited the UV-induced expression of metalloelastase and thus may inhibit the development of solar elastosis, the hallmark of sun-induced damage.

However, clinical studies are required to assess the effectiveness of these antioxidants, including the flavonoid silymarin, α-lipoic acid, and the GSH precursor *N*-acetyl-*L*-cysteine, on skin inflammatory disorders. Using high-throughput procedures such as the cDNA arrays for instance (84), the evaluation of the antioxidants on the whole genome is henceforth possible. These studies will only confirm the hypothesis that antioxidants are responsible for a much broader action spectrum than their antioxidant functions per se and extend their role on more subtle regulatory mechanisms of the gene expression.

PERSPECTIVES

The general role of antioxidants in the protection against oxidative stress is well established. In skin applications, antioxidants are a promising tool to mitigate oxidative injury. Even though a growing amount of literature deals with skin protection by antioxidants, there is still a need for investigation. Especially, clinical human studies need to be carried out to demonstrate the efficacy of antioxidants in topical formulations.

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29 UV Filters

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INTRODUCTION

The presence of UV filters in skin care and cosmetic products represents a key benefit that cosmetics can provide consumers. The hazards of ultraviolet (UV) light exposure are well known. It is estimated that the incidence of non-melanoma skin cancer in the United States exceeds one million cases per year (1). UV-induced or photoaging accounts for most of the age-associated changes in skin appearance (2). UV radiation (UVR) damages the skin by both direct effects on DNA and indirectly on the skin's immune system (3).

In animal models, sunscreens prevent the formation of squamous cell carcinomas of the skin (4). The regular use of sunscreens has been shown to reduce the number of actinic or precancerous keratoses (5) and solar elastosis (6). Daily sunscreen use on the hands and face reduced the total incidence of squamous cell carcinoma in an Australian study (7). Sunscreens also prevent immunosuppression (8). Double-blind photoaging studies show consistent improvement in the "untreated" control groups partly because of the use of sunscreens by all study subjects (9).

The effect of sunscreen use on melanoma is less clear. A meta-analysis of population-based studies of population-based case control studies found no effect of sunscreen use on risk for melanoma (10). Nevertheless, observational studies suggest that intermittent or intense sun exposure is associated with increased risk for melanoma (11), supporting the hypothesis that preventing sunburn especially in childhood may reduce the lifetime risk of melanoma.

The cosmetic formulator has an expanding menu of active sunscreen ingredients for incorporation into a variety of cosmetic formulations. Selection is restricted by regulatory agencies in the country in which the final product is to be marketed. This chapter will concentrate on reviewing available UV filters.

DEFINITIONS

UVR reaching the Earth's surface can be divided into UVB (290–320 nm) and UVA (320–400 nm). UVA can be further subdivided into UVA I (340–400 nm) or far UVA and UVA II (320–340 nm) or near UVA.

The sun protection factor (SPF) is defined as the dose of UVR required to produce 1 minimal erythema dose (MED) on protected skin after application of 2 mg/cm^2 of product divided by the UVR to produce 1 MED on unprotected skin. A "water-resistant" product maintains the SPF level after 40 minutes of water immersion. A "very water-resistant" or "waterproof" product is tested after 80 minutes of water immersion. If the SPF level is diminished by immersion, a separate SPF level may be listed. A "broad-spectrum" or "full-spectrum" sunscreen provides both UVB and UVA protection. Ideally, this includes both UVA I and UVA II coverages.

HISTORY

Acidified quinine sulfate was proposed for use as a chemical sunscreen in the 1890s (12). At the beginning of the 20th century, Unna found aesculin, a chestnut extract used in folk medicine for many years, to be more effective. Two UV filters, benzyl salicylate and benzyl cinnamate, were first incorporated into a commercially available sunscreen emulsion in the United States in 1928 (13). In the early 1930s, phenyl salicylate (salol) was used in an Australian product (14).

Para-aminobenzoic acid (PABA) was patented in 1943, leading to the development of PABA derivative UV filters. During World War II, red veterinary petrolatum (RVP) was used by the U.S. military, encouraging the development of further UV filters in the post-war period.

In the 1970s, increased interest in commercial sunscreen products led to refinements and consumer acceptance of these products over the next two decades. Facilitated by growing awareness as to the hazards of UVR, higher SPF products became the norm. Daily use consumer products containing UV filters, including moisturizers, color cosmetics, and even hair care products, have become more prevalent in the past decade. Concerns related to the adequacy of sunscreen protection for the prevention of melanoma and photoaging in the last few years have led to greater interest in broad-spectrum sunscreen UV protection throughout the entire UVA range.

REGULATORY

United States

Sunscreen products in the United States are regulated by the FDA as over-the-counter (OTC) drugs. The final monograph for sunscreen drug products for OTC human use (Federal Register 1999: 64: 27666–27693) established the conditions for safety, efficacy, and labeling of these products. A recently proposed amendment (Federal Register 2007: 72: 49070–49122) further elaborates on UVB (SPF) and UVA testing and labeling. As active ingredients in drug products, they are listed by their United States Adopted Names (USAN). There are 16 approved sunscreen ingredients (Table 1). All permitted UV filters can be used with any other permitted filters except avobenzone. The latter cannot be used with PABA, octyl dimethyl PABA, meridamate, and titanium dioxide (TiO_2). Maximum allowable concentrations are provided. Minimum concentration requirements were dropped, providing that the concentration of each active ingredient is sufficient to contribute a minimum SPF of not less than 2 to a finished product. A sunscreen product must have a minimum SPF of not less than the number of active sunscreen ingredients used in combination multiplied by 2.

The proposed recent amendment allows products with SPF values above 50, but the SPF declaration for sunscreens with SPF values above 50 are limited to SPF 50+. The term "sunblock" is prohibited. The term "UVB" is to be included before the term "SPF" on the principal product display panel. Newer labeling requires the listing of UV filters not only as active ingredients but also their concentration in the product. To address the inadequacies of any single UVA rating system, the FDA proposes a combination of spectrophotometric (in vitro) and clinical (in vivo) UVA testing procedure to allow for a nonnumerical UVA protection four-star rating system, with one star being low protection and four stars being the

Table 1 FDA Sunscreen Final Monograph Ingredients

Drug name	Concentration (%)	Absorbance
Aminobenzoic acid	Up to 15	UVB
Avobenzone	2–3	UVAI
Cinoxate	Up to 3	UVB
Dioxybenzone	Up to 3	UVB, UVAII
Ensulizole	Up to 4	UVB
Homosalate	Up to 15	UVB
Meradimate	Up to 5	UVAII
Octocrylene	Up to 10	UVB
Octinoxate	Up to 7.5	UVB
Octisalate	Up to 5	UVB
Oxybenzone	Up to 6	UVB, UVAII
Padimate O	Up to 8	UVB
Sulisobenzone	Up to 10	UVB, UVAII
Titanium dioxide	2 to 25	Physical
Trolamine salicylate	Up to 12	UVB
Zinc oxide	2 to 20	Physical

Abbreviations: FDA, Food and Drug Administration; UV, ultraviolet.

highest. The in vivo study to be used is the persistent pigment-darkening (PPD) method (15). The Boots adaptation of the Diffey/Robson method was proposed for in vitro testing in the recent amendment.

Europe

In Europe, sunscreen products are considered to be cosmetics, their function being to protect the skin from sunburn. The Third Amendment of the European Economic Community (EEC) Directive provides a definition and lists the UV filters that cosmetic products may contain. Table 2 lists UV filters that are fully permitted as amended most recently through commission directives 2003/83/EC and 2005/9/EC. As cosmetic products, cosmetic or *International Cosmetic Ingredient Dictionary* (INCI) nomenclature is used as listed in the *CTFA International Cosmetic Ingredient Dictionary*. The European Union (EU) allows, at the date of covering this chapter, several ingredients not available in the United States (see discussion below). The EU has added TiO_2 to the approved list. Zinc oxide (ZnO) is not included in this list but may be used as a cosmetic ingredient.

A more recent commission directive 2006/647/EC provides further guidance on UVA/UVB efficacy claims. The PPD method is clinically recommended. For in vitro testing, the critical wavelength method (16) is to be used, in contrast to the Boots' adaptation recommended by the FDA.

Australia

Sunscreens in Australia are regulated as therapeutic goods. The latest edition of Australian Standard 2604 was published in 1993 as a joint publication of Australia and New Zealand. Sunscreen products are classified as either primary or secondary, depending on whether the primary function of the designated product is to protect from UVR as opposed to a product with a primary cosmetic purpose. SPF designations greater than 30 are not permitted (SPF 30+ represents the maximum designation). In general, Australian Approved Names (AAN) for allowed active sunscreen ingredients are the same as FDA drug nomenclature with a few differences.

Other Countries

Most non–EEC countries follow the EEC directive. Many other countries follow U.S. trends with their own provisions. In Japan, sunscreens are classified as cosmetics. Regulations for each individual country need to be consulted for selection of the various UV filters for incorporation into a sunscreen product to be marketed in a given jurisdiction (17).

MECHANISM OF ACTION

UV filters have been traditionally divided into chemical absorber and physical blockers on the basis of their mechanism of action. Chemical sunscreens are generally aromatic compounds conjugated with a carbonyl group (13). These chemicals absorb high-intensity UV rays with excitation to a higher-energy state. The energy lost results in conversion of the remaining energy into longer lower-energy wavelengths with return to ground state. The evolution of modern sunscreen chemicals represents a prototype study in the use of structure-activity relationships to design new active ingredients and has been well reviewed elsewhere (18).

Physical blockers reflect or scatter UVR. Newer microsized forms of physical blockers may also function in part by absorption (19). Sometimes referred to as "non-chemical" sunscreens, they may be more appropriately designated as inorganic particulate sunscreen ingredients.

NOMENCLATURE

Sunscreen nomenclature can be quite confusing. They may be referred to by their chemical or trade name. In the United States, individual sunscreen ingredients are also assigned a drug name by the OTC monograph. Annex VII of the EU may use either a drug or chemical name. Australia has its own approved list of names (AAN). Table 3 lists the most commonly used names, including their primary listing in the INCI designation (20).

Table 2 List of UV Filters That Cosmetic Products May Contain (EEC Directive Annex VII – Part 1)

Reference number	Substance	INCI name	Maximum authorized concentration (%)
1	4-Aminobenzoic acid	PABA	5
2	*N,N,N*-trimethyl-4-(2-oxoborn-3-ylidenemethyl) anilinium methyl sulfate	Camphor benzylkonium methosulfate	6
3	Homosalate (INN)	Homosalate	10
4	Oxybenzone (INN)	Benzophenone -3	10
6	2-Phenylbenzimidazole-5-sulfonic acid and its potassium, sodium, and triethanolamine salts	Phenylbenzyimidazole sulfonic acid	8 (expressed as acid)
7	3,3'-(1,4-Phenylenedimethylene)bis[7,7-dimethyl-2-oxo-bicyclo-(2,2,1)hept-1-ylmethanesulfonic acid] and its salts	Terephthalidene dicamphor sulfonic acid	10 (expressed as acid)
8	1-(4-Tert-butylphenyl)-3-(4-methoxyphenyl) propane-1,3-dione	Butyl methoxy dibenzoyl-methane	5
9	α-(2-Oxobron-3-ylidene)toluene-4-sulfonic acid and its salts	Benzylidene camphor sulfonic acid	10 (expressed as acid)
10	2-Cyano-3,3-diphenyl acrylic acid, 2-ethylnexyl ester	Octocrylene	10 (expressed as acid)
11	Polymer of *N*-(2 and 4)-[(2-oxoborn-3-ylidene)methyl] benzyl acrylamide	Polyacrylamidomethyl benzylidene camphor	6
12	Octyl methoxycinnamate	Ethyl hexyl methoxy-cinnamate	10
13	Ethoxylated ethyl-4-aminobenzoate	PEG-35 PABA	10
14	Isopentyl-4-methoxycinnamate	Isoamyl *p*-methoxy cinnamate	10
15	2,4,6-Trianilino-(*p*-carbo-2'-ethylhexyl-1'-oxy)-1,3,5-triazine	Octyl triazone	5
16	Phenol,2-(2H-benzotriazol-2-yl)-4-methyl-6-(2-methyl-3-(1,3,3,3-tetramethyl-1-(trimethylsilyl)oxy)-disiloxanyl)propyl) (drometrizone trisiloxane)	Drometrizone Trisiloxane	15
17	Benzoic acid, 4,4-((6-(((1,1-dimethylethyl)amino)carbonyl)phenyl) amino)-1,3,5,triazine-2,4-diyl)diimino) bis-cbis(2-ethylhexyl)ester)	Diethylhexyl butamido triazone	10
18	3-(4'-Methylbenzylidene)-d-t camphor	4-Methylbenzylidene camphor	2
19	3-Benzylidene camphor	3-Benzylidene camphor	2
20	2-Ethylhexyl salicylate	Octyl salicylate	5
21	4-Dimethyl-amino-benzoate of ethyl-2-hexyl	Octyl dimethyl PABA	8
22	2-Hydroxy-4-methoxybenzophenone-5-sulfonic acid and its sodium salt	Benzophenone-5	5 (of acid)
23	2,2'-Methylene-bis-6-(2H-benzotriazol-2yl)-4-(tetramethylbutyl-1,1,3,3,-phenol)	Bisoctyltriazol	10
24	Monosodium salt of 2-2'-bis-(1,4-phenylene) 1H-benzimidazole-4,6-disulphonic acid	Bisamidazylate	10 (of acid)
25	(1,3,5)-Triazine-2,4-bis ((4-(2-ethyl-hexyloxyl)-2-hydroxy)-phenyl)-6-(4-methoxyphenyl)	Anisotriazine	10
26	Dimethicodiethylbenzalmalonate (CAS No 207574-74-1)		10
27	Titanium dioxide		25
28	Benzoic acid, 2-(4-(diethylamino)-2 – hydrobenzoyl)-, hexylester	Dithylamino hydroxybenzoyl hexyl benzoate	10

Abbreviations: EEC, European Economic Community; UV, ultraviolet; PABA, para-aminobenzoic acid; INCI, International Cosmetic Ingredient Dictionary.

Table 3 Sunscreen Nomenclature

CAS #	Drug name (FDA)	INCI name	Colipa #	EU reference #	Trade names	Solubility	Spectrum
150-13-0	Aminobenzoic acid	PABA	S 1	1	4-Aminobenzoic acid	Hydrophilic	UVB
70356-09-1	Avobenzone	Butyl methoxydibenzyl methane	S 66	8	Parsol 1789	Lipophilic	UVA I
104-28-9	Cinoxate	Cinoxate				Lipophilic	UVB
92761-26-7	Ecamzule	Terephthalydene dicamphor sulfonic acid	S 71	7	Mexoryl SX	Hydrophilic	
27503-81-7	Ensulizole	Phenylbenzimidazole sulfonic acid	S 45	6	Eusolex 232, Neo Heliopan Hydro	Hydrophilic	UVB
118-56-9	Homosalate	Homosalate	S 12	3	Eusolex HMS	Lipophilic	UVB
134-09-8	Meradimate	Menthyl anthranilate			Dermoblock MA, Neo Heliopan, Type MA	Lipophilic	UVA II
6197-30-4	Octocrylene	Octocrylene	S 32	10	Escalol 597, Eusolex OCR, Uvinul N-539-50	Lipophilic	UVB
5466-77-3	Octyl methoxycinnamate	Octyl methoxycinnamate	S 28	12	Neo Heliopan AV, Parsol MCX, Eusolex 2292	Lipophilic	UVB
88122-99-0	Octyl triazone	Octyl triazone	S 69	15	Uvinul T-150	Lipophilic	UVB
118-60-5	Octisalate	Octyl salicylate	S 20	8	Escalol 587, Eusolex BS, Uvinul O-18	Lipophilic	UVB
131-57-7	Oxybenzone	Benzophenone-3	S 38	4	Eusolex 4360, Neo Heliopan, Uvinul M40	Lipophilic	UVB, UVA II
21245-02-03	Padimate O	Octyl dimethyl PABA	S 78	17	Escalol 507, Eusolex 6007	Lipophilic	UVB
4065-45-6	Sulisobenzone	Benzophenone-4	S 78	17	Escalol 577, Uvinul MS 40	Lipophilic	UVB, UVA II

Abbreviations: FDA, Food and Drug Administration; INCI, International Cosmetic Ingredient Dictionary; PABA, para-aminobenzoic acid.

INDIVIDUAL UV FILTERS

Sunscreen ingredients may be considered by dividing them into larger overall classes by chemical structure. They may also be classified by their absorption spectrum. Although the lists of UV filters approved by the various regulatory agencies may seem quite extensive, fewer are used with any degree of frequency. The discussion, which follows, will concentrate on those listed in Table 3.

UVB

PABA and Its Derivatives

PABA was one of the first chemical sunscreen chemicals to be widely available. Several problems limited its use. It is very water soluble, was frequently used in alcoholic vehicles, stained clothing, and was associated with photodermatitis. Ester derivatives of PABA, mainly octyl dimetyl PABA or padimate O, became more popular with greater compatibility in a variety of more substantive vehicles and a lower potential for staining or adverse reactions. Amyl dimethyl PABA and glyceryl PABA (glyceryl aminobenzoate) are no longer used.

Padimate O or octyl dimethyl PABA is a most potent UV absorber in the mid-UVB range. Because of problems with PABA formulations, marketers have emphasized the "PABA-free" claim. Although still used (21), it is confused with PABA, limiting its use. The decline in the use of this PABA derivative along with the demand for higher SPF products has led to the incorporation of multiple active ingredients in a single product to achieve the desired SPF.

Cinnamates

The next most potent UVB absorbers allowed by the FDA monograph, the cinnamates, have largely replaced PABA derivatives. Octinoxate or octyl methoxycinnamate is the most frequently used sunscreen ingredient (21). Octyl or ethylhexyl methoxycinnamate is in order of magnitude less potent than padimate O and requires additional UVB absorbers to achieve higher SPF levels in a final product. Cinoxate (ethoxy-ethyl-*p*-methoxycinnamate) is less-widely used. Isoamyl *p*-methoxycinnamate (EU no. 14) is available in Europe.

Salicylates

Salicylates are weaker UVB absorbers. They have a long history of use but were supplanted by the more efficient PABA and cinnamate derivatives. They are generally used to augment other UVB absorbers. With the trend to higher SPFs, more octisalate or octyl salicylate (ethylhexyl salicylate) is being used followed by homosalate or homomenthyl salicylate. Both materials have the ability to solubilize oxybenzone and avobenzone. Trolamine or triethanolamine salicylate has good water solubility.

Camphor Derivatives

Not approved by the FDA for use in the United States, there are six camphor derivatives approved in Europe, and 4-methylbenzylidene camphor (EU no. 18) is the most widely used one (21).

Octocrylene

2-Ethylhexyl-2-cyano-3,3 diphenylacrylate or octocrylene is chemically related to cinnamates. It can be used to boost SPF and improve water resistance in a given formulation. Octocrylene is photostabile and can improve the photostability of other sunscreens. It is expensive and can present difficulties in formulation.

Phenylbenzimadazole Sulfonic Acid

Phenylbenzimadazole sulfonic acid or ensulizole is a water-soluble UVB absorber that can be used in the water phase of emulsion systems, in contrast to most oil-soluble sunscreen ingredients, allowing for a less-greasy, more aesthetically pleasing formulation such as a daily use moisturizer containing sunscreen. Phenybenzimidazole sulfonic acid boosts the SPF of organic and inorganic sunscreens. It can also be used in clear gels owing to its water solubility.

Triazones

Octyl or ethylhexyl triazone (EHT) is a UVB filter available in Europe (EU no. 15). It is eligible to enter the FDA sunscreen monograph through the FDA time and extent application (TEA) process (22), but not yet available. Diethylhexyl butamido triazone (DBT) (methylene bis-benzotriazol tetramethylbutylphenol—EU no. 23) or Tinasorb M is a newer, more efficient UVB filter with improved solubility over EHT. (23). An anisotriazine or bis-ethylhexyloxyphenol methoxyphenyl triazine (BEMT) or Tinasorb S (EU no. 25) is a new broadband filter, which also provides UVA protection (22), as does Tinasorb M, methylene-bis-benzotriazolyl tetramethyl-butyl phenol (MBBT) or bisoctyltriazol (EU no. 23). Both Tinasorb ingredients have also been submitted for the FDA approval through the TEA process.

UVA

Benzophenones

Although oxybenzone or benzophenone-3 absorbs most efficiently in the UVB range, absorption extends well into the UVA II range. It is used primarily as a UVA absorber, but boosts SPF values in combination with other UVB absorbers. Oxybenzone is supplied as a solid material and has poor solubility and a relatively low extinction coefficient. Sulisobenzone or benzophenone-4 is water soluble, somewhat unstable, and used with less frequency.

Menthyl Anthanilate

Meradimate or menthyl anthralate is a weak UVB filter, with absorption mainly in the near UVA portion of the spectrum. It is less effective than benzophenones in this range and is less widely used.

Butylmethoxydibenzoylmethane

Avobenzone or Parsol 1789 is the only current pure UVA filter approved by the FDA for use in OTC sunscreens in the United States. It has been used in Europe for a considerably longer period. It provides strong absorption in the UVA I range with peak absorption at 360 nm. Since standards for measuring UVA protection in the United States have only been proposed, a minimum use concentration has been set at 2% with a maximum of 3%.

Photostability refers to the ability of a molecule to remain intact with irradiation. Photostability is potentially a problem with all UV filters. This issue been raised specifically with avobenzone (24). This effect may degrade other sunscreens in a formulation, including octyl methoxycinnamate. Octocrylene and some of the newer suncreens, including BEMT, stabilized avobenzone (25). Non-UV filters such as diethylhexyl 2,6 napthalate may also be used (22). Overall formulation with avobenzone is therefore critical in this regard.

Tetraphthalydine Dicamphor Sulfonic Acid

3,3′-(1,4-phenylenedimethylene)bis[7,7-dimethyl-2-oxo-bicyclo-(2,2,1)hept-1-yl methanesulfonic acid (EU no. 7) or Mexoryl SX is a UVA blocker more recently available in Europe with comparable (26) or superior efficacy to avobenzone (27). Only specific formulations by the patent holder with this ingredient have been approved in the United States.

Physical Blockers

Some of the original sunblocks were opaque formulations reflecting or scattering UVR. Color cosmetics containing a variety of inorganic pigments function in this fashion. TiO_2 and ZnO are chemically inert and protect through the full spectrum of UVR. They offer significant advantages. Poor cosmetic acceptance limited the widespread use of these two ingredients until microsized forms became available. By decreasing particle size of these materials to a microsize or ultrafine grade, it is less visible on the skin surface.

Micropigmentary sunblocks function differently than opaque sunblocks of pigmented color cosmetics by absorbing and not simply reflecting or scattering UVR (19). By varying and mixing particle sizes, differing levels of photoprotection are achieved throughout the UV spectrum. In addition to avobenzone, micropigmentary TiO_2 and ZnO offer the best available protection in the UVA II range.

Photoreactivity has been raised as an issue with these materials. Both TiO_2 and ZnO are semiconductors potentially absorbing light and generating reactive species (28). These effects have been demonstrated in vitro (29). Coating these materials reduces their photochemical reactivity. The in vivo relevance of these effects has not been demonstrated, and both materials have a long history of safe use. Physical blockers also have the significant advantage of lowered skin irritancy potential.

Titanium Dioxide

TiO_2 was the first micropigment extensively used. Advantages include a broad spectrum of protection and inability to cause contact dermatitis. The use of rutile as opposed to anastase crystal forms of TiO_2 lessens photoactivity. Newer materials are amphiphilic designed to be dispersed in both water and oil emulsion phases. Particle size and uniformity of dispersion is key to achieving SPF. Primary particle size may be 10 to 15 nm with secondary particle assembly to 100 nm. Particle size needs to be less than 200 nm to achieve transparency.

Despite advances in the technology and understanding of these materials, whitening remains a problem secondary to pigment residue. Adding other pigment-simulating fleshtones may partially camouflage this effect. The net effect may be that the user is inclined to make a less-heavy application of product effectively lowering SPF (30). "Hybrid" formulations employing a combination of chemical absorbers with inorganic particulates may represent a practical compromise.

Zinc Oxide

ZnO was added as an active sunscreen agent for the FDA OTC sunscreen monograph with avobenzone. Reduced to a particle size of less than 200 nm, light scattering is minimized and the particles appear transparent in thin films (31). ZnO has a refractive index of 1.9, as opposed to 2.6 for TiO_2, and therefore causes less whitening than TiO_2. ZnO attenuates UVR more effectively in the UVA I range (32) with a peak at 360 nm. Microfine TiO_2 at an equal concentration offers somewhat more protection in the UVB range. Fine particle ZnO is not approved as a sunscreen ingredient in the EU. It is approved as a cosmetic colorant and as a general cosmetic ingredient.

FORMULATION

A detailed discussion of incorporating UV filters into various vehicles to achieve defined goals for efficacy and aesthetics is beyond the scope of this chapter and has been well reviewed elsewhere (33). Briefly, the first step is to determine the type of product, SPF and UVA efficacy levels, aesthetics, and nonsunscreen claims desired. Sunscreen actives are chosen realizing that most products use multiple actives comprising up to 35% of the final formulation. Sunscreen ingredients are among the most expensive used in cosmetic formulation. Vehicle type determines which actives can be used on the basis of polarity and solubility characteristics of individual filters. The most commonly used sunscreen actives are oils significantly affecting greasiness. Inorganic particulates such as ZnO and TiO_2 tend to make products feel dry and drag on application. Emulsions are the most popular vehicles to ideally incorporate active ingredients into both water and oil phases for greater efficiency. The final products, rheological profile and polymer levels, determine that a uniform film coats the surface of insure UV efficacy. Water resistance needs to be considered (34). Lastly, a photostable system needs to be designed.

ADVERSE REACTIONS—TOXICITY

In a longitudinal prospective study of 603 subjects applying daily either an SPF 15+ broad-spectrum sunscreen containing octyl methoxycinnamate and avobenzone or a vehicle cream, 19% developed an adverse reaction (35). Interestingly, the rates of reaction to both the active and vehicle creams were similar, emphasizing the importance of excipient ingredients in the

vehicle. The majority of reactions were irritant in nature. Not surprisingly, a disproportionate 50% of the reacting subjects were atopic. Less than 10% of the reactions were allergic, with none of the subjects patch tested actually found to be allergic to an individual sunscreen ingredient.

Subjective irritation associated with burning or stinging without objective erythema from some organic UV filters (36) is the most frequent sensitivity complaint associated with sunscreen use. This is most frequently experienced in the eye area. Longer-lasting objective irritant contact dermatitis may be difficult to distinguish from true allergic contact dermatitis. In a post-market evaluation of sunscreen sensitivity complaints in 57 patients, 20 patients had short-lasting symptoms, 26 long-lasting symptoms, and 11 mixed or borderline symptoms (37). Half of the patients were patch and photopatch tested, and only three showed positive reactions to sunscreen ingredients.

Contact and photocontact sensitivity to individual sunscreen ingredients has been extensively reviewed (38). Considering their widespread use, the number of documented allergic reactions is not high (36). PABA and PABA esters accounted for many of the early-reported reactions, but with a decrease in their use, an increase in reactions to benzophenones was reported (39). Skin reaction is probably higher with benzophenones than other UV filters (40). Fragrances, preservatives, and other excipients account for a large number of the allergic reactions seen (38).

Virtually all sunscreen ingredients reported to cause contact allergy may be photoallergens. Although still relatively uncommon, sunscreen actives seem to have become the leading cause of photocontact allergic reactions (41,42). Individuals with preexisting eczematous conditions have a significant predisposition to sensitization associated with their impaired cutaneous barrier. The majority of individuals who develop photocontact dermatitis to sunscreens are patients with photodermatides (38).

Contact sensitivity is not an issue with the use of physical blockers. However, concerns with dermal penetration with the use of nanoparticles have more recently been raised. Several studies examining the dermal penetration of pigmentary particles greater than 100 mm and microfine TiO_2 and ZnO would indicate that they do not penetrate the skin and remain in the stratum corneum (40).

Some organic sunscreens show estrogenic activity in screening toxicological assays (43). In vivo studies would question the relevance of this in humans (44). Clearly, the benefits of sun protection outweigh these risks.

CONCLUSION

A limited menu of UV filters for incorporation into sunscreen products is available to the formulating chemist, depending on regulatory requirements in an individual country or jurisdiction. With the demand for higher SPFs, the trend has been to use more individual and a wider variety of agents in newer products. Recent research in sunscreen efficacy has emphasized the need for products protecting against the full UV spectrum with a limited number of available agents. Regulatory agencies are often slow to approve new ingredients. Rules governing the approval of new ingredients by the EEC are more flexible.

Sunscreen efficacy remains very dependent on vehicle formulation. Solvents and emollients can have a profound effect on the strength of UV absorbance by the active ingredients and at which wavelengths they absorb (45). Film formers and emulsifiers determine the uniformity and thickness of the film formed on the skin surface, which in turn determines SPF level, durability, and water resistance (46). Lastly, product aesthetics play a large role in product acceptance, particularly with sunscreens being incorporated into daily use cosmetics. These constraints provide the sunscreen formulator with significant challenges in developing new and improved formulations.

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30 Sun Protection and Sunscreens

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INTRODUCTION

Visible sunlight is only a tiny part of the whole radiation spectrum emitted by the sun (400–700 nm). By increasing wavelength greater than 700 nm, one enters the invisible radiation range called infrared radiation (IRR); by decreasing wavelength lesser than 400 nm, one enters the ultraviolet radiation (UVR) domain. Overexposure to the sun's invisible rays can be harmful to human skin. The damage can be immediate with visible effects, such as erythema and sunburn, leading to cell and tissue degradation. The damage can also be long term, and the cumulative effects of prolonged exposure are now increasingly recognized to be the possible cause of degenerative changes in the skin such as premature wrinkling and skin cancer. Indeed, many skin changes that often are identified with aging actually result from damage by too much sun exposure (1,2).

DEFINITIONS

The shorthand notations for wavelength ranges in the UVR and IRR defined by the Commission Internationale de l'Éclairage (CIE) are closely related to the absorption depth of radiation in tissue. UVR from both sunlight and artificial sources is subdivided into three sections termed "UVA," "UVB," and "UVC" from the longer to shorter wavelengths: UVA from 400 to 320 nm, UVB from 320 to 290 nm, and UVC less than 290 nm. The UVA section is further divided in two subsections: UVA I (longer wavelengths 400–340 nm) and UVA II (shorter wavelengths 340–320 nm). The longer the wavelength and the higher the number, the deeper the UV penetrates the skin. The shorter the wavelength and the lower the number, the greater the energy level of the light and the more damage it can do. UVC, e.g., is highly efficient in causing sunburn and could destroy the skin but, fortunately, it is completely absorbed by ozone in the highest part of the earth's atmosphere. The sunlight's UVR at the surface of the earth is therefore constituted by variable proportions of UVB and UVA passing right through the atmosphere, even on a cloudy day. This variability is due to different factors such as latitude, height of the sun above horizon (time of the day), altitude, atmospheric conditions, etc. As a rule, the amount of UVA reaching the earth's surface may be considered to be 10 to 30 times greater than that of UVB (3,4).

IRR is also subdivided into three sections termed "IRA," "IRB," and "IRC," but from the shorter to the longer wavelengths: IRA (or near-IR) from 780 to 1400 nm, IRB from 1400 nm to 3000 nm, and IRC from 3000 nm to 1 mm. Contrary to UVR, the longer the wavelength and the higher the number, the less deep the IRR penetrates the tissue. Deep-penetrating IRA does not cause any strong sensation of heat. But this is the case with the longer wavelengths IRB and particularly IRC, which, at sufficiently high intensities, may damage or destroy the skin (2).

BASICS ON PROTECTION

Ultraviolet Radiation

Skin Effects of UVR

It has long been known that UVB is the principal cause of acute sunburn and tanning (4–6). Therefore, sunscreen efficacy is predominantly directed against UVB radiations (the relative contribution of UVA radiation to sunburn is considered to be only 15–24%). In addition, exposure to UVB radiation is immunosuppressive, mutagenic, and carcinogenic.

Meanwhile, the importance of the biological effects of UVA has been recognized (5,6). UVA induces significant photobiological reactions, mostly of indirect nature and requiring the presence of oxygen, such as immediate and delayed tanning reactions and new melanin formation. Importantly, there is now considerable evidence that UVA definitively contributes to long-term degenerative changes of the skin such as significant connective tissue damage (premature skin aging) and cancer formation, and may also contribute to UVB-induced carcinogenesis (6,7).

UV Filters, Sunscreens, Photostability

Protection against the effects of UVR in the skin is achieved by specially designed molecules (i.e., UV filters) incorporated in suitable formulations (sunscreens) such as creams or lotions, oils, gels, sticks, etc. (8). However, in view of the growing photobiological knowledge about the mechanisms of UVR-induced effects in the skin, this definition must now be revised to include new concepts. Besides providing a minimum sunburn protection, modern sunscreens are now required to provide a significant protection against UVA-induced effects (5,6). Further, they may not only contain chemicals that absorb, reflect, or scatter UVR but also chemicals that interfere with secondary reactions such as generation of free radicals and reactive oxygen species (ROS) in the skin, generation of inflammatory mediators, photoexcitation of different molecules, etc., and ultimately exert long-term protective effects on degenerative skin damage (8). Consequently, non-UVR-absorbing, UVR-reflecting, or UVR-scattering molecules with a pharmacological action in the skin may also be incorporated in sunscreens, endowing the corresponding products with expanded properties (8–12).

UV filters used in cosmetic sunscreen formulations are roughly considered belonging to two groups (13): organic molecules deliberately selected for their UVR-absorbing capacities (i.e., organic UV filters) and particles that absorb, reflect, or scatter UVR. Particles may be inorganic (i.e., metal oxides) or organic (microfine polymeric molecules) (8,13,14).

Both classes aim at preventing UVR from striking the skin. By absorbing UVR, UV filters are transferred to an excited electronic state from where the energy may dissipate into molecular vibrations (organic molecules) and into heat via collisions with surrounding molecules. However, when an efficient dissipation of the absorbed energy is not possible via, e.g., fluorescence, phosphorescence, heat, or internal conversion (isomerization) of an organic molecule, the UV-filter may break and irreversibly change its chemical structure. The molecule is not photostable. Consequently, the performance of the sunscreen may be altered (8,15–17).

Free Radicals, Free Radical Scavengers, and Antioxidants

Photochemical reactions due to UVR are inextricably coupled with the chemistry of free radicals. The photostability of UV filters is only one aspect of the reaction that may occur in cosmetic products applied on sunlight-exposed skin. The role of ROS and, among these, of free radicals has now been recognized as a possible cause of skin damage (4,7,18–20). Exposure of the skin to the UVR leads to the generation of a multitude of ROS. These cause injury by reacting with molecules such as lipids, proteins, nucleic acids; and by depleting the skin of its natural endogenous antioxidant defenses, a condition now arises termed "oxidative stress" (4,18,21). Thus, incorporation of molecules being able to control ROS in sunscreens should be beneficial in terms of the so-called photodamage or actinic damage of the UV-exposed skin.

Infrared Radiation

Skin Effects of IRR

Interest in investigating the effects of IRR on the skin is growing, and the current understanding of the biological effects of IRR is evolving rapidly notwithstanding controversies (22,23). IRR is inseparably linked to sunlight and perceived as heat. In the skin, heat is implicated in erythema "ab igne" and elastotic degeneration, and may even be the consequence of modern way of living (24).

Chronic exposure to IRR is growing because of the increased popularity of outdoor activities combined with the lack of protection of sunscreens in the IRR range and increased

use of IRR for wellness or therapeutic purposes, including promotion of healing of acute and chronic wounds (22,23). Recent publications, however, have reported controversial results concerning the biological effects of IRR in the skin. IRR, and particularly IRA, seems to be involved in premature photoaging and also in photocarcinogenesis (2,22). On a molecular basis, upregulation of endopeptidases in dermal fibroblasts [matrix metalloproteinases (MMPs)], which are responsible for the degradation of the extracellular matrix (ECM), seems to be at the origin of these deleterious effects. Induction of ROS is also involved. On the other side, protective effects of IRR against UVB-induced changes have been reported, together with a lack of upregulation of MMPs after IRA irradiation of dermal fibroblasts. The mechanisms of this protection are not completely elucidated and seem to implicate ferritin and/or heat shock proteins (5,22,23,25–29), although protection has also been observed without the involvement of heat shock proteins (27,30,31). A general view of these results points to temperature-induced protection against oxidative stress due to UV, a mechanism that could represent a natural process of cell protection acquired and preserved through evolution (23,27,28).

Protection Against IRR

In the past, some sunscreens claimed to protect against IRR (32). However, this has been rapidly abandoned because of the lack of a proper investigation method and the obvious lack of spectral absorption of UV filters in the IRR wavelength range. However, given the possible protective effect of the IRR exposure against UVR damage, the question, nature, and mode of IRR protection should be thoroughly investigated.

Nutritional protection using targeted micronutrients with ROS scavenging activities is one such possibility. Several studies have already demonstrated that it is possible to modify some properties of the skin (33,34) or reactions to sunlight by the administration of suitable nutritional complements (35–37). Presently, nutritional protection against skin damage from sunlight is increasingly advocated to the general public, but its effectiveness is controversial (35–37). Investigation of an interaction (whatever direction) with IRA effects in the skin is presently only possible by analyzing gene expression of key enzymes in skin biopsies (7,23,30,31). Clearly, much more studies are presently needed about this controversial issue, which is not explicitly addressed in the most recent regulatory monographs on sunscreens (5,6).

SPECIFIC LEGISLATION CONCERNING SUNSCREENS

Sunscreens are subject to specific regulations in almost every country of the world. In particular, UV filters allowed to be used in sunscreens and their maximal concentrations are listed in specific regulatory documents issued by the authorities. However, the spirit of the regulations varies between countries. For example, in the United States, UV filters are considered as over-the-counter drugs, and the monograph of the final over-the-counter sunscreen drug products (6,38) issued by the Food and Drug Administration (FDA) lies down conditions for the safety, efficacy, and labeling of sunscreens. It went into effect in 2001. UVA labeling and testing was deferred until 2007, and a proposal has now been recently released (6). In Europe, UV filters are cosmetic ingredients and are regulated by the Cosmetic Directive last amended for the seventh time in 2003 (39).

However, in every country, and notwithstanding a cosmetic status, sunscreens and the basis on which their efficacy is being claimed are viewed as important public health issues. For example, the European Commission has recently issued a recommendation on the efficacy of sunscreen products and the claims made relating thereto, which sets out claims that should not be made in relation to sunscreen products, precautions to be observed including application instructions, and the minimum efficacy standard for sunscreen products to ensure a high level of protection of public health (5). The same concerns are addressed in the proposed rules for sunscreens, which were recently issued by the FDA (6).

Safety requirements for the registration of chemical UV filters are important and are based on a stringent risk/benefit assessment (8,40). The safety dossier of a UV filter is evaluated and approved by national and international health authorities such as the Scientific Committee on Consumer Products (SCCP) in Europe and the FDA in the United States.

EVALUATION OF THE EFFICACY CLAIMS

Protection Against UVB: the Sun Protection Factor

The protective efficacy of a sunscreen against UVB is expressed as the sun protection factor (SPF). This is universally accepted and acknowledged in the most recent regulatory issues (5,6,38,41). The SPF is a number representing the ratio of the time required for a given irradiation to produce minimal perceptible erythema (MED: minimum erythemal dose, the UVR dose necessary to produce the minimal sunburn or minimal perceptible erythema 16–24 hours after exposure) in sunscreen-protected skin to the time required in unprotected skin. It should be remembered that it is not a ratio of doses, as at each lecture the skin has received the same dose (the MED). Only the irradiation times are different (38,41).

The SPF measurement method is now well established. Detailed guidelines concerning the experimental procedure have been published in many different countries (6,38,41,42). The SPF measurement methods may now be considered worldwide as close to harmonization, although minor differences in the experimental design may subsist (43). The SPF value of a given sunscreen is now considered to be the same within acceptable limits regardless of the country where the product is sold.

Protection Against UVA

The situation is different concerning the evaluation of the protection afforded by a sunscreen in the UVA range, because contrary to the measurement of the SPF, no universally accepted method exists to evaluate UVA protection. However, most recent regulatory recommendations or requirements consider the following methods for the evaluation of UVA protection of a sunscreen (5,6,44):

- in vivo measurement of afforded protection with the persistent pigment darkening method
- in vitro absorbance measurements
- in vitro determination of the critical wavelength
- in vitro determination of a UVA protection factor (UVAPF)

The requirements differ slightly between different regulatory authorities. The FDA has proposed a two-step testing [in vivo persistent pigment darkening testing and in vitro the ratio of long-wavelength UVA absorbance (UVA I) to the total UVR absorbance (UVB + UVA)] (6). In addition, if the products claim water resistance (WR), the UVA protection should be tested after the appropriate period of water immersion. The European Union (EU) has recommended the use of in vivo persistent pigment darkening test and the in vitro determination of the critical wavelength (5). In addition, the EU clearly stated that in vitro testing methods delivering equivalent results should be preferred, as in vivo methods raise ethical concerns. At the time of writing, the testing methods referred to in the European recommendation are subject to standardization by the European Committee for Standardization.

The determination methods are published and are freely available on the Internet. The Colipa (the European cosmetic, toiletry and perfumery association) has recently issued a detailed guideline on the in vitro determination of the UVAPF of sunscreen products, which now may be used instead of the in vivo persistent pigmentation darkening method, thus complying with the recommendations of the EU Commission (44).

Both the FDA proposed rules and the Colipa guidelines addressed on the photostability issue and incorporation of a pre-irradiation step to the in vitro test method. Concerning the pre-irradiation dose, the FDA proposes a dose of UVR corresponding to the SPF of the product, but reduced by a factor of one-third to represent a reasonable exposure (6,43). The Colipa method requires the determination of a UVAPF on a non-irradiated sample and then to irradiate the product with a UVA dose of 1.2 J/cm^2 multiplied by the UVAPF. The value of 1.2 J/cm^2 results from a Colipa round robin test (44). The UVAPF determined after irradiation is the one used to calculate the ratio of SPF to UVAPF, which should be less than 3 (5).

The Australian method was the first official method for evaluating the UVA protection of a sunscreen (42). It is an in vitro method involving the determination of transmission values between 320 and 360 nm (UVA II). This method was frequently used by sunscreen manufacturers to claim UVA protection for their products at a time when no real alternative

existed. However, because the Australian method takes no account of the long-wave UVA I, it has not been considered by the EU for UVA protection claim of sunscreen products.

Protection Against IRR

There is presently no standard method to evaluate efficacy claims concerning IRR. This results on one side from the current debate concerning the question if IRR effects on the skin are deleterious, or not, and on the other side from the absence of defined criteria for measurements of IRR effects in the skin, particularly concerning IRA.

Water and Sweat Resistance

WR characterizes the property of a sunscreen to maintain its degree of protection under adverse conditions, such as repeated water immersion or sweating. Because of the outdoor use of sunscreens in conditions where water immersion is usual and abundant sweating may be encountered, water and sweat resistance is very important (45). Human testing is considered to be the most acceptable and definitive method for claiming WR, and several guidelines have been published for estimating the WR of a sunscreen (38,42,45).

FDA Guideline

A product is claimed to be "WR" if it retains the same category description after 40 minutes of water immersion (2×20 time periods separated by a 20-minute rest period without toweling). It may be claimed "very WR" if this is the case after four immersion periods of 20 minutes each (80 minutes). The claim "waterproof" is no longer allowed. Products carrying the claims WR or very WR may also claim to be "sweat resistant" because the FDA concluded that the immersion test is a more severe test than a sweating test (45,46). It is now proposed that the time spent in water (40 or 80 minutes) would be added on the product label to ensure frequent reapplication (6).

Australian/New Zealand Guideline

The SPF of the sunscreen is determined after water immersion for not less than 40 minutes (2×20 minutes' time periods separated by a 5-minute rest period without toweling) (42). If WR of greater than 40 minutes is to be tested, the schedule of alternating 20 minutes immersion per 5 min rest is continued. The SPF measured after immersion determines the category classification of the sunscreen. Any claim of WR is to be qualified by a statement of the time for which the WR has been tested, up to the maximum claimed time. No statement is made about sweat-resistance.

The Colipa Guideline

The Colipa guideline for testing WR was published recently (45). Contrary to the FDA and to the Australian method, the SPF is determined on dry skin as usual before immersion. The watering conditions are ensured by 2×20 minutes' (40 minutes) immersion separated by a 15-minute drying period, which is very similar to the FDA requirements. The SPF is then measured again after the immersion period and the so-called mean percentage water resistance retention (%WRR) is calculated. The %WRR is the mean of the individual ratios:

$$\frac{[\mathrm{SPF\ (wet\ skin)} - 1]}{[\mathrm{SPF\ (dry\ skin)} - 1]} \times 100$$

A product may claim WR if the value for the 90% lower unilateral confidence limit of the %WRR is greater than or equal to 50% and the 95% confidence interval on SPF (dry skin) was within 17% of the mean SPF.

Finally, one must be aware that an important difference remains concerning the labeling of the products: A water-resistant sunscreen in the United States or Australia is labeled with the SPF measured after water exposition, whereas in Europe the SPF indicated is still the SPF measured on dry skin.

SUN PROTECTION WITH NUTRITIONAL COMPLEMENTS

As already quoted, nutritional protection against skin damage from sunlight is increasingly advocated to the general public. Several reasons are at the origin of these marketing campaigns, besides the fact that the nutritional complement market is a fast-growing area with a big development potential.

Recent results have been published showing that the mean yearly UVR exposition of the skin amounts to 200 SED (standard erythemal dose) in Europe and to 250 SED in the United States, and occurs during all-day life. Holiday exposition is shown to be only 30% and the weekend exposure 40% (summer) and 20% (winter). The remaining 10% are the daily amount, whereas children may be exposed to higher than 10% of the yearly SED (47–49). Therefore, up to 70% of the yearly exposure is all-day exposure.

In addition, recent analyses have shown that 25% to 30% of the lifelong UVR exposition occurs before 18 years of age (19,47,48). In this context, a systemic lifelong protection of the whole body (and not only the treated areas) through oral administration of targeted micronutrients makes sense and is considered by some experts as contributing to the general public health (4,19,49).

However, the protection offered by the nutritional complements is difficult to assess. In the majority of the published studies, it was measured using a standard MED determination as practiced for the measurement of the SPF. All measured protection factors did not exceed 2.

The investigated micronutrients are more or less potent antioxidants and/or free radical scavengers: vitamins or provitamins with antioxidative properties (carotenoids such as β-carotene, vitamin A, vitamin C, and vitamin E), plant extracts enriched or containing polyphenols as the most potent antioxidants known (polycopodium leucotomos, pycnogenol, green tea extract), ω-3 fatty acids, and trace elements such as selenium and zinc (5,19,33–37).

In view of these developments, the following points must be borne in mind:

- Protection measurements with the standard MED determination always showed results around 2. This raises the question if this experimental approach is the right one. But in any case, a thorough risk-benefit assessment must be conducted, as risks of long-term administration of such micronutrients in high doses are not really appreciated.
- Recent investigations have shown that a mixture of different micronutrients may show a far better activity than each micronutrient administered alone (50). Therefore, some people may face a huge amount of supplemented substances, depending on their way of living. For β-carotene, it has been demonstrated that a category of persons is definitively at risk if the amount administered is above a daily threshold (51).
- The current legislation concerning nutritional supplements is rapidly evolving, at least in Europe.

CONCLUSION

The increasing awareness about the damaging effects of sunlight has led to a significant demand for more protection from sunscreens and to an enlargement of the concept of sun protection toward global photoprotection. A controversial issue is the enlargement of this concept to the IRR wavelengths, as it is not entirely clear if IRA is damaging the skin or exerts a protective effect against UVB damage.

The demand for more protection is clearly reflected by the steady increase in the SPF. The SPF values of the majority of the sunscreens found in the market are now between 10 and 30, compared with 2 and 8 at the end of the 1980s (52). Sunscreens with SPF greater than 50 are now available. However, failure to use the sunscreen appropriately is a major concern and a great educational effort is needed concerning the amount of product applied and the frequency of use as consumers are still getting sunburned despite high-SPF sunscreens (51). This, and recent results concerning the lifelong exposition to sunlight, has led some experts to advocate systemic sun protection through administration of micronutrient supplements. This is still another controversial issue, notwithstanding the missing risk-benefit analysis for this kind of sun protection.

Finally, the traditional "sunscreen" concept has been further enlarged to "photoprotection," including UVA radiation and its consequences as a general public health issue (5,6).

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31 After-Sun Products

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THE HISTORY BEHIND AFTER-SUN PRODUCTS

It was at the beginning of the 20th century when vigilance against significant sun exposure (a relic of the 19th century) started to erode, and the roots of later attitudes toward sunbathing were already manifested. The first clinical observations associating long-term sun exposure with beneficial effects (phototherapy) as well as with skin cancer were also reported during this time (1).

The role of sunlight as a major cause of skin cancer was increasingly mentioned in popular magazines in the 1940s and 1950s. However, rapid growth of the sunscreen and later of the after-sun industry has taken place, allowing sun tanning to remain popular (2). Sunburn treatment and after-sun products were already used at the end of the 19th century. Examples of such products are lactic acid with glycerine and rose water, hydrogen peroxide, iodine, lemon or cucumber juice, and buttermilk baths (1).

AFTER-SUN-RELEVANT EFFECTS OF SUNLIGHT ON THE SKIN

Sunlight is highly energetic. Upon interaction with the skin, sunlight can be reflected, scattered, or absorbed. To initiate a physical or chemical process, light needs to be absorbed by an atom or molecule. Human skin is an abundant source of numerous chromophores with strong absorption, particularly in the UVB, UVA, and blue visible region, e.g., porphyrin, bilirubin 2, and pheomelanin (3).

Irradiation of the skin with ultraviolet radiation (UVR) is today known as a major cause of skin cancer and of local and systemic immunosuppression and as a contributor of cutaneous photoaging (4). Major changes concern all parts of the dermis and the dermal-epidermal junction (5).

UVR is proved to produce DNA damage directly and indirectly through oxidative stress. It provokes increased production of reactive oxygen species (ROS), leading to local inflammation and connective tissue degradation. Increased production of endopeptidases such as matrix metalloproteinases (MMPs) results in destruction of extracellular matrix (ECM). Alterations of the ECM result in wrinkle formation with loss of moisture and elasticity, increased skin fragility, and impaired wound healing (5). Furthermore, UVR induces synthesis of various neuropeptides (substance P, calcitonin gene-related peptide, propiomelanocortin, etc.) leading to immunosuppression and photoaging (6). UVR supports additionally epidermal thickening, reduced skin barrier function, and breakdown of tissue homeostasis (3).

Sunlight affects human skin not only via UVR but also via infrared radiation (IRR) and the combination of them. IRR is perceived as heat. It has been observed that the temperature of human skin measured inside the dermis increases to 40°C to 43°C within 15 to 20 minutes after exposition to direct summer midday sunlight (7). Heat is one of the environmental factors that amplify the effects of UVR mentioned above, reinforcing at the same time angiogenesis and dehydration (7). The skin lipids may as well get thermally destroyed, leading to further damage of the epidermal barrier. Additionally, thermal degradation of carbohydrates leads to modified osmolar environment and further dehydration. Even if the skin does not seem to have been affected by sun irradiation, increased transepidermal water loss (TEWL) is a fact and irritation of nerve endings as well (8). Other climatic conditions can reinforce dehydration. On the beach, wind is always blowing accelerating water evaporation from the skin. Because of the cooling effect of the wind, heat perception is diminished or absent, and beachgoers may consequently be staying longer in the sun without any discomfort. With increased evaporation,

the skin looses both moisture and the important water-soluble natural moisturizing substances too. In this respect, particular attention should be paid to elderly sunbathers. It is known that elderly skin is dry, exhibiting an overall dermal atrophy and reduced amounts of fibrillar collagens and elastic fibers. Thus, prolonged sunbathing may worsen the already present skin condition, leading to accelerated photoaging (9).

Under the influence of the sun, the human body increases sweat production. The horny layers of the skin swell and loosen up, facilitating penetration of the radiation into the epidermis. This may furthermore favor the effects described above (9).

Most of the effects described above are not immediately perceived by sunbathers. Rather acute reactions to sunlight irradiation include erythema, edema followed by exfoliation, tanning, and epidermal thickening, depending on exposure dose. Erythema appears 2 to 4 hours after irradiation, reaches its maximum by 24 to 48 hours, and then gradually disappears (9). Pain, in the form of tenderness to touch, is usually delayed for several hours after sun exposure (10).

The skin can manage the sunlight radiation stress only up to a limited grade of irradiation. Sunscreen protection, although greatly limiting the effects of UVR in the skin, does not completely protect against DNA damage. Small damage may be immediately restored, but repair takes time. Therefore, a sufficient resting period, of at least 12 hours, should be taken before going back to the sun (9).

SKIN CARE AFTER SUN EXPOSITION

Following intensive sun exposition, the skin needs appropriate care or treatment. Even after a sunbath without any signs of erythema, an appropriate skin care is recommended. In case of sunburn, treatment is necessary (8,9).

As a first step after every sun exposure, the skin should be washed with lukewarm water to remove sweat and superficial mud particles. Thereafter, the skin should be treated in accordance with the general schema of skin care: cleanse, vitalize, and maintain the status of the skin.

For cleansing, surfactants showing little interaction with both skin lipids and skin proteins are suitable. Products of pH, which are neutral for the skin, mildness enhancers, and moisturizing agents such as lipids, occlusives, and humectants minimize aggressive interactions with the epidermal barrier, thereby reducing skin damage (11). Recent clinical testing on leading facial cleansers shows that non-foaming, emollient-based cleansers are extremely mild to the skin while still being efficacious (12).

To vitalize and maintain the healthy status of the skin, appropriate skin care is most important after sun exposition. Restoring the hydrolipidic skin barrier is mandatory (8). Formulations containing moisturizers such as sodium lactate, urea, glycerine, panthenol, etc., are most suitable. Slightly occlusive water-in-oil formulations may further enhance the moisturizing effect by impairing TEWL (13,14). However, they should be applied after first cooling and calming the sun-irritated skin with an indicated oil-in-water formula (9,15). More recently, use of skin-like physiologic lipids or naturally occurring lipid complexes of precursor barrier lipids in the appropriate emulsion and molecular ratio has been shown to favorably influence skin repair, barrier function, and moisturization (16,17).

AFTER-SUN PREPARATIONS AND THEIR ACTIVE INGREDIENTS

Most of the after-sun formulations are emulsions (lotions, creams, and sprays) or gels containing moisturizers and actives known for their anti-inflammatory and antioxidative effects. Only cosmetic, nondrug actives will be considered in this section. Thus, active ingredients are usually of plant origin such as azulene or bisabolol (from chamomile), glycyrrhizin (from the root of liquorice), hamamelis distillate (witch hazel), or extracts from aloe vera or chamomile. Further compounds such as allantoin, panthenol, menthol, jojoba, collagen, silk amino acids, unsaturated fatty acids, and fat-soluble vitamins such as retinol (vitamin A) and vitamin E are also found (9). Formulations containing vasoconstrictive and/or tanning or astringent agents (tannins) are used to alleviate heat and tension sensations due to erythema. However, owing to the potent astringent

effect (protein precipitation), use of such preparations on irregular pigmented skin or skin of elderly people is not recommended. New developments have led to preparations facilitating or supporting repair mechanisms (9). Recent studies demonstrated the effect of specific molecular photoprotective agents. Prototype agents that antagonize, modulate, or reverse the chemistry of skin photodamage hold promise in delivering therapeutic benefits (18–20).

In general, the active components used in after-sun preparations can be divided into moisturizers, anti-inflammatory substances, antioxidants, and other ingredients.

Moisturizers

The name "moisturizer" is poorly defined and may be used to define the formula indented to be applied to the skin or the ingredient(s) incorporated in the formulation (14,21). Moisturizing substances are classical, well-known cosmetic ingredients used "to reduce the signs and symptoms of dry, scaly skin" (21). Certainly, effective moisturizers are important components of after-sun preparations for the reasons delineated previously. There is a huge amount of literature available on different moisturizers and their mechanisms of action, thus the reader is referred to very recent pertinent monographs (22–24) and to chapter 10 of this book.

Anti-Inflammatory Substances

Ingredients such as dexpanthenol, azulene, glycyrrhetinic acid, bisabolol, allantoin are incorporated into after-sun formulations to relieve erythema and its symptoms of pain, redness, and burning. The effects of these substances are generally weak. For example, dexpanthenol was shown to alleviate dry, inflamed skin in an experimental model of skin irritation due to repetitive washing. It improved stratum corneum hydration, stabilized the epidermal barrier function, and showed an anti-inflammatory effect (25–27). The combination of skin hydrating and anti-inflammatory properties in the same molecule is of course very interesting for after-sun preparations.

However, more powerful are the tanning or astringent agents. They alleviate itch and possess a local anesthetic effect (9).

Hamamelis (Witch Hazel)

For a long time hamamelis (witch hazel) has been used in the natural medicine. In United States, hamamelis is approved as an over-the-counter astringent in the external analgesic (pain relieving) and skin-protecting categories. In Germany, hamamelis extract–containing preparations are approved among others for the treatment of minor skin injuries and local skin inflammation. Extracts from the leaves or from the bark, but also flower distillate, are mainly used. Main components of the distillate are tanning agents as well as flavonoids and essential oils. Gallotannine and proanthocyanidine act constrictive at the outer membranes of the skin (28,29). Flavonoids are known for their anti-inflammatory effects on the skin (30). In several studies, the anti-inflammatory and erythema-suppressing effect of hamamelis has been demonstrated (27,31–33), even if lower than the one of hydrocortisone-containing formulations (31). However, the efficacy of the hamamelis distillate lotions has been shown to be similar to an antihistamine gel containing 0.1% dimethindene maleate. The low toxicity of hamamelis and the absence of known undesirable effects underline its favorable risk-benefit ratio supporting thus its usage in after-sun formulations.

Polyhydroxy Acids

Polyhydroxy acids (PHAs) are a special type of AHAs (α-hydroxyl acids) known to modulate keratinization, normalize stratum corneum exfoliation and thickness, and condition the barrier integrity due to the effects mediated by the α-hydroxy group. In addition, PHAs have been found to be less irritating to the skin than traditional AHAs mainly due to their larger size (34). For this reason, PHAs offer a distinct advantage over conventional AHAs (glycolic acid and lactic acid) when used on sensitive or irritated skin. In addition to the fact that many PHAs are strong humectants, several PHAs including gluconolactone, lactobionic acid, and glucoheptonolactone have also been shown to function as antioxidants. Through chelating of oxidation-promoting metals, PHAs may prove beneficial in protecting the skin from UVR-induced damage (35).

Cooling Compounds

Menthol and related "cooling agents" such as camphor, alcohol, and other compounds are widely used in cosmetics and particularly in after-sun products. The cooling and carminative actions of menthol have been repeatedly shown (36–39). Green (37) demonstrated that menthol intensified paradoxical cutaneous sensations. In the study of Yosipovitch et al. (39), menthol had a subjective cooling effect lasting up to 70 minutes in most of the volunteers. However, it did not affect cold and heat pain threshold. Recently, Green and Schoen (40) found that dynamic contact can suppress steady-state cold sensations from menthol, proving the bimodal action of menthol.

Alcohol induces an immediate short cold sensation and lowers the threshold of cold sensation. This is mainly due to its rapid evaporation.

Thus, incorporation of cooling agents such as menthol or alcohol in after-sun preparations is meaningful, as they soothe and alleviate the sensations of warmth and tenseness of sun-irritated skin, at least for a short time. This clearly improves their cosmetic acceptance.

Antioxidants

The cutaneous antioxidant defense system is complex, multilayered, and far from being completely understood. The human skin contains various antioxidants such as lipophilic ones [vitamin E with its active components tocopherol and tocotrienol, ubiquinones (coenzyme Q10), carotenoids, and vitamin A] as well as hydrophilic ones (vitamin C, uric acid, and glutathione) and enzymatic antioxidants (catalase, etc.) (41,42). Being the most environmentally exposed skin layer, the stratum corneum may particularly benefit from an increased antioxidant capacity due to topical supplementation as shown in the example of vitamin E (43,44).

While efficacy of topical antioxidant application before UVR is demonstrated in several studies (35), the effect of these agents after irritation is less obvious (44,45). Hence, use of antioxidants in after-sun preparations aims at replenishing the depleted antioxidant pool and/or at boosting or reinforcing the antioxidant defenses of the horny layer and not necessarily at a direct effect on sun-exposed skin. For this purpose, substances such as vitamins of the A, C, and E series, ubiquinones (coenzyme Q10), and/or plant polyphenols from green tea (catechins), vine (resveratrol), pomegranate (anthocyanidins and hydrolizable tannins) or soybeans (genistein) are now incorporated in many after-sun preparations (46).

Green tea extract is particularly interesting in this respect because several studies are available regarding photoprotection and anti-inflammatory activities after topical application. A protection against UVA has also been demonstrated (47–49). The principal chemical constituents of green tea are polyphenols containing (−) epigalocatechin (EGC), (−) epicatechin (EC), (−) epicatechin-3-gallate (ECG), and (−) epigalocatechin gallate (EGCG). Topical EGCG has been shown to reduce UV-carcinogenesis in mice (50–52). Furthermore, recent studies suggest that green tea polyphenols mediate almost probably their UV-protective effects via induction of DNA repair (53).

Other Ingredients

Shea Butter

Shea butter may be incorporated in the lipid fraction of after-sun formulations. Shea butter contains a highly unsaponifiable fraction consisting of terpenic alcohols and sterols found almost exclusively as cinnamic acid esters. They give shea butter its known healing properties, e.g., the elimination of superficial irritation and erythema. The unsaponifiables also contain 5% to 10% phytosterols, which are known to activate cellular growth stimulation. Moreover, shea butter contains the potent antioxidant butylated hydroxytoluene as well as various catechin compounds (54). Shea butter is a nontoxic and nonirritating material. It has a highly unsaturated glyceridic fraction, which makes it useful as UV screen. It also contains linoleic acid (55).

Clinical studies were performed with shea butter showing protecting, regenerating, wound-healing, and wrinkle-reducing effects (56). It has moisturizing, soothing, antiageing, and anti-inflammatory properties and contributes to an efficient release of active ingredients (56).

Olive Oil
Extra virgin olive oil (EVOO), which is obtained from whole fruit, is rich in phenolic compounds having potent anti-ROS activity. Recently, it has been found that EVOO painted on mouse skin immediately after UVB radiation, significantly retarded the onset and reduced the number of skin cancer and ROS-induced DNA damage. Interestingly, pretreatment with EVOO and pre- or posttreatment with regular olive oil neither retarded nor reduced skin cancer in UV-irradiated mice. Of the olive oil components tested, oleuropein has been found to reduce ROS-induced skin damage (57). These results strongly suggest that topical use of olive oil after sun bathing in humans may prevent skin cancer formation by reducing ROS-induced DNA damage. However, further human studies are needed to support recommendations in the public.

Molecular Photoprotective Agents
On the basis of the causative involvement of skin chromophores in skin photodamage, it is to be expected that molecular antagonism of photoexcited states offers a potential therapeutic opportunity for skin photoprotection. Reactivity-based approaches are widely followed for the design of photoprotective agents, including FDA-approved drugs, cosmeceuticals, and experimental therapeutics in preclinical development.

Two classes of reactivity-based agents for skin photoprotection can be distinguished on the basis of their mechanism of action: *direct antagonists of photooxidative stress* (sunscreens, quenchers of photoexcited states, antioxidants, redox modulators, and glycation inhibitors) and *skin photo-adaptation inducers* [nuclear factor erythroid 2-related factor 2 (Nrf2) activators, heat-shock response inducers, and metallothionein inducers] (20). Of the first class, quenchers are worth to be mentioned more explicitly as many of them are allowed to be used in cosmetics.

Quenchers are compounds capable of inactivating photoexcited states by direct chemical and/or physical interactions. Physical quenchers that can undergo repetitive cycles of excited state quenching without chemical depletion or the need of metabolic regeneration represent a very attractive class of compounds. Examples of such quenchers are vitamin E, ascorbate, L-proline, L-proline methylester, carotenoids (lycopene, lutein, and zeaxanthin), the microbial osmoprotectant ectoine, etc. (20). Ectoine, which can be used, as well as a strong moisturizer is already included in various kinds of marketed products (58,59).

Two further molecular photoprotective concepts are worth to be mentioned.

Few studies demonstrated that when exogenously applied, the bacterial DNA incision repair enzyme T4 endonuclease V (T4N5) is able to protect human skin in vivo from UVR-induced DNA damage. Although neither erythemal response nor microscopic sunburn cell formation were influenced by T4N5 treatment, UVR-induced upregulation of interleukin 10 and tumor necrosis factor-α were prevented (19). Interestingly, the liposomal lotion was applied after irradiation, thus as an after-sun product would be. A further experiment in xeroderma pigmentosum patients showed that the T4N5 enzyme clearly lowered the annualized rate of new lesions in these patients with defects in their DNA repair mechanisms. An interesting and perhaps fundamental finding was that the rate of new actinic keratoses and basal cell carcinomas did not increase during the six months of follow-up after discontinuation of treatment (60). This may imply that T4N5 treatment reverses a fundamental and common source of these neoplasms and is not just a cosmetic treatment (61).

The second approach is similar to the first one but uses a different DNA repair enzyme. Photolyases are repair enzymes found in fish or prokaryotic organism and normally not present in human skin. They bind to cyclobutan-pyrimidine dimers after their formation due to UVR exposure. The complex enzyme plus dimer is activated by light in the range of 300 to 500 nm, and the repair process gets induced. Here too, the photolyase from the blue algae Anacystis nidulans prepared from genetically engineered *Escherichia coli* was packed in liposomes and applied topically after irradiation. After a new irradiation with activating light of 365 nm, a reduction of 40% to 45% of the cyclobutan-pyrimidine dimers was noted. Similarly, a clear effect on immunosuppression was seen and on the expression of the pro-inflammatory ICAM-1, which was clearly decreased by UVR, but the decrease was antagonized by topical enzyme treatment (18).

Topical application of molecular photoprotective agents is a very promising approach to skin cancer prevention and totally compatible with the principle of after-sun products. Thus, one

may think of a pre-sun preparation combining antioxidants to replete the horny layer with the T4N5 enzyme to prevent formation of cyclobutan-pyrimidine dimers. Then, an after-sun preparation could provide moisturization and skin care, including photolyase to repair the last DNA changes. A liposomal lotion containing photolyase is already on the market in Germany (62).

CONCLUSIONS

Appropriate after-sun skin care is necessary to support the natural regeneration process of sun-irritated skin. Mild cleansing, cooling, moisturizing, and caring of the skin belong to the basis of a good after-sun treatment. In the last years, a lot of research has been successfully conducted to find ways to inhibit or repair sun-induced skin damage and to prevent skin cancer. Of the investigated compounds, antioxidants and molecular photoprotective agents seem to be the most promising for future application in after-sun products. However, ingredients with dual action showing UV filter properties as well as repair properties are certainly a valuable alternative. Recent studies have shown that topical caffeine as well as caffeine benzoate application induces apoptosis, thus decreasing the probability of neoplasmic transformation–irradiated cells, whereas having at the same time a sunscreen effect (63).

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32 Skin Care Products: Artificial Tanning

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INTRODUCTION

The desire for a tanned appearance along with increasing awareness of the hazards of ultraviolet (UV) light exposure has generated a renewed interest in artificial tanning products. Better formulations of sunless or self-tanners with improved aesthetics are more widely available. As consumer experience with the newer products has grown, this category has become more popular, and there has been an increasing proportion of overall sun care sales. Over 20% of young adults in both United States and Australia reported using these products in the preceding year (1,2). Individual users were also more likely to have sunburned consistent with higher use of these products in fairer Caucasians. In other studies, exclusive users of sunless tanners were more likely to practice overall sun protection (3,4) and decrease their use of tanning beds (5).

Dihydroxyacetone (DHA) is the active ingredient in sunless or self-tanners, and is responsible for darkening the skin by staining. DHA is classified in the *International Cosmetic Ingredient Dictionary and Handbook* (6) as a colorant or a colorless dye. Other agents with the potential to enhance skin pigmentation, including tan accelerators containing tyrosine and other ingredients. Tanning promoters containing psoralens, which require UV exposure, will not be discussed here (7).

HISTORY

The first mention of DHA as an active ingredient in medicine appeared in the 1920s, when it was proposed as a substitute for glucose in diabetics. In the 1950s, the oral administration of DHA was restudied as a diagnostic procedure for glycogen storage disease when it was given in large doses orally (8). When the children spit up this sweet concentrated material, the skin became pigmented in areas splattered on the skin, without staining clothing. Aqueous solutions were then applied to the skin directly, and the pigmentation reproduced (9). In the late 1950s, cosmetic tanning preparations first appeared in the marketplace. Cosmetic acceptance of these initial products was limited because of the uneven orange-brown color they imparted to the skin. With the availability of improved formulations in the 1990s, sales of sunless tanners grew exponentially as a total proportion of sun care product sales. In the last several years, operator-assisted spray tans using DHA have become popular in spas and salons.

CHEMISTRY

DHA ($C_3H_6O_3$) is a white, crystalline hygroscopic powder. This 3-carbon sugar forms a dimer in freshly prepared aqueous solution (Fig. 1). With heating to effect a solution in alcohol, ether, or acetone, it reverts to the monomer. The monomeric form is less stable, but more important in the browning reaction, which leads to the skin color change (10). DHA is stable between pH 4 and 6, but above pH 7, efficacy is lost with the formation of brown-colored compounds. A buffered mixture at pH 5 is most stable. Heating above 38°C for long periods of time will also affect stability. DHA needs to be stored in a cool, dry place, ideally 4°C and low atmospheric humidity (11). Glyceraldehyde, the isomer of DHA, is also present in the solution. Glyceraldehyde may degrade into formaldehyde and formic acid. In acidic solution (pH 4), this isomerization and therefore these latter undesirable ingredients are minimized.

Monomer Dimer

Figure 1 Chemical structure of DHA. *Abbreviation*: DHA, dihydroxyacetone.

The Maillard or browning reaction has been defined as the reaction of an amino group of amino acids, peptides, or proteins with the glycosidic hydroxyl group of sugars. DHA in the context of this reaction may be considered a 3-carbon sugar, reacting with free amino groups available as amino acids, peptides, and proteins supplied by the keratin to form products or chromophores referred to as melanoidins (12). Melanoidins have some physicochemical properties similar to naturally occurring melanin (13). Electron spin resonance has recently shown that free radicals are produced in vivo by the Maillard reaction (14).

FORMULATION

The concentration range of DHA in self-tanning products can range from 2.5% to 10%, with the usual concentration being 5% (10). Lower concentration products allow the consumer greater latitude with application since they tend to be more "forgiving" of uneven application or rough surfaces. Labeling products as light, medium, or dark can be particularly helpful with the depth of shade a function of the DHA concentration.

DHA is predominantly formulated in oil-in-water emulsions. Formulating with silicones allows the formulator to obtain the spreadability of oils, which potentially reduces streakiness with application to the skin. Minimizing particle size of the micelles in the emulsion chosen also improves uniformity of spreading the formulation on the skin surface. On the basis of the chemistry of DHA, formulations should be buffered to an acidic pH (4 to 5) and not heated in manufacturing to temperatures higher than 40°C.

After incorporation of DHA into a formulation, the pH may drop during storage, suggesting that stability may actually be increased when the pH is kept between 3 and 4 (15). The use of nonionic emulsifiers as opposed to ionic emulsifiers may also improve stability. Some thickeners such as carbomers, sodium carboxymethylcellulose, and magnesium aluminum silicate can cause rapid degradation of DHA. Hydroxyethylcellulose, methylcellulose, and silica as well as xanthan gum and polyquaternium-10 for thickening DHA-containing emulsions are better choices.

DHA can react with oxygen and nitrogen-containing compounds, collagen, urea derivatives, amino acids, and proteins. They should be avoided in the formulation of the DHA-containing vehicle. Non-nitrogen-containing sunscreen should be used if sun protection is desired. Attempts have been made to take advantage of this effect by adding amino acids to speed up the skin-darkening process, but with less substantive color results. Methionine sulfoxide, a sulfur-containing amino acid, has been used as an excipient applied before the application of the DHA-containing cream (16). Two compartment systems have been patented on the basis of this reaction.

As with moisturizing products in general, lotions are more readily accepted by consumers than are creams with ease of spreadability and aesthetics. Creams can produce a more intense tan owing to greater applied film thickness. Products may be formulated for dry-skin types by the addition of emollients and humectants. Products formulated in gel or alcoholic vehicles may be more suitable for oily skin. Newer vehicles include sprays, foams, mousses, and wipes.

MECHANISM OF ACTION

The site of action of DHA is the stratum corneum (17). Tape stripping of the skin quickly removes the color (18), as does mechanical rubbing. Deeper staining in areas with thicker stratum corneum and no staining of mucous membranes without a stratum corneum are also

consistent with this being the site of action. DHA may be used as a substitute for dansylchloride as a measure of stratum corneum turnover time (19,20). Microscopic studies of stripped stratum corneum and hair reveal irregular pigment masses in the keratin layers (21) consistent with melanoidins. These melanoidins are formed via the Maillard reaction with DHA as a sugar reacting with the amino groups supplied by the keratin.

APPLICATION

Following application of a typical DHA-containing self-tanning lotion, color change may be observed within an hour (22). This color change may be seen under Wood's light (black light) within 20 minutes. Maximal darkening may take 8 to 24 hours to develop. Individuals can make several successive applications every few hours to achieve their desired color. Color may last as long as five to seven days with a single application. Depending on anatomical application, the same color can be maintained with repeat applications every one to four days. The face requires fewer applications but more frequent reapplication to maintain the color than the extremities. Depth of color varies with the thickness and compactness of the stratum corneum. Palms and soles stain deepest, necessitating washing of hands after application to avoid staining. Hair and nails will color but not mucous membranes lacking a stratum corneum or keratin layer. Rougher hyperkeratotic skin over the knees, elbows, and ankles will color more unevenly as will older skin with keratoses and mottled pigmentation. Color will also be maintained longer in these areas.

As in the formulation, the pH of the skin before application may have an effect on the tonality of the skin color (10). Alkaline residues from soaps or detergents may interfere with the reaction between DHA and the amino acids on the skin surface, resulting in a less natural-appearing (more yellow) color. Wiping the skin surface with a hydroalcoholic, acidic toner just prior to DHA application may improve results. Ex vitro epidermal studies suggest that skin hydration (23) and relative humidity (24) influence the development of coloration.

Careful directions provided with these products are therefore quite important in determining consumer satisfaction. The skin may be prepared with a mild form of exfoliation. Even application is required with lighter application around elbows, knees, and ankles to avoid excessive darkening in these areas. Care also needs to be taken around the hairline where lighter hair may darken. Hands need to be washed immediately after use to avoid darkening of the palms, fingers, and nails. Skill and experience are necessary with using these products resulting in greater user satisfaction.

ADDITIVES

As commonly occurs, growth in this category has compelled both formulators and marketers to seek points of differentiation between their product and that of their competitors. Besides formulating for specific skin types, active treatment ingredients may be incorporated into DHA-containing formulations. Vitamins, botanical extracts, antioxidants, anti-irritants, and even α-hydroxy acids may be added to broaden the claims made with a given product. Addition of antioxidants can shift tonality to a more natural coloration (25). The addition of sunscreen ingredients to self-tanners warrants a more detailed discussion in the section that follows.

Some newer formulations have included colorants as used in bronzers, including dyes and caramel, to achieve an immediate makeup effect. Similarly, tinting with titanium oxide or iron oxides can provide immediate color and allow the user to more easily visualize the evenness of application. Metal oxides may however induce degradation of DHA (15). To compensate for less red absorption by the products of the Maillard reaction, erythrulose may be added as a colorant to add red to the tone.

SUNSCREEN ACTIVITY

In the United States, the FDA Tentative Final Over-the-Counter Monograph on Sunscreens (Fed. Reg. 1993) listed DHA as an approved sunscreen ingredient when used sequentially with lawsone (2-hydroxy-1,4-napthoquinone). The final monograph (Fed. Reg. 1999) removed this

combination from the approved list. The European Economic Community Directive does not list DHA as a permitted UV filter.

DHA itself has at most a modest effect on SPF (26), providing perhaps SPF 3 or 4. SPF increases with DHA concentration and number of applications (27). Low-level SPF persists for several days decreasing with loss of color (28). The brown color obtained on the skin does absorb in the low end of the visible spectrum with overlap into long UVA and may provide some UVA I protection (29). Melanoidins can act as free-radical scavengers as they demonstrate an electron spin resonance signal (14). Superficial skin coloration induced by frequent topical application of DHA in high concentrations may delay skin cancer development in hairless mice irradiated with moderate UV doses (30).

Individuals using DHA-containing tanning products need to be cautioned that despite visible darkening of their skin, these products provide minimal sun protection. Confusion may be compounded by the addition of UV filters to the formulation providing significant sun protection. The stated SPF for the product is applicable for a few hours after application, but not for the days during which the skin color change may remain perceptible.

INDICATIONS

Even with recent improvement in DHA formulations, the color achieved remains dependent on skin type. Individuals of medium complexion with skin phototypes II or III (31), as opposed to those who are lighter or darker, will obtain a more pleasing color. Individuals with underlying golden skin tones will achieve better results than individuals with rosy, sallow, or olive complexion. Older consumers with roughened, hyperkeratotic skin, or mottled pigmentation with freckling may be less pleased with their use.

Dermatologists regularly recommend these products for tanning as a safe alternative to UV exposure. They may be used to camouflage some skin irregularities such as leg spider veins. Light to medium complected patients with vitiligo who show increased contrast with the vitiliginous areas with natural or unavoidable tanning in their normal skin may also benefit (32,33). They may even provide some protection for individuals with certain photosensitivity disorders (34). Protection of uninvolved skin by DHA during psoralen-UVA treatment (PUVA) allows higher UVA exposures to be tolerated, with fewer treatments resulting in faster clearing know as turbo-PUVA (35).

SAFETY

The visible color change associated with the use of artificial tanning products might suggest to some users that these products are hazardous. On the basis of the chemistry of DHA and its toxicological profile, it can be considered nontoxic. It reacts quickly in the stratum corneum, minimizing systemic absorption. The acute toxicity of DHA was investigated for diabetics in the 1920s with oral intake well tolerated (15). The phosphate of DHA is found naturally as one of the intermediates in the Krebs cycle.

Contact dermatitis to DHA has only rarely been reported (36). As with other topical products with active ingredients, such as sunscreens, much of the reported sensitivity is secondary to other ingredients in the vehicle (37). Adverse reactions are more likely to occur on the basis of irritation and not true allergy. Ultimately, all claims related to product safety are based on testing the final formulation.

ALTERNATIVE TANNING AGENTS

Lawsone found that henna plant and juglone (5-hydroxy-1,4,-napthoquinone) derived from walnuts also stain hair, skin, and nails. They have been used for centuries for hair coloring. Both substances lack skin substantivity and readily discolor clothing (38). The skin color they produce does not resemble a natural tan.

On the basis of the underlying principle of the Maillard reaction, other molecules with a ketone function have been investigated (39). An α-hydroxy group attached with electron-withdrawing groups can also increase reactivity. Substances such as glyceraldehyde and

glyoxal (40) have been described but found ineffective. Mucondialdehyde as described by Eichler (41) is an effective agent, but associated toxicity mitigated against its use (39). Although several other aldehydes have been shown to have better color properties, stability issues limit their use (39).

CONCLUSION

Increasing consumer awareness to the hazards of UV light should fuel ongoing interest in self-tanning products. The benign toxicologic profile of DHA reinforces the notion that these products represent a safe alternative to a UV-induced tan. The results obtained with these products are dependent on the final formulation, individual application technique, and the consumers' complexion type. Greater experience in formulation combined with increasing sophistication on the part of the consumer should lead to continuing growth and satisfaction with the use of these products.

Consumers need to be clearly informed that these products do not offer significant protection against UVB. If formulated with standard sunscreens, consumers should be cautioned that the duration of UV protection is more short lived than the color change.

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33 Reconstructed Human Skin and Skin Organ Culture Models Used in Cosmetic Efficacy Testing

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INTRODUCTION

The use of animals for research purposes and especially for cosmetic efficacy testing has been a sensitive matter for several decades. Regulatory agencies and political as well as scientific communities have put constant pressure to ban the use of animals for such purposes. In Europe, this has forced the Council of the European Union to approve legislation for banning the use of animals for testing cosmetic products, starting March 2009. This implies that alternative methods require validation by the European Center for the Validation of Alternative Methods (ECVAM). To date, only a few alternative testing methods have been validated for toxicological testing using reconstructed human skin models. Nevertheless, human skin equivalents, such as reconstructed human epidermis (RHE), full-thickness (FT) skin, or skin organ culture (SOC) models have already been adopted by cosmetic laboratories as alternatives to animal experimentation for quite some time. These models not only allow to comply with the demands of regulatory authorities, animal welfare organizations, and consumers but also provide a means to improve and extend our knowledge on skin biology. Moreover, they have proven to be reliable, efficient, informative, and predictive tools for screening, bioavailability, and efficacy testing of active ingredients.

One of the main reasons for the development of reconstituted human skin (RHS) models is the fact that they mimic more closely human skin in vivo, which includes the presence of a stratum corneum, allowing topical application of both aqueous and oily solutions as well as final formulations. Three-dimensional (3D) skin models are composed of either the epidermal compartment or both the epidermal and dermal compartments. Various cells types can be incorporated within each compartment, including melanocytes and Langerhans cells in the epidermis and fibroblasts and endothelial cells in the dermis. Because the use of RHS models requires a substantial amount of expertise, including internal benchmark validation, which often requires considerable financial resources, and given the fact that these models have some inherent limitations, especially an impaired barrier function, skin organ culture models are also being used as alternatives for cosmetic efficacy testing.

In this chapter, we first present a review about the development of reconstructed skin models, followed by an overview of the commercially available ones. We review a selection of published literature using commercially available models as well as homemade models developed in research laboratories used for in-house testing of cosmetic formulation. This overview will mostly focus on studies in oxidative stress photoprotection evaluation and pigmentation assessment. A brief review of more complex and integrated skin models for clinical use is also discussed. Then, we present the results of two sets of experiments carried out using both RHE and SOC models. The first experiment deals with vitamin E prodrug, its diffusion and metabolism in RHE and human SOC models, and also its efficacy in the prevention of UV-induced lipid peroxidation in RHE. The second experiment studies the response of RHE and a pig SOC model to solar radiation–induced cytotoxicity and apoptosis and tests the efficacy of a sunscreen formulation. In conclusion, these studies on cosmetic efficacy testing confirm that both RHS and SOC models represent complementary models with a genuine added value for the cosmetic testing.

SKIN EQUIVALENTS USED IN COSMETIC EFFICACY TESTING

Basic Research on Reconstructed Human Skin

New and stricter regulations have led researchers and companies to develop in vitro tissue models to be used in the study of cutaneous biochemistry and physiology. An excellent guideline enumerates the processes used in developing these cell culture models (1). Briefly, Bell's model (2), the first in vitro reconstructed model, developed in 1979, was a dermal equivalent composed of fibroblasts in a collagen lattice. Rheinwald and Green (3) fortuitment found that under certain culture conditions a teratogenic cell lines undergoes a maturation that mimics epidermal keratinization (3). Later, the major breakthrough was the culture of keratinocytes (KCs) at the air-liquid interface (4), leading to the formation of a multilayered and differentiated epidermis, a characterisitc of all types existing in 3-D skin models. This differentiation program provided the crucial stratum corneum (SC) layer, which constitutes a barrier function for reconstructed models. As cell culture undergoes constant evolution, various models were subsequently designed by differentiating KC cultures on different supports: inert filters (5–7), dermal substrate such as collagen matrices (8,9), lyophilized collagen-GAG membranes (10), de-epidermized dermis (DED) (11), and fibroblast-populated dermal substrates (12,13). Some of the above mentioned commercially available skin models are listed in Table 1.

Morphological studies (14,15) have shown that RHS models form a multilayered epithelium, displaying characteristic epidermal structure and expressing markers of epidermal differentiation. Ultrastructurally, keratohyalin granules, lamellar bodies, and lamellar structures filled with epidermal lipids are present in the stratum granulosum and SC. The SC layer, a corneified barrier for RHS models (Fig. 1), is composed of multiple lipid lamellae located in the intercellular spaces between keratinized cells, a corneocyte lipid envelope, and desmosomal structures. This barrier is associated with an epidermis calcium gradient, similar to that found in native human skin (16). However, it has been established that there is a difference in the lipid organization, with a hexagonal SC lipids packing in RHS, whereas in native skin it is orthorhombic (17). This difference may account for the 5- to 50-fold higher penetration rate observed in RHS models for most of the substances tested (18). In addition, the recent scientific committee on consumer products (SCCP) guidelines in dermal absorption

Table 1 Commercially Available RHS and SOC Models

Company/laboratory	Skin models	Web site
Bioprédic, Rennes, France	Skin organotypic culture Natskin® (human skin), 1.0 and 1.6 cm^2	www.biopredic.com
CellSystems, St Katarinen, Germany	Reconstructed human epidermis (EST 1000) Full-thickness skin (AST 2000)	www.cellsystems.de
MatTek Corp. Ashland, MA, U.S.	Reconstructed human epidermis (EpiDerm®), 0.9 cm^2 Full-thickness skin (EpiDermFT®), 1.2 cm^2 Reconstructed pigmented epidermis, (Melanoderm®), 0.9 cm^2 Melanoma skin model, 0.6 cm^2	www.mattek.com
Phenion GmbH & Co. KG (Henkel), Düsseldorf, Germany	Reconstructed human epidermis (OS-REP) 0.63 cm^2 Full-thickness skin (Phenion FT), 1.3 cm^2	www.phenion.com
SkinEthic, (L'Oréal), Nice, France	Reconstructed human epidermis, 0.5/4 and 0.11/0.33 cm^2 in 24/96 well plate Reconstructed pigmented epidermis, 0.5 cm^2 Full-thickness skin (Episkin®), 0.38/1.07 cm^2	www.skinethic.com
StratiCell, Gembloux, Belgium	Reconstructed human epidermis Genetically modified reconstituted human epidermis Reconstructed human 3-D dermis Reconstructed full-skin equivalent	www.straticell.com

Technical specifications and ordering informations for these skin models can be found in the respective Web site. Some of these companies also supply reconstructed epithelia models (buccal, vaginal, pulmonary etc.).

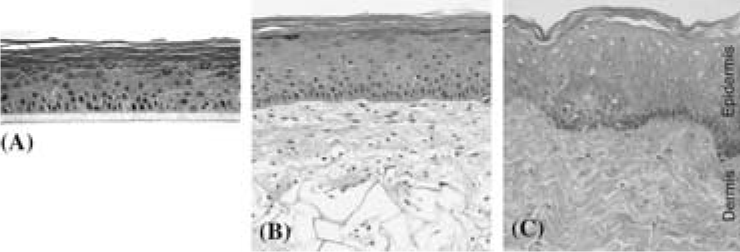

Figure 1 Histological section of different skin models. (**A**) A reconstructed human epidermis (SkinEthic Laboratories, Nice, France[a]); a polycarbonate membrane supports a stratified epidermis. (**B**) A full-thickness skin model (Phenion®[a]) with a stratified epidermis on a dermal equivalent. (**C**) Human SOC model from personal data.

of cosmetic ingredient (19) consider that RHS models as alternative membranes are inadequate for use in dermal absorption study because of this impaired barrier function. In spite of these differences (18,20), the presence of the SC on skin equivalents makes it possible to apply a great variety of active ingredients topically. In addition, through metabolism studies performed on 3-D human reconstructs and reviewed in details (21), most of 3-D skin equivalents (RHE, FT skin, or SOC) are metabolically representative of human skin and therefore can be used as a good surrogate model for human skin. For instance, similar enzymatic activities, such as phase I and phase II enzymes were found in RHE (22), making them efficient models for toxicological assessment. Other enzymatic activities, such as esterase, β-glucocerebrosidase, or 5α-reductase are also found in skin equivalents. Accordingly, reconstructed skin models were used successfully in the study of the controlled release of pro-vitamin E into free tocopherol (as referred to in details in section "Skin Absorption, Metabolism, and Antioxidant Efficiency of Vitamin E Prodrugs) (23,24), and of testosterone metabolism with androgen modulators (25).

Table 1 lists the commercially available skin models used for pharmaco-toxicological trials. Reconstructed epidermis models are first used for irritation testing, and some have already been validated or are in the process of being validated for this use. Providers now offer pigmented epidermis as well as FT skin models. However, the fact that only a handful companies are capable of producing skin models in sufficiently large quantities demands for some careful consideration. For instance, in 2006, Episkin® (L'Oréal group) acquired SkinEthic Laboratories. The resulting situation created a kind of monopoly situation in Europe (15). In addition, all models currently validated are either protected by patents or being commercialized using proprietary tissue culture procedures by only a few private companies; hence, their continued availability is dependent only and entirely on the corporate strategies of these companies and therefore cannot be guaranteed for the long-term future. In addition, although it is generally accepted that the reconstructed epidermal models currently available are all "comparable," intrinsic differences inherent to their proprietary manufacturing procedures have been causing reasonable concern in the scientific community; for instance, the test protocol transferability from one model to another is often limited and considered a bottleneck for method acceptance both at industry and regulatory levels.

Not only for regulatory toxicology testing but also in cosmetic efficacy testing, the use of these models requires great care and must include the appropriate controls and an analysis of reliable and reproducible endpoints. Accordingly, multiple endpoint analysis (MEA) including tissue viability, morphology, and the release of proinflammatory mediators should be used to determine the efficacy of a cosmetic product (26). One of these endpoints is the MTT [3-(4,5-dimethylthiazol)-2,5-diphenyltetrazolium bromide] toxicity test (27), which measures either cellular viability and therefore the cytotoxic effects of external stress or a cosmetic formulation. We recently determined, for instance, that the release of lactate dehydrogenase (LDH) into the culture medium is a more relevant endpoint than MTT assay to study irritation and solar-induced cellular damages in SOC models (28).

Applications of Skin Equivalents in Cosmetic Efficacy Testing

Below we describe some experiments carried out to determine the effects of UVB-UVA irradiation as inducer of oxidative stress and photoaging. Studies performed to measure photoprotection and monitor the pigmentation process in a pigmented skin model are also

discussed. Finally, new and "clinical use" models of skin equivalents are presented as potentially useful tools for the testing of future cosmetic formulations.

Oxidative Stress and Photoprotection

The skin is directly exposed to external oxidative stress, and in vitro RHE models have been used in the evaluation of ozone- (29) or UVA- (30) induced lipid hydroperoxides (LPOs). Ex vivo pig and human SOC models have been also developed as tool for use in investigating short-term UV-induced damage (31) and time course and spatial distribution of UV effects (32). Such experiments have demonstrated that skin models are sensitive to UV and that topical application of various antioxidants could successfully be evaluated against UV-induced oxidative stress by reducing apoptotic response and DNA damages: combination of vitamins on a pig SOC model (33), topical application of genisteine on a human SOC model (34), or epigallocathecinegallate (35) and salicylic acid (36) in RHS. In addition, UVA dermal alterations in an human SOC model and topical application of retinoids (37) induced the formation of newly synthesized collagen, suggesting dermal repair on this "photoaged" skin model.

Moreover, although in vivo assays are essential for sunscreen testing [determination of SPF (sun protection factor) and PFA (protection factor in UVA)], in vitro techniques based on skin equivalents were developed and have shown to be very useful for these types of tests. Using an RHS model, Augustin (38) showed the deleterious effects of both UVA and UVB irradiation by measuring viability and Il-1α release assay, which were abolished following the application of sunscreens. Also the apoptotic response was also assessed following UV irradiation with and without photoprotection in RHE (39), RHS (40), and pig SOC (41) (see experimental details and results in section "Deleterious Effects of Solar Radiation and Sunscreen Efficacy Testing on Both RHE and Pig SOC"). In general, all these skin models demonstrate their usefulness and ability to model UV-induced damages, which can be reversed in presence of photoprotectant formulations.

Pigmentation

Pigmented RHS are cocultures of both KCs and melanocytes. The integration of melanocytes from different ethnic regions results in a pigmented epidermis (42) or skin equivalent (43) reflecting Caucasian, Asian, and African-American skin phenotypes. These models provide an interesting alternative to animal testing for evaluating the regulation of mammalian pigmentation by melanogenic factors and for elucidating the mechanisms of action of these factors. Using these models, it has been demonstrated that the protease-activated receptor-2 (PAR-2) pathway (44) regulates pigmentation via melanosome transfer, but only when KCs and melanocytes are in contact. Gibbs has shown (45) that a complete program of melanogenesis occurs following UVB irradiation and supplementation with 3-isobutyl-1-methyl-xanthine: melanosome synthesis, melanosome transport to KCs, supranuclear capping of KC nuclei, and tanning of the epidermis. Dysfunctionnal pigmentation can also be assessed through the use of in vitro recontructed models. A xeroderma pigmentosa skin model was reconstrcuted to study genetic hyperphotosensitivity (46). Recently, an RHS model was engineered with normal nonsegmental nonlesional vitiligo cells (47), and tested under stressed conditions (H_2O_2). First results seem to support the melanocytorrhagic hypothesis of vitiligo.

Additionally, pigmented skin tissue models provide a useful tool in the comparison of the inhibitors (kojic acid, arbutin, and hydroquinone) and activators (α-MSH and dihydroxyphenylalanine) of melanogenesis (43) after repeated topical application or systemic delivery (48). Pigmented skin tissue is also useful in the assessment of the antipigmentation effects of sunblocks.

New and "Clinical Use" Skin Models

Tissue engineering attempts to reconstruct complete skin tissue, integrating different cellular types. Most of the previously mentioned skin models are comprised of KCs, fibroblast, melanocytes, and endothelial cells. However, the skin is an immune organ, displaying an elaborated enervated system. Immunoreactive reconstructed skin containing Langerhans cells has been reconstructed. The immune response of this RHS was demonstrated by a reduction in the number of Langerhans cells and by a modification in their dendritic morphology (49) after

exposure to sensitizers or UV irradiation. Another immunocompetent reconstructed model was developed, comprising epidermal Langerhans cells, dermal dendritic cell, and endothelial cell activated to acquire HLA-DR expression (50). This model provides a complex environment-integrating vascular components to study the differentiation of interstitial dendritic cells in the dermis. Also a "neuronal" epidermal model was developed to evaluate the regeneration of sensory neurons on injured skin (51), which was mainly influenced by the extracellular matrix molecules, matrix-binding growth factors, and trophic factors.

In addition to these "dermo-cosmetic" models, tissue engineering has led to the developement of complex reconstructed models for clinical indications such as grafting ulcers, treatment of burn patients, and wound repair. Some of these models are commercially available: Apligraf™, Alloderm™, Integra™, Dermagraft™, and Orcelf™ (52). Last, but not the least, a model comprising of endothelial cells was created for testing potentially angiogenic molecules (53) as well as for treating recalcitrant leg ulcers through split-thickness skin autografting (54). As perspective, additional development in the two integrative aspects of neovascularization and reinnervation is necessary for such skin reconstructs to reach their full therapeutic potential. Clearly, such "complete" skin models would be of great interest for integrated pharmaco-toxicological trials of the future cosmetic formulations.

EXAMPLES OF COSMETIC EFFICACY TESTING USING SKIN MODELS

After this brief literature review, we report our results obtained on two sets of experimentations using both RHE and SOC models. The first one deals with a skin diffusion and metabolism study of two vitamin E prodrugs on RHE and human SOC models (24), followed by determination of the antioxidant protection on RHE. The second example (41,55) shows the biological response to UV irradiation through cytotoxicity, DNA damage, and apoptosis in both RHE and pig SOC models treated with and without photoprotection.

Skin Absorption, Metabolism, and Antioxidant Efficiency of Vitamin E Prodrugs (24)

The development of prodrug technology is intended to avoid certain undesirable properties of topically applied drugs, such as instability and pro-oxidant effects. Accordingly, cutaneous metabolism could be considered as a critical determinant of the efficacy of these topically applied molecules, especially for the delivery of a pharmacologically active molecule through the skin. A gluco-vitamin E conjugate, δ-tocopherol glucoside (δ-TG) has been synthesized by making use of β-glucocerebrosidase activity, which hydrolyzes amphiphilic β-glucocerebrosides into ceramides. The resulting glycosidic bond cleavage allows the release of active vitamin E into the SC. The skin penetration and metabolism of δ-TG were evaluated in 18-hour studies in both RHE (SkinEthic Laboratories, Nice, France) and human SOC (Biopredic, Rennes, France) models. In addition, the efficacy of delivery of free tocopherol in a 0.5% δ-TG solution was tested by means of an lipid hydroperoxide (LPO) assay prior to solar irradiation (105 mJ/cm^2) on the extracted lipids. All these experiments were assessed in comparison with α-tocopherol acetate (α-TAc), the most common vitamin E prodrug known to be converted in the skin (23,56).

Following an infinite dose prodrug solution applied on RHE, a better diffusion was observed for α-TAc. However, no metabolism was detected with α-TAc. In contrast, 20% and 50% of δ-TG was bioconverted into free tocopherol in both SC and epidermis, respectively. A kinetic study with a 0.05% finite dose of δ-TG solution applied on RHE showed that about 90% of the prodrug was converted at 18 hours. A similar experiment on viable human skin confirmed that no free tocopherol was detected from α-TAc, in spite of the fact that the amount diffused was four times higher than the amount of δ-TG (Table 2). The compartmental distribution study confirmed a delivery of free tocopherol from δ-TG. The highest amount was found in the SC, then in the epidermis and also in the dermis (Table 2). Antioxidant efficacy is obtained only if the prodrug is metabolized into free tocopherol, and this was confirmed by a 70% decrease in LPO concentration after treatment with δ-TG, in contrast to treatment with α-TAc (Fig. 2) or the positive control (irradiated lipids). After topical application, δ-TG had a reservoir effect, associated with gradual delivery of free tocopherol. This experiment enabled us to demonstrate that RHE and human SOC models are complementary. Indeed, RHE can be used to confirm the metabolism and the efficacy of an active ingredient, as a result of its low barrier function, offering better bioavailability of the molecule being tested. Finally, human

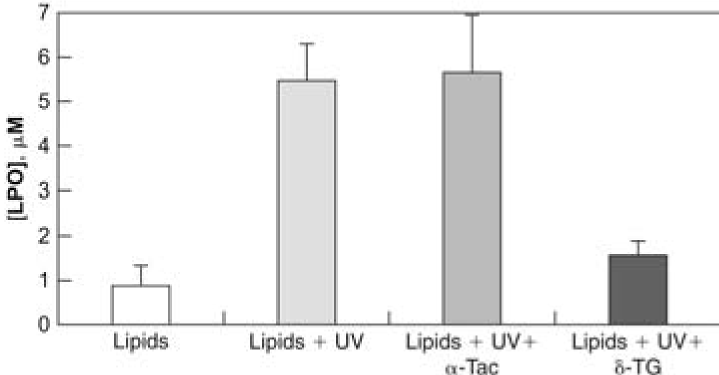

Figure 2 Concentration of lipid hydroperoxide (LPO) in lipids extracted from RHE, after 18-hour topical application of a 0.5% solution of the prodrugs with or without UV irradiation ($n = 4$) showing an antioxidant activity only when the skin model was treated with δ-TG (*last bar*).

Table 2 Compartmental Analysis [Surface, Stratum Corneum (10 Tape Strippings), Epidermis, and Dermis] of Two Prodrugs (δ-Tocopherol-Glucoside and α-Tocopherol Acetate), Their Conversion into Free Tocopherol, and Percentage of Metabolism After 18 Hours' Incubation in Viable Human Skin [Mean (S.E.)], ($n = 6$)

Analyte	Surface in μg/cm^2	Stratum corneum in μg/cm^2	Epidermis in μg/cm^2	Dermis in μg/cm^2
α-TAc	0.17 (0.04)	2.98 (0.18)	0.80 (0.27)	0.79 (0.45)
α-Toc	Not detected	Not detected	Not detected	Not detected
% of metabolite	/	/	/	/
δ-TG	3.44 (0.45)	0.78 (0.27)	0.35 (0.24)	0.16 (0.09)
δ-Toc	0.04 (0.04)	0.12 (0.07)	0.10 (0.04)	0.02 (0.02)
% of metabolite	1.1	15.3	29.2	13.1

SOC on the other hand, having a more efficient barrier function, can be used either as preclinical model or in the optimization of an active ingredient concentration to be included in the formulation.

Deleterious Effects of Solar Radiation and Sunscreen Efficacy Testing on Both RHE and Pig SOC (41,55)

Solar radiations are a major concern for human health since they have been recognized as an environmental human carcinogen, and they also contribute to the photoaging process (57–59). Today, sunfilters provide a good protection against the harmful effects of UVA and UVB radiations. However, the ban of animal testing in cosmetic industry urges to develop new alternative models to discover innovative strategies of skin photoprotection and to assess the photoprotective capacities of a sunblock formulation. In this context, we recently developed a short-term ex vivo skin organ culture model from domestic pig ears (Fig. 3) and also investigated solar-induced cytotoxicity and photoprotection. Furthermore, the results were compared to data obtained with in vitro RHE (SkinEthic Laboratories, Nice, France).

Skin samples were irradiated by using a suntest apparatus and received a single solar-simulated radiation (SSR) dose up to 275 kJ m^{-2}. This acute UV dose corresponds to about fivefold minimal erythemal doses (MEDs). The sunburn cells (SBCs) corresponding to apoptotic KCs following UVB exposure were easily detected by hematoxylin staining 24 hours postirradiation in pig skin and RHE models. These dying cells were mainly located in the basal layers of the epidermis. Furthermore, the deleterious effects of SSR were associated with the formation of thymine dimers (Fig. 4) and DNA fragmentation (Fig. 5) in both the epidermal and dermal compartments in both tissue models, as respectively assayed by immunohistochemistry and TUNEL technique (TdT-mediated dUTP nick-end labeling). None of these

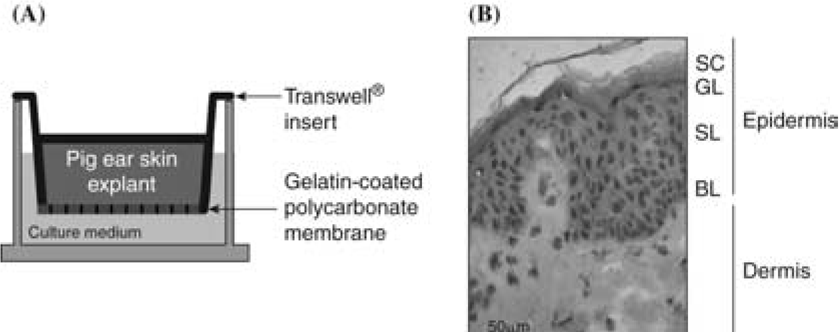

Figure 3 Pig ear SOC. (**A**) Schematic representation of the organ culture system. Skin was excised from domestic pig ears, sectioned at 500-μm thickness, and punched into 12-mm diameter discs. The explants were then seeded in gelatin-coated polycarbonate Transwell® inserts in 12-well plate prefilled with culture medium. (**B**) The multilayered structure of the porcine epidermis. Hematoxylin staining. *Abbreviations*: SC, stratum corneum; GL, granular layer; SL, spinous layer; BL, basal layer.

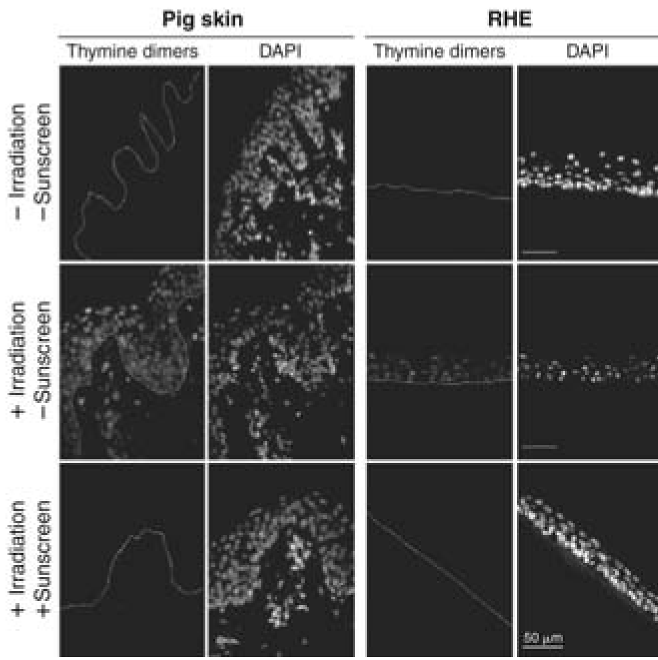

Figure 4 Thymine dimer formation after solar irradiation in pig skin and RHE. Skin was pretreated with or without sunscreen, and then exposed to solar-simulated radiation. Thymine dimers were identified by immunofluorescence 24 hours postirradiation. Note that topical application of sunscreen prevented the formation of DNA lesions in keratinocyte nuclei stained with a DNA fluorescent staining (DAPI). Dashed lines correspond to the dermal-epidermal junction and filter surface in pig skin and RHE, respectively. *Abbreviation*: RHE, reconstructed human epidermis.

cellular responses was observed in nonirradiated skin. The DNA damage was clearly correlated to SSR-induced cytotoxicity since a significant level of LDH activity and extracellular signal-related kinase 2 (ERK2) protein were recovered in the culture supernatant from the irradiated skin models (Fig. 6). The SSR-induced apoptosis involved the upregulation of the p53 tumor suppressor and the activation of the caspase-3 protease. Interestingly, caspase-3 activation was detected mainly in the basal epidermis after irradiation in pig skin organ culture. Thus, basal KCs might be more sensitive to UV exposure than suprabasal KCs and dermal fibroblasts.

To explore whether pig SOC and RHE models are suitable for investigating photoprotection, a broad-spectrum UVB+A sunscreen formulation, was topically applied on skin samples at a dose of 2 mg cm^{-2}. The experiments showed that the sunfilter provides good photoprotection without affecting skin viability in both tissue models. Indeed, sunscreen application completely abrogated not only SBCs and DNA damage formation (Figs. 4 and 5) but also the LDH/ERK2 leakage and caspase-3 activation in irradiated models. These data are

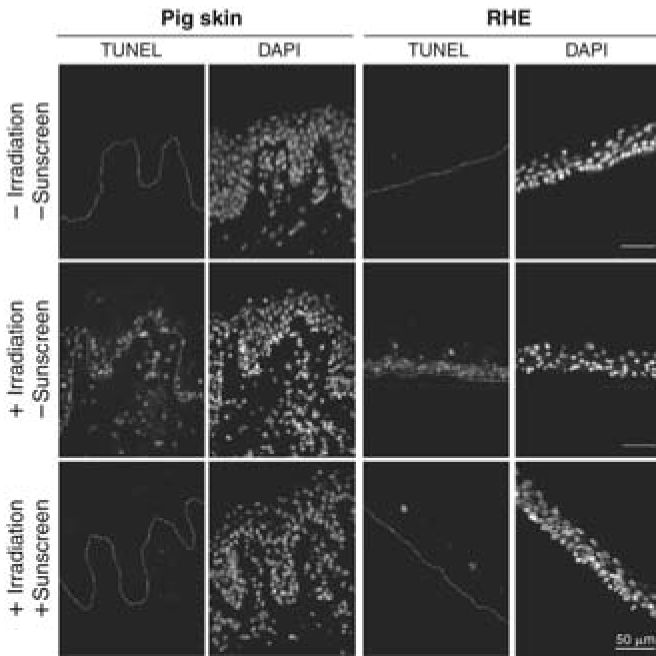

Figure 5 DNA strand breaks after solar irradiation in pig skin and RHE. Skin was pretreated with or without sunscreen, and then exposed to solar-simulated radiation. DNA strand breaks were identified 24 hours postirradiation by TUNEL reaction using fluorescein-dUTP. Note that topical application of sunscreen prevented the formation of DNA lesions in both dermal fibroblast and keratinocyte nuclei stained with DAPI. Dashed lines correspond to the dermal-epidermal junction and filter surface in pig skin and RHE, respectively. *Abbreviations*: TUNEL, dT-mediated dUTP nick-end labeling; RHE, reconstructed human epidermis.

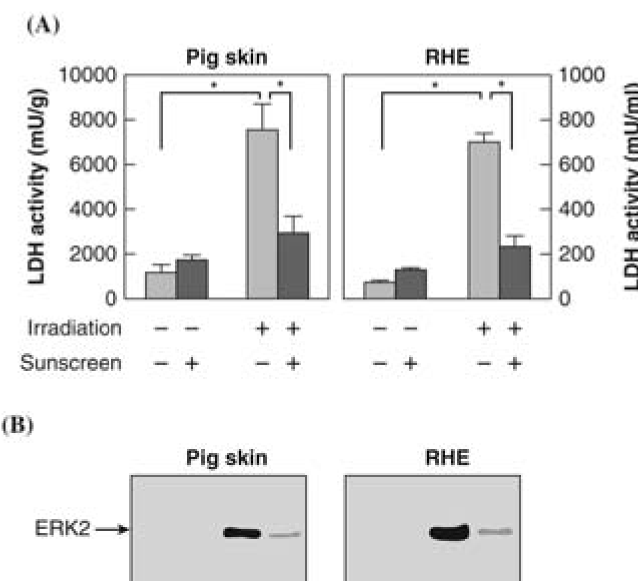

Figure 6 Cytotoxicity after solar irradiation in pig skin and RHE. Skin was pretreated with or without sunscreen, and then exposed to solar-simulated radiation. Culture media were harvested 24 hours later and tested for the presence of cytosolic proteins released from the explants. (**A**) LDH activity was measured by colorimetry using tetrazolium dye (*mean* ± *SD*, $n = 3$, $^*p < 0.01$). (**B**) ERK2 expression was analyzed by immunoblotting. Note that topical application of sunscreen reduced the leakage of both LDH and ERK2 from UV-irradiated pig skin and RHE. *Abbreviations*: RHE, reconstructed human epidermis; LDH, lactate dehydrogenase.

in agreement with previous results obtained in various skin models (31,32,39,40) and in clinical studies with sunscreen-treated volunteers (60–62).

Altogether, our results indicate that both pig SOC and RHE models are good surrogates to human tissue and that these 3-D alternative models are relevant tools to better understand SSR-induced phototoxicity and to evaluate sunscreen efficacy against UVB and UVA damage.

GENERAL CONCLUSION

A substantial amount of effort is being put into the development and validation of skin models, including reconstructed skin equivalents and skin organ culture models. Even if most of these skin equivalents are not fully validated, they are already frequently being used to prove cosmetic product effectiveness, and hence they represent a genuine added value for the cosmetics industry. The increasing availability of complex reconstructed models offer a wide range of possibilities for use in the efficacy evaluation of cosmetic ingredients, in spite of some limitations such as low barrier function. In addition, these should be provided preferentially by multiple and independent skin model suppliers as a guarantee of their availability to the cosmetic industry. The skin organ culture models are less expensive than the in vitro reconstructed models, are easy to obtain, and possess good barrier function. Accordingly, the combination of RHE and SOC models currently provides a very useful and complementary test system and represents an essential step in the pharmaco-toxicological trials needed in the development of cosmetic products, from screening on cellular models to final validation in clinical trials.

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34 Skin Feel Agents

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INTRODUCTION

Skin feel additives are substances conferring sensorial properties to a skin care product, triggering pleasant perception during application onto the skin and after use. Effectiveness of sensory triggers is governed by their substantivity to the skin that occurs either by hydrophobic interaction, or by electrostatic interaction, or a combination of these two factors. A large variety of cosmetic ingredients function as skin feel/conditioning additives comprising lipophilic materials, silicones, water-soluble polymeric substances (including proteins) and their cationic derivatives, humectants, etc. The Cosmetic, Toiletry, and Fragrance Association (CTFA) divides skin-conditioning agents into several groups: emollients, occlusive materials, and miscellaneous substances including, among others, cationic macromolecules and various surfactants.

This chapter focuses on skin feel agents for rinse-off products and more particularly for surfactant-based skin-cleansing products: facial cleansers, soap and syndet bars, shower gels and body washes, foam baths (or bubble baths), and bath oils.

Shower gels, bars, and facial cleansers first contact the skin, before being rinsed during the cleaning process; the persistence of the conditioning agents onto the skin is crucial to ensure sensory performances otherwise they are washed off and the end benefit is not perceived by the user. For bath products intended to be heavily diluted for use, it is difficult for skin feel agents to be really effective, except perhaps in case of bath oils. Indeed, when bath oils are diluted in water, they either float at the water/air interface or lead to a coarse unstable o/w emulsion; when the body emerges from the bath, oils spontaneously stick onto the skin because of their water incompatibility, excluding them out of the "bathing liquor."

The advent of emollients in body-cleansing liquids occurred with the emergence in the early 1990s of the "body washes" referring to "2 in 1" foaming emulsions; before the development of this new product niche, cationic polymeric materials were the most used skin-conditioning agents in skin-cleansing products.

Sensorial performance profile of a body-cleansing product comes in a variety of signal attributes:

- Feeling on the skin during use: spreading of the liquid (also related to product rheology), feel of the bar (slipperiness or roughness), foam feel related to foam quality (creaminess, density);
- Skin feel during rinsing under running water: for example, slipperiness, roughness of the skin, "clean feel" (squeaky feel) left by soaps;
- Feel while drying the skin with a towel and feel on damp skin: softness, roughness, stickiness; and
- After feel and long lasting of skin sensations: smoothness, softness, moisturization, etc.

All these product attributes are governed at first by the composition of the cleansing base: the surfactant nature (amphoterics, nonionics, and anionics), their total and relative concentrations, the clinical mildness for the skin of the surfactant mixture, etc. The sensorial profile of the product can be further influenced or improved by judiciously chosen skin-conditioning agents.

Besides physical, clinical, and organoleptic characteristics of the body-cleansing product, several other imponderable parameters can act upon the skin feel performance and perception: environmental conditions, usage habits, water hardness, skin condition of the user, pilosity, etc.

Also, consumer expectations in terms of sensorial profile of a product depend on climatic (relative humidity, temperature) and socio-demographic parameters (sex, occupation, lifestyle, running water availability, etc.), skin type and concerned body part (face, whole body, etc.), product positioning (sport, moisturizing, nourishing, others), etc.

Criteria of selection and technical constraints to be taken into account when choosing skin feel agents are as follows:

- Solubility and compatibility with the surfactant system
- Sensitivity to electrolytes and pH
- Product physical form: bar, liquid
- Processability (bars) and easiness of formulation
- Sensitivity to temperature
- Impact on finished product performance profile:
  - on the foam: foam feel, volume and stability, creaminess, bubble size, texture, etc.
  - on the product rinseability
  - induction of undesirable and unexpected secondary effects on skin feel when skin is damp (e.g., stickiness)
- Impact on finished product esthetic:
  - on base odor, fragrance character, perception, and stability
  - on product clarity when relevant
  - on viscosity, rheological profile
  - on color
- Origin: animal or vegetal, natural, or synthetic
- Risk of skin sensitization
- Cost

EMOLLIENTS AND REFATTENERS

Introduction

CTFA dictionary defines *emollients* as "cosmetic ingredients which help to maintain the soft, smooth, and pliable appearance of the skin; emollients function by their ability to remain on the skin surface or in the stratum corneum to act as lubricant, to reduce flaking, and to improve the appearance of the skin."

Emollients are also described as *refattening additives* or *refatteners* in case of bath products. The word "refattener" refers to substances improving the lipid content of the upper layers of the skin; they prevent defattening and drying out of the skin.

Several emollients showing strong lipophilic character are identified as *occlusive* ingredients; they are fatty/oily materials that remain on the skin surface and reduce transepidermal water loss. CTFA dictionary defines "occlusives" as: "cosmetic ingredients which retard the evaporation of water from the skin surface; by blocking the evaporative loss of water, occlusive materials increase the water content of the skin."

Overall, emollients and refatteners are oils and fats derived from natural origins or obtained by chemical synthesis; they are classified as nonpolar (paraffin's and isoparaffin's) and polar substances (esters and triglycerides); their chemical structure influence the interaction with the skin surface and affect their sensorial properties. As a class, they comprise lipids, oils and their derivatives, fatty acid esters, lanolin derivatives, and silicones and their organo-functional derivatives.

Originally emollients were developed for use in leave-on skin care products. Formulation technology can aid the deposition of refattening additives on the skin from wash off products and avoid that they rinse off with the surfactants; nevertheless, the large dilution factor in bath products remain a significant hurdle for skin end benefit perception (except in bath oils).

Emollients and refatteners will provide after feel but will also influence skin feel during usage, foam feel, and most of the time foam quantity and quality. The more hydrophobic is the refattening additive, the more deleterious its impact on flash foam generation, foam quantity, and stability.

In other respects, the more oily/fatty the material, the better is its deposition onto the skin and its substantivity, and the easier the efficacy documentation. Proof and substantiation of claims is of more and more importance in the frame of European legislation for cosmetics and toiletries and in the context of the competitive environment.

Lipophilic Emollients and Occlusives

Occlusive materials comprise among others vegetable oils, triglycerides, mineral oil, natural or synthetic waxes, fatty acid esters, lanolin oil and its derivatives, polydimethylsiloxanes, etc. (Table 1).

They form an occlusive layer on the skin, keeping water inside the upper stratum corneum (SC) layers and consequently acting as moisturizers.

Mineral oil and vegetable oils as well as waxes generally produce heavy and greasy feeling on the skin. *Hydrophobic fatty acid esters* are an almost unlimited source of synthetic emollients and refatteners; they provide lighter and more pleasant skin feel than oils and waxes. Any fatty acid can be esterified either by ethylene glycol or propylene glycol, or

Table 1 Emollients and Refatteners

		INCI names
Fats/oils (triglycerides); hydrocarbons; waxes		Petrolatum
		Ceresin
		Mineral oil
		Wheat germ oil/wheat germ glycerides
		Almond/peach oil
		Coconut oil
		Jojoba oil
		Rape seed/olive/sesame oil
		Sunflower/corn/safflower oil
Fatty acid esters: hydrophobic emollient esters	Ethylene glycol esters	Glycol stearate, or palmitate, or oleate
	Polyethylene glycol esters	PEG-5 octanoate
		PEG-5 isononate and trideceth-9
	Propylene glycol esters	Propylene glycol myristate or laurate
	Polypropylene glycol esters	PPG-36 Oleate
	Isopropyl esters	Isopropyl myristate, or laurate, or palmitate
	Polyglyceryl esters	Polyglyceryl-10- laurate or myristate
	Alkyl esters	Octyl octanoate
		Cetearyl octanoate
		Octyl hydroxystearate
		Castoryl maleate
Fatty acid mono and diglycerides		Glyceryl oleate
		Glyceryl laurate
Ethoxylated triglycerides		PEG-6 caprylic capric triglycerides
		PEG-4 caprylic/capric glycerides
		PEG-45 palm kernel glycerides
		PEG-20 almond glycerides
		PEG-60 corn glycerides
		PEG-18 palm glycerides
		Olive oil PEG-7 esters
		Hydroxylated milk glycerides
Ethoxylated mono and diglycerides: hydrophilic emollient esters	Ethoxylated glyceryl esters	PEG-7 glyceryl cocoate
		PEG-8 glyceryl laurate
		PEG-15 glyceryl laurate
		PEG-30 glyceryl cocoate
		PEG-78 glyceryl cocoate
		PEG-20 glyceryl oleate
		PEG-82 glyceryl tallowate
		PEG-200 glyceryl tallowate

Abbreviations: INCI, International Nomenclature of Cosmetic Ingredients; PEG, polyethylene glycol.

glycerin polymers, or isopropyl alcohol, or any longer chain alcohol, etc. The feel they impart and their impact on foam is related to the fatty acid chain length; short chains (e.g., isopropyl myristate and octyl octanoate) deliver dryer feel and have lesser impact on foam than longer ones (e.g., stearates and isostearates) that are greasier and detrimental to foam quantity and stability (1).

Hydrophobic emollients are efficacious skin refatteners but not easy to incorporate in surfactant mixtures commonly used in liquid skin-cleansing products. They must be emulsified that most of the time necessitates hot process. They have a detrimental impact on foam speed, quantity, and stability. In order to circumvent this weakness of lathering capacity, manufacturers sometimes provide a mechanical foaming device with the body-cleansing product: a puff or massage flower (2).

Highly hydrophobic refattening additives are not meant for foaming preparations but rather for bath oils. Soaps and syndet bars can easily accommodate waxes and oils without impairing their basic foaming and cleaning functions. Besides beeswax, petrolatum or ceresin, lanolin and jojoba oil, cocoa butter or mineral oil are other examples of skin conditioners commonly used in bars. Paraffin wax is often used in soaps and syndets not only for the smooth feel they impart to the finished bar and the mildness they bring to the formulation but also for the role of plasticizer they play, adding firmness to the bar. Vegetal oils are included as skin nourishing/refattening agents (e.g., almond, wheat germ, olive oils).

Fatty acid mono and diglycerides (1,3) are prepared either by transesterification of triglycerides with glycerin or reaction of alkanoates with glycerin. Lipophilic character remains predominant in these esters; depending on chain length, they are soluble in surfactant solutions or they must be emulsified. Besides the improved skin feel they induce, they also reduce defatting of the skin possibly caused by surfactant-based cleansers. Monoglycerides of stearic, lauric, and palmitic acids (glyceryl mono stearate, laurate, and palmitate) are part of the composition of natural lipids of the skin. They adsorb and can be detected on skin after application through a skin-cleansing product (4).

Several mixtures of monoglycerides and mild foaming surfactants are commercially available; they claim improved foam qualities (bubble sizes, creaminess, and stability) and documentable skin-refattening properties (5,6). On top of skin feel improvement, they also reduce degreasing effect of cleansers, thanks to their lipophilic character, and improve the compatibility of the surfactants with the skin (1). An example of improvement in the skin barrier function and in skin tactile sensations has been demonstrated for glyceryl oleate in a model shower gel composition (5).

Hydrophilic Lipids

Hydrophilic lipids are (Table 1) (1) preferred for foaming skin-cleansing preparations. Ethoxylation and propoxylation make lipids more compatible with water and more easily soluble in aqueous surfactants solutions.

One has to find the right balance between ethoxylation and skin substantivity: the more the lipids are ethoxylated, the more they are soluble and the less the impact on foam but also the less their persistence on the skin, the weaker their refattening properties.

Ethoxylated glycerides are obtained either by reaction of natural triglycerides with ethylene oxide (a complex end mixture is then obtained) or by ethoxylation of monoglycerides. They are often referred to as "water soluble vegetable oils"; their solubility in water will depend on the carbon chain length of starting glycerides and on the degree of ethoxylation.

Low ethoxylates triglycerides are still enough lipophilic to provide good refattening properties, leading to very pleasant skin feel, perceivable at quite high use levels.

Ethoxylated mono and diglycerides generally associate various properties beneficial to the skin. They are more or less refattening the skin, depending on chain length and ethoxylation ratio. They act as anti-irritant or mildness additives and confer slipperiness to the foam. Depending on chain length and ethoxylation degree they are either water dispersible or soluble. Among the low ethoxylates monoglycerides, PEG-7 glyceryl cocoate is one that is mostly used. This emollient depresses skin irritation induced by anionic surfactants and shows minimum impact on lathering profile. Higher ethoxylates of longer carbon chain length (PEG-200 glyceryl tallowate) are still substantive to the skin because of their high-molecular weight; they provide a smooth feel, but because of their stronger hydrophilic character, their refatting properties are less obvious to evidence (7).

Table 2 Emollients and Refatteners

		INCI names
Lecithin		Propylene glycol (and) lecithin (and) sodium lauryl sulfate (and) disodium sulfosuccinate (and) cocamidopropyl hydroxysultaine (and) isopropyl alcohol
Lanolin and its derivatives		Lanolin oil
		Lanolin alcohol
	Ethoxylated lanolin	PEG-75 lanolin
	Ethoxylated lanolin alcohols	Laneth-16
		Laneth-25
	Propoxylated lanolin alcohols	PPG-30 lanolin alcohol ether
		PPG-12 PEG-50 lanolin
	Alkoxylated lanolin	PPG-40 PEG-60 lanolin oil

Abbreviations: INCI, International Nomenclature of Cosmetic Ingredients; PPG, polypropylene glycol; PEG, polyethylene glycol.

Ethoxylated/propoxylated fatty alcohols are useful light emollients: through an appropriate selection of optimum combination between parent alcohol chain length and propoxylation/ethoxylation degree, these emollients can be formulated up to 2% to 3% in surfactant solutions with minimum impact on foam volume.

Lanolin

Lanolin is extracted from sheep wool grease; it is a complex mixture of esters of high-molecular-weight lanolin alcohols (aliphatic alcohols, sterols, and trimethyl sterols) and of lanolin fatty acids. Free lanolin alcohols, acids, and lanolin hydrocarbons are minors. Lanolin alcohols and lanolin oil are recommended as superfatting agents in soaps. *Ethoxylation* of the hydroxyl groups *of lanolin* or of its *derivatives* leads to hydrophilic, water-soluble lanolin compounds, offering a broad range of useful emollients to the formulator (Table 2) (8,9). Some moderately to highly ethoxylated derivatives, recommended for their good emolliency and moisturization properties, are processable in liquid skin cleansers with limited impact on foam profile; as an example, *the 75 moles ethoxylated lanolin* does not depress foam and is recommended as skin conditioner in soaps, liquid body-cleansing products, and bubble baths. *Medium ethoxylates lanolin alcohols* have limited impact on foam performances of body-cleansing liquids; *lower ethoxylates* can be formulated in bars. *Propoxylated lanolin alcohols* are lipophilic emollients used in soap bars and in other cleansers on the basis of synthetic surfactants.

Alkoxylated lanolin derivatives are obtained by reaction with mixtures of propylene and ethylene oxides in various ratios; they are more soluble than ethoxylated lanolin. They serve as refattening and foam-stabilizing agents.

Esterification of *lanolin fatty acid* with isopropyl alcohol provides a range of *esters* of various molecular weights. Medium-molecular-weight esters are used as superfattening agents in soaps.

The use of animal-derived ingredients in cosmetics is regulated in Europe. Lanolin is part of those ruminant-derived ingredients considered as noninfective with regard to BSE, according to World Health Organization (WHO).

Lecithin

Lecithin (Table 2) is a natural mixture of polar and neutral lipids; the word "lecithin" is also used as the trivial name of a particular phospholipid: phosphatidylcholine.

Main vegetable sources of lecithin used in personal care products are soybean and maize; egg yolk is practically the only animal source of lecithin used in cosmetics and toiletries. The percentage of polar lipids and their fatty acid pattern are characteristic of the lecithin source.

Bare lecithin, a secondary product of soybean oil extraction, typically contains 60% to 70% polar lipids (mainly phospholipids, namely phosphatidylcholine and glycolipids), and a

remaining, 25% to 35% soybean oil. This raw lecithin is further fractionated, purified, and chemically modified to allow easier processing and formulation in toiletries products. Emollient, refattening, and moisturizing properties of lecithin are guided by its content in phospholipids.

Lecithin softens, nourishes, and refattens the skin; it provides a nongreasy, long-lasting skin feel and improves foam feel and quality (creaminess, slipperiness, richness).

Ready to use mixtures of phospholipids in surfactant solutions, free of residual soybean oil, are commercially available for an easy incorporation in liquids or bars; some of these compounds allow formulation of clear products.

Silicone Derivatives

Only major materials used in body-cleansing products will be briefly discussed here (10,11).

Predominant silicones used overall in personal care products are polydimethyl siloxane also named *dimethicones*. They are not soluble in water or in surfactant solutions; their incorporation into liquid cleansers requires an emulsification process. The length of dimethylsiloxane polymer chain dictates its molecular weight and hence its viscosity. Most commonly used materials have viscosity ranging from about hundred to several thousands centistokes. High- to medium-molecular-weight dimethicones are occlusive, skin protective emollients; lower molecular weights are dryer emollients, generally preferred for use in skin cleansers. Dimethicones have detrimental effect on foam profile but are good film-forming agents, lubricant, imparting a nongreasy, nontacky, and silky feel as compared with "heavier" mineral or vegetable oils. They are used in soap bars, where they also aid mold release, and in 2-in-1 shower gels (foaming emulsion body washes). More recently, emulsions of ultra high-molecular-weight silicone were developed that are easily incorporated in aqueous systems such as body washes and facial cleansers; they impart soft residual film, moisturized skin feel even from a rinse-off product.

Polymethylcyclosiloxanes or *cyclomethicones* are tetrameric or pentameric oligomers of the same backbone as polydimethylsiloxane and show the same chemical and physical properties; they are low viscosity fluids with relatively high volatility because of their low molecular weight and the weak intermolecular attractivity. Because they are not substantive, cyclomethicones are often identified as dry emollients; they deliver light, transient, and dry skin feel during product use.

Formulation of these nonpolar insoluble silicones requests hot emulsification process (nonionic emulsifiers) and proper emulsion stabilization.

Dimethicones are modified or functionalized with other organic groups to modulate their solubility in water or in surfactant solutions (and consequently make them easier to formulate) and their skin substantivity properties. By adjusting the type and proportion of hydrophilic substituents, the resulting copolymer is soluble or dispersible in aqueous cosmetic products. The combination of the dimethicone structure with polyoxyalkylated substituents (ethylene or propylene oxide) yields *dimethicone copolyols*: copolymers more soluble in water with surface activity. They are foam boosters and stabilizers; even if they are less film forming than parent polydimethylsiloxanes, they significantly participate to skin sensations during application (use) and provide excellent smooth and silky after feel (12). They can be used to formulate clear aqueous products.

Blends of polydimethylsiloxanes with volatile and/or water-soluble derivatives are used to design a sensorial profile adapted to the finished product and its end use.

HUMECTANTS

CTFA dictionary defines humectants as "cosmetic ingredients intended to increase the water content of top layers of the skin" (Table 3).

Humectants are hygroscopic substances generally soluble in water; these "moisture attractants" maintain an aqueous film at the skin surface.

The primary humectant used in personal care products is glycerin; it tends to provide heavy and tacky feel, which can be overcome by using it in combination with other humectants such as sorbitol.

Table 3 Humectants

Humectants	INCI names
	Glycerin
	Glycereth-26 and glycereth-7
	Propylene glycol
	1,3 butylene glycol
	PEG-8 to about PEG-200
	Sorbitol
	Sorbeth-6 to sorbeth-40
	Xylitol
Ethoxylated methyl glucose	Methyl gluceth -10/methyl gluceth-20
	Amino acids
	Lactic acid/sodium lactate
	Sodium PCA
Substantive conditioning humectants	Steardimonium panthenol
	Lauryl methyl gluceth-10 hydroxypropyl dimonium chloride
	Chitosan-PCA

Abbreviations: INCI, International Nomenclature of Cosmetic Ingredients; PCA, pyrrolidone carboxylic acid; PEG, polyethylene glycol.

Less expensive than glycerin, propylene glycol is the second most widely used humectant in cosmetics and toiletries products; it reduces viscosity of surfactant solutions and tends to depress the foam.

Low-molecular-weight polyethylene glycols (PEGs from about 10–200 PEG units), amino acids, and other constituents of skin natural moisturizing factors (NMFs) such as sodium pyrrolidone carboxylic acid (PCA) and sodium lactate are also applicable for use in surfactant-based skin-cleansing products.

Humectants are not substantive to the skin, they are easily rinsed-off after cleaning; consequently, skin feel improvement is not obvious to perceive, and their efficacy in terms of skin moisturization is difficult to document.

Glycerin, propylene glycol, 1,3-butylene glycol, or sorbitol are typically used in body washes, bubbles baths, shower gels, or soaps to prevent the desiccation of the product itself and the formation of a dry layer (a skin) at the surface. They also ensure stability and clarity of liquid cleansers at cold temperatures.

Few substantive humectants can be mentioned. They are cationic in nature, which makes them adsorbing to the negatively charged skin surface. In the quaternized polyalkoxylated methyl glucose derivative (lauryl methyl gluceth-10 hydroxypropyldimonium chloride), the hydrophilic moiety delivers humectant properties; the hydrophobic chain at the cationic end of the molecule ensures both substantivity and skin conditioning.

Another example is chitosan-PCA; it is a polycationic (at acidic pH) high-molecular-weight polymer produced by deacetylation of chitin, the major constituent of invertebrate exoskeletons. Combining chitosan with PCA leads to a highly substantive, film-forming humectant material.

POLYMERS

Polymeric materials can interact both with protein of skin surface and with skin lipids. Parameters influencing the interaction between skin surface and polymers are as follows:

- The positive charge density: the more cationic the character of the polymer, the better the polymer interaction with negatively charged skin surface.
- The hydrophobicity of polymer: grafting of fatty moieties on the polymer backbone favors hydrophobic interactions with hydrophobic areas of the keratin.
- The molecular weight of the polymer: the higher the polymer size, the more its substantivity to the skin (film-forming properties). However, very low-molecular-weight polymers can easily penetrate the skin surface chinks and as such adsorb into the superficial stratum disjunctum.

- The nature of surfactants surrounding the polymer in the finished product: the polymer can interact with surfactants either through their charges or through hydrophobic interactions; also competition between polymer and surfactants for skin-anchoring sites can occur. In both cases, deposition and adsorption of polymer onto the skin surface is weakened.

Natural Polymers and Their Chemically Modified Derivatives

Proteins

Proteins differ by (*i*) the source; (*ii*) the molecular weight; (*iii*) the amino acids (AA) composition, AA side groups, and electrical charge (more of cationic or of anionic AA); and (*iv*) the chemically attached moieties (quats, fatty chains, silicone, etc.) on the peptide backbone (Table 4) (13–15).

Proteins can be from vegetable or animal origin. The use of animal-derived ingredients in cosmetics is regulated in Europe because of the emergence of BSE infection in ruminants. Hydrolyzed proteins, among other collagens, are considered as safe in cosmetics provided that the supplier implements and certifies the application of an adequate production process.

Collagen from pork or beef is still one of the most widely used *animal* proteins; "marine collagen" (fish) is used as alternative source of collagen to traditional bovine-derived materials. Milk proteins, keratin, and elastin are also considered in cosmetics and toiletries. Concerns related to BSE have initiated a shift away from animal-derived ingredients and an increased interest in plant-derived proteinic materials.

Vegetable/plant proteins are most of the time associated with significant amounts of soluble and insoluble carbohydrates because of extraction process; soluble carbohydrates confer dark color and strong odor to the raw material; in some commercial grades carbohydrates have been removed. The combination of hydrolyzed vegetable proteins and oligosaccharides produces conditioning additives with synergistic moisturizing action and

Table 4 Natural Polymers and their Chemically Modified Derivatives

		INCI names
Native proteins		
	Solubilized in anionic surfactants	Native wheat protein/lauryl ether sulfate complex
Protein hydrolyzates	Animal source	Hydrolyzed animal protein
		Hydrolyzed collagen
		Hydrolyzed milk protein
	Plant derived	Hydrolyzed vegetal protein
		Hydrolyzed wheat protein/oligosaccharide complex
		Hydrolyzed wheat protein and hydrolyzed wheat starch
		Hydrolyzed oats
		Hydrolyzed wheat gluten
Quaternized protein hydrolyzates	Animal source	Hydroxypropyl trimonium–hydrolyzed collagen
	Plant derived	Hydroxypropyl trimonium–hydrolyzed wheat protein
Fatty side chains grafted on protein backbone	Native protein	Wheat extract (and), stearic (and), sodium chloride
Quaternized fatty chains grafted	Protein hydrolyzate	Steardimonium-hydrolyzed wheat protein or collagen
		Lauryl or cocodimonium hydroxypropyl–hydrolyzed collagen
		Alkyl quaternary–hydrolyzed soya protein
Copolymers	Protein-PVP	Hydrolyzed wheat protein/polyvinyl pyrrolidone copolymer
	Protein-Silicone	Hydrolyzed wheat protein hydroxypropyl polysiloxane copolymer
	Quaternized copolymer	Hydroxypropyl trimonium–hydrolyzed wheat protein polysiloxane copolymer

Abbreviations: INCI, International Nomenclature of Cosmetic Ingredients; PVP, polyvinyl pyrrolidone.

film-forming properties. Major vegetal starting materials are wheat gluten, almond meal, rice, oat, soy, maize, etc.

Proteins are functional over a wide range of pH; nevertheless, as they are amphoteric materials, below their isoelectric point they carry a net positive charge, which makes them substantive to the negatively charged skin surface.

Film-forming properties of proteins and hydrolyzates are related to their molecular weight (the higher, the better). Overall, proteins convey smoothing and moisturizing effect, they produce soft and silky feel to the skin. They have positive effect on foam profile: they increase foam stability, creaminess, and density as well as confer slipperiness to the foam.

Proteins and hydrolyzates are also known for their ability to reduce the irritation caused by anionic surfactants and to combat skin dryness induced by detergents (16–19).

Some native proteins such as elastin, keratin, or vegetable proteins are insoluble. There exists soluble native collagen species; their use is restricted to some specialized applications. In order to make native proteins suitable for a wide range of applications, they are converted into soluble hydrolyzates by chemical or enzymatic degradation. The sizes of resulting peptides depend on the hydrolysis process used: chemical processes give rise to broader-molecular-weight distributions and enzymatic digestion to narrower ones. Besides that, native proteins solubilized in various anionic surfactants (by formation of a protein-surfactant complex), are commercially available, allowing easy formulation of these film forming, moisturizing, skin mildness additives.

A wide range of protein hydrolyzates molecular weights is available, ranging from 500,000 down to 1000 Da. Protein hydrolyzates of intermediate molecular weight (average 3000–5000 Da) are the most widely used; they are less substantive than high-molecular-weight proteins but still provide smooth skin feel, slippery feel during use, and sensation of skin hydration.

Hydrolyzates are readily soluble and compatible with all classes of surfactants.

Most of commercially available proteins and derivatives have characteristic odor and color. Furthermore, products formulated with proteins or hydrolyzates should be adequately preserved.

Chemically Modified Protein Derivatives

In order to increase interaction of proteinic material with skin surface, proteins or hydrolyzates are functionalized or chemically modified (Table 4). Protein possesses reactive side chain amino and carboxyl groups, which are sites for further modification of their intrinsic properties.

Hydrophobic interactions with the skin surface are favored and reinforced by grafting fatty carbon chains; ionic interactions are maximized by grafting cationic moieties onto the protein backbone.

Hydrolyzed protein copolymers combine substantivity and film-forming properties of parent proteins with characteristic sensorial properties of companion conditioning agents; these macromolecular proteinic complexes offer greater moisturizing and conditioning potential as compared with the individual components (20).

1. *Native proteins coupled with fatty acids* lead to macromolecular entity with dual hydrophilic/hydrophobic characteristics and physicochemical properties. Skin substantivity is guided both by the size of the starting protein and by the chain length (the hydrophobicity) of the fatty acid. The macromolecules are surface active and can be formulated in bars or liquids; they produce smooth long-lasting skin feel. Long chain fatty acid derivatives tend to decrease foam volume but confer creaminess, richness, and slipperiness to the lather.
2. *Copolymers of silicone and proteins* are obtained by covalent bonding of low-molecular-weight polydimethylsiloxanes on amino groups of (vegetable) protein hydrolyzate. They combine beneficial properties of proteins (anti-irritant effect, substantivity, film forming, soft after feel) with lubricity of silicone (21,22). Quaternized protein–silicone copolymers are also commercially available.
3. *PVP-protein copolymers*: proteinic component imparts substantivity and polyvinyl pyrrolidone (PVP) maximizes the moisture retention and film-forming properties of

the resulting copolymer. PVP/protein ratio will modulate the profile of performance on the skin and the influence on lathering characteristics of surfactant-based skin cleanser.

4. *Quaternized protein hydrolyzates*: cationic protein hydrolyzates are obtained by reaction of the primary amine sites on the protein backbone with a tertiary amine: hydroxypropyl or propyl trimethyl ammonium or alkyl trimethyl ammonium (23).

   Covalent attachment of quaternary groups strongly increases the cationic character of the protein hydrolyzate, making it further skin substantive and resistant to rinsing with water.

   Covalent attachment of fatty quaternary groups (alkyl dimethyl ammonium) on peptides greatly enhances both ionic and hydrophobic interactions with the skin. Alkyl chain can be lauryl, myristyl, and stearyl. Alkyl trimonium-hydrolyzed proteins are still water soluble and compatible with all classes of surfactants. These hydrophobically modified cationic protein hydrolyzates are highly adsorbing to skin surface at all pH levels and offer skin substantivity at minimum concentration. They impart pronounced conditioning effect; the lipophilic moieties provide emollient feel.

   Overall, quaternized versions of a protein are many times more substantive than the parent protein hydrolyzate. Quaternization of protein hydrolyzates raises their isoelectric point (IP) to pH 10 regardless of their initial IP values.

Cationic Guar Gum

Guar gum is a galactomannan polysaccharide derived from the endosperm of cyamopsis tetragonolobus seeds (Table 5).

Depolymerization of the gum by enzymatic or chemical processes allows to modulate its molecular weight and consequently to impact its solubility, thickening properties, and the transparency of the finished product. Free hydroxyl groups on the polysaccharidic backbone can participate in esterification and etherification reactions. Hydroxypropyl (HP) side groups improve guar compatibility with electrolytes. Cationic guar derivatives are obtained by reaction of HP guar with epoxypropyltrimethyl ammonium chloride; positive charge density of resulting guar hydroxypropyl trimonium chloride depends on substitution degree. Cationic guar derivatives are film forming and impart soft, smooth, and silky feel to the skin; moreover

Table 5 Natural Polymers and their Chemically Modified Derivatives

	INCI names	Comments
Cationic cellulose derivatives		
	Polyquaternium 10	Polymeric quaternary ammonium salt of HEC reacted with trimethyl ammonium-substituted epoxide
	Polyquaternium 24	Polymeric ammonium salt of HEC reacted with lauryl dimethyl ammonium-substituted epoxide; average degree of substitution=1
	Polyquaternium 67	Polymeric quaternary ammonium salt of HEC reacted with a trimethyl ammonium-substituted epoxide and a lauryl dimethyl ammonium-substituted epoxide
	PG-hydroxyethyl cellulose lauryl or coco or stearyl dimonium chloride	Average degree of substitution>1
Cationic guar derivatives		
	Guar hydroxypropyl trimonium chloride	
	Hydroxypropyl guar hydroxypropyl trimonium chloride	
Cationic phospholipid polymer	Polyquaternium 64	Cationic phospholipid polymer with phosphorylcholine moiety

Abbreviations: INCI, International Nomenclature of Cosmetic Ingredients; HEC, hydroxyethyl cellulose.

they act as anti-irritant for anionic surfactants and soaps and have positive effect on foam feel and quality (24,25).

Cationic Cellulose Derivatives

Polyquaternium 10 is a range of polymeric quaternary ammonium salts of hydroxyethyl cellulose (HEC) reacted with *trimethyl ammonium-substituted epoxide*. Polyquaternium 10 solutions are non-Newtonian and are commercially available (*i*) in several viscosity grades depending on their molecular weight (they contribute to viscosity of formulations) and (*ii*) with "high" to "moderate" cationic substitution. In vivo tests showed that these cationic cellulosic polymers protect the skin from aggression by anionic surfactants (Table 5) (26,27).

Polyquaternium 24 is a polymeric quaternary ammonium salt of HEC reacted with *lauryldimethyl ammonium-substituted epoxide*; it is a hydrophobically modified polyquaternium 10.

The degree of substitution with quaternary fatty chain is average one in polyquaternium 24; a range of alkyl dimonium hydroxypropyl oxyethyl cellulose with higher proportion of grafted cationic fatty groups (average degree of substitution is 1.2) is also commercially available.

Polyquaternium 67 is high-viscosity quaternized hydroxyethylcellulose incorporating variations in charge level and hydrophobic modification. This family of cationic cellulosic conditioning polymers combines the *trimethyl ammonium* functionality of polyquaternium 10 with various levels of *dimethyl-dodecyl-ammonium* hydrophobic functionality. Their degree of cationic substitution has been fixed at ~0.2, which corresponds to a weight-percentage nitrogen of ~1%. Low levels of hydrophobic dimethyl-dodecyl-ammonium substitution (HS=0.01) were used to impart hydrophobic character to the polyquaternium 10-type polymers. Four polymer grades are available ranging from the lowest to the higher degree of hydrophobic substitution. The whole range enables crystal clear formulations.

The presence of fatty side chains on all these quaternized cellulose ethers makes them surface active and further participates to their very high skin substantivity and film-forming properties. They impart silky smooth after feel.

These alkyl quaternary cellulose polymers are soluble in water (longer carbon chains must be slightly warmed) and compatible with a wide range of surfactants; they have favorable influence on the lathering properties providing creaminess, density, slipperiness, and stability to the foam.

Synthetic Quaternized Polymers

An array of dimethyldiallylammoniumchloride (DMDAAC)-based polymers and copolymers are commercially available. Their substantivity, film-forming properties, and resulting skin feel depend on both the molecular weight (ranging from about 400,000–7 millions) and the density of positive charges, which also dictates the compatibility of the polymer with anionic surfactants. These polymers generally make foam denser and more stable (Table 6) (28).

DMDAAC homopolymer (polyquaternium 6) carries the highest positive charge density and is not compatible with anionic surfactants.

Table 6 Synthetic Quaternized Polymers

		INCI names
Polyquaterniums		
	Polyquaternium 6	Dimethyl diallyl ammonium chloride homopolymer
	Polyquaternium 7	Acrylamide/dimethyl diallyl ammonium chloride copolymer
	Polyquaternium 11	Poly(vinylpyrrolidone/dimethylaminoethyl methacrylate)
	Polyquaternium 22	Acrylic acid/dimethyl diallyl ammonium chloride copolymer
	Polyquaternium 39	Acrylamide/acrylic acid/dimethyl diallyl ammonium chloride terpolymer
	Polyquaternium 70	Polymeric quaternary ammonium salt consisting of an ethoxylated propoxylated stearyl amine condensed with adipic and dilinoleic acid,quaternized with dimethyl sulfate

Abbreviation: INCI, International Nomenclature of Cosmetic Ingredients.

Inclusion of acrylamide into DMDAAC homopolymer decreases the positive charge density, leading to a skin-conditioning polymer more compatible with anionics (polyquaternium 7) (29,30). Polyquaternium 7 is probably one of the most widely used synthetic cationic polymer in body-cleansing products; it is highly substantive to the skin, delivering soft, silky, moisturized after feel (28).

Positive charge density is also decreased by copolymerizing DMDAAC with either acrylic acid (polyquaternium 22) or with both acrylamide and acrylic acid (polyquaternium 39). Another widely used synthetic cationic polymer in liquid skin cleansers and in bar soaps is a quaternized copolymer of PVP and dimethylaminoethyl methacrylate (DMAEM) (polyquaternium 11). This PVP copolymer is available in molecular weights ranging from 100,000 to 10,00,000.

SURFACTANTS

Benefits brought by additional skin-conditioning agents are sometimes hidden by a mild or very mild cleaning surfactant system delivering by itself very good skin feel properties; the sensorial baseline is high to start with, and the increment in performance brought by skin feel agent is leveled off, and sometimes even not perceivable (Table 7).

Table 7 Surfactants

		INCI names
Nonionics		
	Polyhydric alcohol esters	
	Sucrose esters	Sucrose laurate or cocoate
	Methyl glucose esters	PEG-120 methyl glucose dioleate
		PEG-80 methyl glucose laurate
	Glucose ethers	Alkyl polyglucosides
	Fatty acid alkanolamides	Cocodiethanolamide
Amphoterics		
	Ampholytes	Cocamidopropyl betaine
		Olivamidopropyl betaine
		Sesamidopropyl betaine
		Isostearamidopropyl betaine
		Cocamidopropyl hydroxysultaine
		Cocamidopropyldimethyl aminohydroxypropyl hydrolyzed collagen
		Dimethicone propyl PG-betaine
	Propionates	Alkylamino propionates
		Alkyliminodipropionates
	Imidazoline derivatives	Acylamphoacetate
Anionics		
	Phosphoric acid esters and salts	C9-C15 alkyl phosphate
		PPG-5 ceteth-10 phosphate
		Oleth-3 phosphate
	Acyl amino acids and salts	
	Acyl peptides	Sodium cocoyl hydrolyzed protein
		Sodium lauroyl oat amino acids
		TEA or sodium lauroyl animal collagen amino acids
	Acyl glutamates	Sodium cocoyl glutamate
	Sarcosinates	Sodium cocoyl or lauroyl sarcosinate
	Taurates	Sodium methyl cocoyl taurate
	Sulfonic acids and salts	
	Sulfosuccinates	Disodium laneth-5 sulfosuccinate
		Disodium ricinoleamido MEA-sulfosuccinate
		Disodium laureth sulfosuccinate
		Disodium PEG-8 palm glycerides sulfosuccinate
	Isethionates	Sodium cocoyl isethionate
	Carboxylates	Alkyl glucose carboxylate
		Sodium PEG-7 olive oil carboxylate

Abbreviations: INCI, International Nomenclature of Cosmetic Ingredients; PPG, polypropylene glycol; PEG, polyethylene glycol.

Table 8 Exfoliants/Scrubbing Agents

Apricot/walnut shells powder or flour
Corn cob
Jojoba beads
Polyethylene/styrene beads
Almond meal
Apricot/peach seed powder
Loofah
Maize scape powder
Kaolin

It is, however, important to notice that several mild anionic and most of the nonionic surfactants, if they provide a pleasant after feel, are characterized by a "water feel" (feel in solution) often unpleasant, with rough and drag feel sensations.

Amphoteric surfactants are amino acid derivatives, and their net charge varies with the pH in solution; at pH below the IP, they are positively charged in aqueous solution and can consequently adsorb more easily onto the skin. Alkyl chain length also significantly acts on the skin feel; some betaines based on C16/C18 cuts provide greasier refattened feel, but also have detrimental effect on foam. Polydimethylsiloxane grafted with a betaine moiety leads to an amphoteric surfactant combining substantivity, refattening properties, as well as silicone typical skin feel profile.

Some *nonionics* are used for their emollient properties and excellent after feel: sucrose and methyl glucose esters as well as sucrose ethers are mentioned as examples. Fatty acid alkanolamides are often referred to as refatteners; these are not lipids but they confer a greasy slippery feel to the foam and impart a particular after feel on the skin, which subjectively compares to refattening.

Several mild *anionic surfactants* are known to provide improved skin feel (after feel) by themselves: sarcosinate, taurate, acylglutamate, and isethionate are cited as examples. Fatty acids-protein condensate salts and also act as a conditioning aid imparting a pleasant smooth feel to the skin. The inclusion of fatty acids in soap and syndet bars contributes to enhance skin feel during and after use and produce creamier lather. Phosphoric acid fatty esters deliver soap-like skin feel: slipperiness during use and very good rinseability, leaving skin feeling "clean" and powdery like after feel.

EXFOLIATING AGENTS

Skin scrub agents or body polishers are solid materials from natural origin (clay, fine powder of seeds or shells of different vegetables) or obtained by chemical synthesis (tiny beads of styrene or polyethylene) (Table 8). When the scrub agent containing body-cleansing product is rubbed or massaged onto the skin, fine solid particles remove superficial skin horny layer by mechanical abrasion, leaving behind a fresh smooth skin surface. They are the easiest additives for the consumer to perceive. Scrubbing particles can be suspended in liquid body cleanser, thanks to structuring polymers such as xanthan gum or carraghenan, which build a viscoelastic network in the surfactant matrix. The scrubbing agent must be very carefully selected when formulating facial cleansers. Skin of the face is more sensitive or delicate than the rest of the body. For facial application, formulator should orientate his choices toward soft clays or melting jojoba beads for example.

CONCLUSIONS

The overall skin feel profile provided by a skin-cleansing product is conditioned by the huge variety of composition constituents. Many of them have been described in this chapter, but not exhaustively. Other factors can influence the sensations perceived by the consumer such as the presence of electrolytes, or of thickening polymers in the product as well as the water hardness in the user dwelling. It will be the responsibility of the formulator to consider all the potential synergisms or antagonisms in his finished product in order to deliver the desired skin feel.

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35 Silicones—A Key Ingredient in Cosmetic and Toiletry Formulations

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UNIQUE MATERIALS

Silicone is a generic name for many classes of organo-silicone polymer that consist of an inorganic siloxane (Si–O) backbone with pendant organic groups (usually methyl). It is this structure that gives silicones their unique combination of properties and, in particular, their surface properties (Fig. 1 and Table 1).

SILOXANE BACKBONE

The prime role of the siloxane backbone is to present the available methyl groups to their best advantage, and it does this by virtue of its unique flexibility. In most hydrocarbons, the bond angles are very fixed, and steric packing considerations often prevent the available methyls from adopting the lowest surface energy orientations. In silicones, the Si–O bond length is significantly longer, and the Si–O–Si bond angle is flatter than the comparable C–C and C–O bonds, resulting in a very low barrier to rotation and making the polymer chains very flexible. This flexibility makes many orientations possible and provides "free space" to accommodate different-sized substituents or to allow easy diffusion of gaseous molecules, a property useful in the formation of "breathable" films. Coupled with the low intermolecular forces between methyl groups, this flexibility also has a profound effect on the bulk as well as the surface properties of silicones, seen in the small variation of physical parameters with temperature and molecular weight, the low freezing and pour points of fluids, the low boiling points, the high compressibility, and the retention of liquid nature to unusually high molecular weights. It also makes a number of structural and compositional variations possible, resulting in many families of silicones, including linear and cyclic structures, a wide range of molecular weights, and varying degrees of branching or cross-linking.

Additionally, the siloxane bond is exceptionally strong, providing the polymer with a high degree of thermal and oxidative stability and ensuring the stability in formulation (1–3).

PENDANT ORGANIC GROUPS

The key function of the organic (methyl) groups is to provide the intrinsic surface activity of the silicones. The order of increasing surface energy for single carbon-based groups is $-CF3 > -CF2- > -CH3 > -CH2-$. Liquid surface tension measurements show that, as expected, the order of increasing surface activity is hydrocarbon, followed by silicone, and then by fluorocarbon. Interfacial tension measurements against water, however, show the order of increasing interfacial activity to be fluorocarbon, hydrocarbon, and silicone. Silicones do not fit the simple pattern that a reduction in surface energy means an increase in hydrophobicity and interfacial tension because of their backbone flexibility, which allows them to adopt various orientations at different interfaces. The interfacial tension of silicone is also independent of chain length indicating high-molecular chain freedom. In addition, the critical surface tension of wetting values for silicones has been found to be higher than their liquid surface tension values, meaning that they are able to spread over their own absorbed film. This has an advantage as silicones achieve complete, uniform surface coverage, facilitate the efficient spreading of other materials, and result in smooth, lubricating films.

In addition, because of the organic groups the solubility parameters of silicones are significantly lower than those of water, and many organic materials make them useful in forming barriers to wash off or wear and increase the substantivity of formulations.

Dimethicone polydimethylsiloxane (PDMS)

Figure 1 Unique chemical structure of silicones.

The introduction of functional groups such as phenyl, alkyl, polyether, and amino onto the backbone expands the properties and benefits of silicones further (1–3).

KEY INGREDIENTS IN THE COSMETICS AND TOILETRIES INDUSTRY

Silicones were first used in the cosmetics and toiletries industry in the 1950s, when low levels of medium-viscosity dimethicone [polydimethylsiloxane (PDMS)] were used to prevent the whitening effect, characteristic of soap-based skin lotions. It was not until the 1970s when formulators were concerned about the use of chloro fluoro carbons (CFCs) in aerosols and silicones were considered more seriously as possible ingredients for cosmetic formulations and their unique properties began to be recognized. Since then, the use of silicones has expanded rapidly to virtually all segments, and today, more than 40% of all new products being introduced into the U.S. market contain silicone, with many different types being used (4).

There are five main families of silicones that are used in the cosmetics and toiletries industry today:

1. Volatile siloxanes [cyclosiloxanes (ring structure) and low–molecular weight linear PDMSs] can be used as such or blended with each other to adjust the volatility. They are good solvents and serve as good carriers for high–molecular weight silicones that would otherwise be very difficult to handle. In addition, they have very low heat of vaporization compared with water or ethanol giving them a non-cooling feel when drying. Cyclomethicones are classified as non-VOCs (volatile organic compounds) in the United States (Fig. 2).
2. Dimethicones (PDMSs) are linear structures with molecular weight ranging from 700 to more than 100,000. The nonvolatile dimethicones exist as fluids with viscosities of 5.0 mm^2/sec up to gums. Dimethicone emulsions make handling of the higher–molecular weight fluids easier. Specialized emulsion polymer technologies allow the production of ultrahigh–molecular weight linear PDMS emulsions with an internal dynamic viscosity as high as 200 million mm^2/sec.
3. Silicone blends consist of dimethiconol or dimethicone gums or trimethylsiloxysilicates (highly cross-linked resins) dispersed in lower–molecular weight dimethicones or cyclomethicones. They have been developed to improve the ease of formulation and compatibility of high-molecular gums or resins used for their substantivity.
4. Dimethicone and vinyl dimethicone cross polymers or blends are silicone elastomers, which are cross-linked to different degrees, resulting in different product forms. They exist in powder form, pure or coated with particles (free flowing or suspended in water), or as elastomeric silicone gels that are swollen with solvent (usually volatile silicone). The introduction of different functionality into such products is also possible. They are used as rheology modifiers in skin care and antiperspirant products, providing a dry, powdery feel to formulations (5).
5. Functional silicones:
   a. Dimethicone copolyols (silicone polyethers) are fluids or waxes in which some of the methyl groups along the siloxane backbone have been replaced with

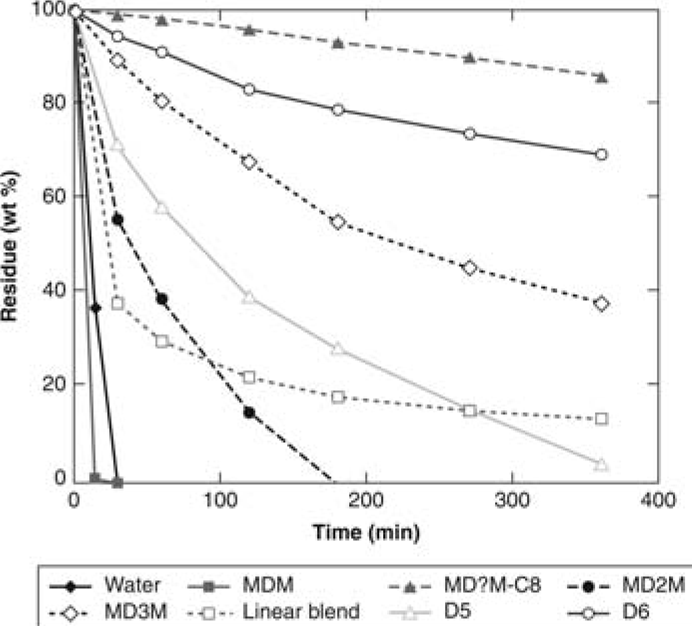

Figure 2 Comparison of volatility for volatile methylsiloxanes (0.1 g, 31°C).

polyoxyethylene or polyoxypropylene groups. The addition of polyoxyethylene substituents increases the hydrophilicity of silicones. Polyoxypropylene substituents are used to balance out this hydrophilicity by increasing the hydrophobic characteristics of the copolymer. Some of these silicone polyethers are also very good water-in-silicone emulsifiers. The addition of alkyl chains results in a material able to emulsify water into low to medium polarity oils.

Among these polyether-modified silicones, a special mention is necessary for silicone carbinol fluid (bis-hydroxyethoxypropyl dimethicone) (6), a very versatile material with good compatibility with organic materials that can act as a wetting agent to stably disperse pigments and actives, while also providing moisturization.

b. Phenyl trimethicones are fluids in which some of the methyl groups have been replaced by phenyl groups. The phenyl groups increase the refractive index and improve compatibility with organic materials.
c. Amodimethicones or trimethylsilylamodimethicones are fluids in which some of the methyl groups have been replaced by secondary and primary amine groups. The polar amine groups have a profound effect on the deposition properties of the silicone, giving it an affinity for negatively charged surfaces, such as the proteinaceous surface of the hair. Emulsions of these fluids are commonly used.
d. Alkyl dimethicones are fluids or waxes in which some of the methyl groups have been replaced by alkyl groups. This results in a family of silicone-hydrocarbon hybrids with possibilities for variations in viscosities, softening temperatures, and rheological characteristics. They have increased compatibility with organic materials.
e. Silicone acrylate copolymers (acrylates/polytrimethylsiloxymetacrylates copolymer). These film-forming copolymers can be delivered from both silicone and organic solvents and provide enhanced durability, wash off resistance, and aesthetic coupled with easy formulation for skin care and color cosmetic formulations (7)

SKIN CARE, SUN CARE, AND DECORATIVE PRODUCTS

Skin Feel/Emolliency

The main reason why the silicones are used in all types of skin care product is because of their sensory properties. Studies on the emollient properties of various materials have shown that

silicones deliver greater emolliency values than many commonly used cosmetic ingredients both during and after application. They are described as smooth, velvety, and nongreasy or oily and are able to impart this feel to cosmetic and toiletry formulations, improving the negative feel associated with other ingredients (8).

Volatile silicones are used for transient effects giving slight lubricity, a light texture, fast spreading, and good distribution of the product on application, while leaving no residual effects. They are often included in formulations to remove the greasy or oily feel of hydrocarbon-based emollients and are the basis for "oil-free"-type claims (9). They are used in light products for daily use such as facial cleansers, day creams, or liquid foundations. Higher–molecular weight silicones such as dimethicone (and) dimethiconol are used to give a more lubricious, longer-lasting effect in richer, more nourishing skin treatment products such as night creams or after-sun products (10). Silicone elastomers are used to give a dry, powdery feel to skin care formulations (11). Silicones are also noncomedogenic/nonacnegenic unlike many occlusive, lipophilic fatty emollients, which can promote comedone/acne formation on the skin (12).

Substantivity (Long Lasting/Durability)

High–molecular weight dimethicone or cyclomethicone (and) dimethiconols form water-resistant films on the skin, which can help prolong the effects of skin care, sun care, or decorative products. This substantivity can be improved further by using alkyl dimethicones such as cetyl dimethicone or C30-45 alkyl methicone (Fig. 3) (13).

The use of silicones to improve the substantivity of other ingredients in cosmetic and toiletry formulations has been demonstrated in sun care products. The addition of 2.5 wt% cetyl dimethicone to an oil-in-water sunscreen formulation shows excellent in vivo resistance to wash off. The formulation has a sun protection factor (SPF) of 21.1 before immersion, which reduces to 19.2 only, after immersion for 80 minutes (14).

Volatile silicones are the basis for long-lasting/nontransfer decorative products, especially lipsticks. They are used to disperse waxes and pigments, improve application, and impart a pleasant skin feel, often replacing nonvolatile hydrocarbon oils. When they evaporate, a uniform film of waxes and pigments remains, which is resistant to transfer and wear (15).

Silicone acrylate copolymers have the ability to form non-occlusive films that resist wash off. This film-forming property is useful in sunscreens, while in lipsticks it pairs with an excellent sensory profile translating into improved nontransfer while maintaining very good wearing comfort (7).

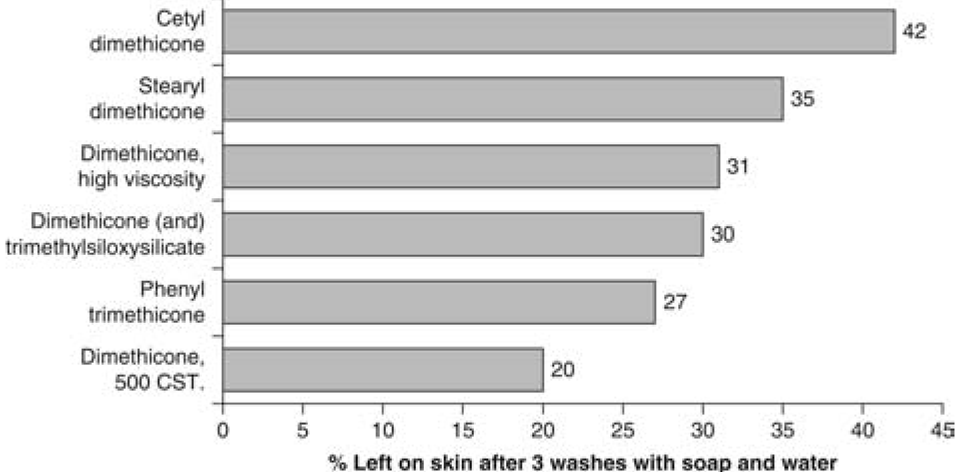

Figure 3 Substantivity of different silicones.

Table 1 Permeability of Different Materials Payne Cup Method

Material	Water vapor permeability g/m^2/hr
Volatile silicone	152
Sunflower oil	125
Silicone gum	121
PDMS 350 cSt	107
Mineral oil	93
C18 Alkylmethylsiloxane	37
C30 Alkylmethylsiloxane	2
Petrolatum	0

Abbreviation: PDMS, polydimethylsiloxane.

Permeability/Controlled Moisturization/Protection Against Dehydration

Because of the flexibility of the Si–O–Si backbone, the majority of silicones are permeable to water vapor, producing breathable films. This is an important parameter for cleansing products or color cosmetics to avoid clogging pores. The presence of an alkyl group in the chain, however, reduces this permeability, resulting in silicones, which can give semipermeable materials, e.g., cetyl dimethicone or occlusive material similar to petrolatum, e.g., C30-45 alkyl methicone (Table 1) (16).

Enhanced Efficacy

Apart from improving the feel and long-lasting benefits of skin care products, silicones can also enhance the efficacy of other ingredients in the formulation. Studies carried out on sun care products have shown that the alkyl methicones can enhance the SPF of products containing either organic or inorganic sunscreens. A 2% addition level of stearyl dimethicone into an oil-in-water silicone containing 11% of organic sunscreens resulted in an in vivo SPF of 49.7, an SPF/UVB ratio of 4.5, thus demonstrating high efficiency (17). For inorganic sunscreens, a 100% increase in SPF was seen with an oil-in-water system containing 2 wt% cetyl dimethicone and a 75% increase in the SPF for a water-in-oil system containing C30-45 alkyl methicone (18).

In antiaging formulations, the addition of silicone elastomer powders, at a level of 4%, has been shown to provide wrinkle-masking benefits in addition to their unique skin feel (19).

Protection

Dimethicone is listed in the FDA (food and drug administration) Monograph for Skin Protectant Drug Products for over the counter (OTC) Human Use in the United States. Because of their hydrophobicity, silicones are used in protective hand creams to provide a water-resistant barrier against waterborne contaminants. Recent studies indicate that cyclomethicone and dimethicone may also prevent irritation caused by sunscreen agents (20).

Cleansing

The excellent spreading characteristics, dry nongreasy/oily feel, and good solvency of volatile silicones make them ideal for use in skin cleansers to help lift and remove dirt without stinging. They can be used alone or in combination with ingredients such as mineral oil. Silicone emulsifiers allow low-viscosity silicone fluids to be present in the continuous phase as well as the incorporation of polar ingredients such as water and glycerin, allowing the formulation of rinseable foaming facial washes (21).

Volatile alkyl silicones (caprylyl methicone) were demonstrated to have superior cleansing properties over cyclopentasiloxane and isohexadecane, ingredients often used in biphasic makeup removers (22).

Powdered silicone elastomers have the capability of absorbing lipophilic materials such as sebum from the skin making them very useful for greasy-skin application (19).

Water-soluble dimethicone copolyols have shown benefits in foaming facial washes. They provide creamy, denser foam as well as improving the foam volume. In liquid body-cleansing products such as foam baths, shower gels, and liquid soaps, they can improve

foaming and foam stabilization. They have also been recognized as additives that reduce eye and skin irritation from anionic surfactants (23).

Emulsions of ultrahigh–molecular weight linear silicones can be perceived on the skin at very low levels because of their very low sensory threshold (24).

Rheology Modification/Structural Integrity (Sticks)

As well as improving the aesthetics of formulations, silicones can also act as rheology modifiers. This is particularly applicable to water-in-oil- or water-in-silicone-type systems. One such silicone rheology modifier is the C30-45 alkyl methicone where 149% and 93% increases in emulsion viscosity have been observed for water-in-silicone and water-in-oil emulsions, respectively, with 2 wt% of the wax (11). Rheology modification using 2 to 4 wt% stearyl dimethicone is believed to be part of the reason for the success of this product in enhancing the SPF of sun care products containing organic sunscreens (17). These waxes are also used in maintaining the structural integrity of stick products, improving their feel and application. Silicone elastomers can also be used to modify the rheology of skin care and antiperspirant formulations. Such elastomers have the capacity to absorb large amounts of solvents such as cyclomethicone or low-viscosity dimethicone without exhibiting any syneresis (extraction of a liquid from a gel). It is this property, which allows them to successfully thicken formulations. The ability of elastomers to significantly modify the rheology of a formulation combined with their unique powdery feel has led to their use in antiperspirant products (5).

Formulating Flexibility

Silicones can be used in all types of skin care products ranging from simple oil-in-water gels or emulsions to water-in-silicone and water-in-oil emulsions, from crystal clear to white in color. Silicone emulsifiers increase this flexibility further. They allow silicones to be present in the continuous phase as well as allowing the incorporation of polar ingredients such as water and glycerin. Matching the refractive index of the water phase with the oil phase in such emulsions makes the formulation of clear gels possible, and adjusting the phase ratio determines the product form from lotions to gels. This technology is the basis for the clear antiperspirant gels seen on the market today. It is also possible to make nonaqueous emulsions using silicones to deliver hydrophilic ingredients or those that are sensitive to hydrolysis. In addition, the benefits of water-in-oil systems such as good sensory profiles, improved wash off resistance, and excellent moisturization have been demonstrated. Silicone emulsifiers offer versatility for low- or high-shear systems as well as cold processing, presenting new opportunities for cost-effective and highly innovative skin care and underarm products (25).

HAIR CARE PRODUCTS

Hair Conditioning/Improved Combing

Various types of silicone are used to give different degrees of hair conditioning. Dimethicone copolyols provide light conditioning due to their solubility in water and low level of substantivity. They can also help reduce eye irritation associated with shampoos and similar products that contain anionic surfactants (23). Higher–molecular weight dimethicones/dimethiconols or trimethylsilylamodimethicones/amodimethicones provide a higher level of conditioning due to their insolubility in water and greater substantivity. The latter have an affinity for negatively charged surfaces such as the proteinaceous surface of the hair that contributes to their substantivity.

Evaluation of the average detangling times of dimethiconol (gum), amodimethicone, and dimethicone (high-viscosity fluid) emulsions at a 4% level in an illustrative two-in-one shampoo formulation indicates that they all show significant improvement over the untreated control tress with the dimethiconol emulsion providing the best conditioning effect (26).

Synergistic effects have been observed between quaternary polymers commonly used in shampoos for conditioning and dimethicone copolyols (Fig. 4). Better detangling results are observed for shampoos containing dimethicone copolyol and quaternary polymers than with the quaternary polymers or dimethicone copolyols alone (27).

Similar evaluation of silicones in conditioners indicates that dimethicone emulsions provide the best conditioning effect in rinse-off products and in permanent waving products;

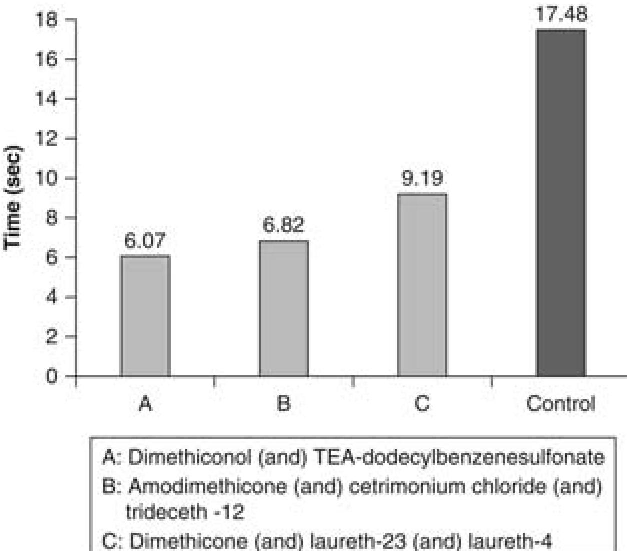

Figure 4 Hand detangling results on slightly bleached hair for diluted emulsions.

an emulsion of trimethylsilylamodimethicone significantly reduces the wet and dry combing force. Combinations of silicones such as cyclomethicone, silicone blends, and phenyl trimethicone are the basis for anhydrous leave-in conditioners, sometimes referred to as "cuticle coat" products (26).

Sensory Enhancement

As in skin care, silicones impart a soft smooth feel to the hair. Sensory evaluations of cuticle coat formulations consisting entirely of blends of silicone showed that, in addition to ease of combing, they improve spreadability, silkiness and softness, gloss, and perceived repair of split ends compared with the control (28).

Silicones as Drying Aids

Silicones such as trimethylsilylamodimethicone can help hair dry more quickly in comparison with drying aids such as stearalkonium chloride, preventing damage due to the use of hair dryers, and others (29,30).

Foam Boosting

Dimethicone copolyols can be used to boost the foaming properties of shampoos as well as provide a light conditioning effect (26).

Reduced Flyaway

Tests comparing shampoo formulations containing quaternary polymers with those with quaternary polymers and dimethicone copolyols show an improvement in static control with the addition of the silicone. Sensory evaluation has also shown a reduction of flyaway with dimethicone emulsions (26).

Improved Shine

Silicones, in particular phenyl trimethicone, are recognized for their ability to enhance hair shine and gloss along with adding softness, manageability, and smoothness to the abraded hair cuticle (26,31).

Natural-Look Fixatives

Because of their low surface tension, silicones spread easily to help fixative products distribute evenly on the surface of hair and improve their effectiveness. They are also used in conjunction with or as a replacement for organic plasticizers. Organic materials tend to be hydrophilic, which diminishes the holding power of a resin. In contrast, the hydrophobic nature of silicones

helps repel water, so there is less opportunity to reduce the resin's holding properties. The use of dimethicone copolyol as a resin plasticizer can also help give hair a more natural look (26).

LONGER-LASTING PERMANENT WAVE AND COLORING PRODUCTS

Silicones, such as trimethylsilylamodimethicone, can be used to provide a more durable conditioning effect and a longer-lasting permanent wave. Pretreatments containing silicone blends help prevent hair damage during the harsh perming process. In hair color products, blends of volatile and nonvolatile silicone (cyclomethicone and trimethylsilylamodimethicone) can be used to seal the hair cuticle and hold color in. The volatile silicone evaporates, leaving behind a smooth, uniform film on the surface of the hair (32).

Amino-functional silicones can prevent degradation of the hair color due to the combination of UV exposure and washing and provide conditioning benefits such as ultra shine, improved drying time, moisturization, and a smooth and light feel without negatively impacting hair body and volume (33).

Evaluation of rinse-off conditioners indicates that silicones show excellent potential to provide color lock properties in permanent hair color products.

HAIR STRENGTHENING

Amino functional silicones leave a medium-weight film deposited primarily at the open edges of the cuticle, resulting in improved hair strength. Two possible mechanisms could explain this effect (34):

- The film may protect the hair cuticle during elongation.
- The film may assist in sealing the hair cuticle, helping prevent moisture penetration into and out the hair cortex, maintaining an optimal moisture level for hair strength.

ANTIPERSPIRANT AND DEODORANT PRODUCTS

In addition to the benefits that silicones bring to skin care products such as improved feel, delivery of actives, low residue, and formulating flexibility, the following advantages are seen in antiperspirant and deodorant formulations (35).

Anti-Whitening

Dimethicones, phenyl trimethicone or alkyl dimethicones have been shown to reduce/mask the whitening effect caused by antiperspirant salts by matching the refractive index (36).

Improved Spray Characteristics

Low levels of cyclomethicone (and) dimethiconol have been demonstrated to reduce the spray width, height, and particle size of antiperspirant pump spray and aerosol formulations, leading to a more directional spray with low mistiness and dustiness (35,37). The silicone blend may also contribute to the substantivity of the antiperspirant active and lubricate the spray valve to prevent clogging.

Non-Cooling

The heat of vaporization of volatile silicones such as cyclomethicone is much lower than that of water or ethanol meaning that much less energy is required for them to evaporate. This leads to a non-cooling effect in formulation (35).

CONCLUSION

The multifunctional benefits of silicones make them invaluable ingredients in today's cosmetic and toiletry formulations, and with the introduction of more and more new silicones, this is a trend that is expected to continue.

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36 Sensory Effects and Irritation: A Strong Relationship[a]

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INTRODUCTION

Most methods for testing the potential skin effects of consumer products rely on an objective evaluation of how the product affects the appearance of the skin, i.e., erythema, as scored on a predefined numerical scale. In contrast, the endpoints that consumers typically describe are subjective in nature and are an evaluation of the sensations the product causes them to experience, i.e., itchy, dry, rough, tight, etc. Even though these subjective sensory effects are the endpoints reported by the consumer and, therefore, arguably the most important, few test methods incorporate a means to leverage these endpoints.

Few reports attempt to either quantitate sensory effects or correlate sensory effects to the degree of irritation. In studies on soap and detergent bars, Simion et al. determined that test subjects differentiated between products on the basis of sensations they felt during exaggerated arm-washing applications (1). In several test methods designed to simulate normal use conditions for detergent and personal cleansing products, the perceptions of the panelists are routinely recorded during the course of the study as an additional endpoint to consider when evaluating the data (2,3). Sting tests have been used in evaluating some cosmetics and leave-on preparations (4,5).

We recently developed the behind-the-knee (BTK) test method as a means for evaluating the irritation potential of catamenial products (6–8). The BTK measures both chemical irritation and the potential for mechanical irritation due to friction. In the course of this development program, we collected subjective sensory data from our panelists in parallel with the outward signs of irritation, i.e., erythema and dryness, to determine if there may be value in attempting to quantitatively study sensory effects to learn more about the relationship between these sensations and irritant effects. We evaluated the results against the fundamental question: are differences in the irritation potential, as defined by objective scores of erythema, consistent with differences in the reported sensory effects?

Subjective sensory data on certain test products tested in the BTK were compared to several different objective endpoints: unaided visual scoring, enhanced visual scoring using polarized light, and the descriptive analysis panel (DAP).

Unaided visual scoring of erythema has been used reliably for a number of years to detect skin irritation in a wide variety of test protocols on a large number of body sites. It requires no special equipment and is easily adaptable to large-scale testing, such as the type that is required to provide safety assurance for consumer products. Trained skin graders can accurately and reproducibly score test sites for erythema and dryness (9). Several authors have demonstrated that trained graders can reliably detect evidence of irritation with equal or higher degrees of sensitivity to that of instrumental measures (10–14).

Enhanced visual scoring using polarized light has been added to existing test protocols recently in an attempt to increase the ability to differentiate between very similar products without requiring other protocol modifications. When skin reactions are scored visually, the grader is seeing a combination of endpoints: the surface changes, which provide information about the shape and texture of the skin surface, and the subsurface changes, which provide information about internal components such as erythema, pigmentation, and the vasculature (15,16). Use of polarized light sources can enable the observer to selectively examine either the surface or subsurface components. Authors have described the use of polarized light as an aid

[a]A portion of this review appeared in Farage MA, Santana V, Henley E. 2005. Correlating sensory effects with irritation. J of Toxicol: Cutan Ocul Toxicol 2005; 24:45–52. With kind permission of Taylor and Francis Publishing Group.

in visualizing various skin conditions, including irritation, acne vulgaris, rosacea, photoaging, lentigo simplex, and basal cell carcinoma (17–20).

The DAP is used in the development of feminine care products to evaluate the intensity of six physical characteristics, including degree of plasticity, compression (loft), scratchiness, glide, cottony feel, and flexibility. We compared the results of the DAP to the results of the sensory effects analysis attempting to determine if certain physical characteristics of the products correlated to adverse sensations experienced by the BTK panelists.

The test design and conduct of the BTK studies have been described in detail by Farage et al. (6). Each study consisted of 9–22 healthy, adult volunteers (male and female) who had signed an informed consent. For each study, the protocol was approved by the test facility's institutional review board. Subjects were excluded from participation for certain skin abnormalities or health conditions that could adversely impact the test, as detailed in the prior publication.

Test materials consisted of currently marketed products and products in development for the marketplace. These included standard catamenial pads (products A, B, C, E, H, M, and N), a pantiliner (product P), a lotioned pad (product NL), intralabial pads (product IL2 and IL25), and tampons (products R and S). For application, a test material was placed horizontally and held in place behind the knee by an elastic knee band (Ace® knee bandage) of the appropriate size and removed by the panelists 30 to 60 minutes before returning to the laboratory for grading and/or reapplication of test materials. Exposures consisted of six hours per day for five consecutive days.

Visual grading of the BTK test sites was conducted by an expert grader under a 100-watt incandescent daylight blue bulb. Scoring was done using a scale of "0" to "4" previously described by Farage et al. (6). In this scale, "0" indicates no apparent cutaneous involvement and "4" is moderate to severe, spreading erythema and/or edema. The same grader was used throughout an experiment, and the grader was not aware of the treatment assignments.

Enhanced visual grading using polarized light has been described in detail by Farage (21). Grading was conducted using a polarized light visualization system (Syris v600® Visualization System, Syris Scientific, LLC, Gray, Maine, or syrisscientific.com), with separate scores recorded for parallel-polarizing illumination (surface mode) followed by cross-polarizing illumination (subsurface mode). On the basis of a communication with the manufacturer, the subsurface mode allows visualization of the site at a depth of 1 mm beneath the surface.

The mean irritation scores (unaided and enhanced visual) were compared using analysis of variance/analysis of co-variance (ANOVA/ANCOVA) or, where model assumptions failed, CMH/stratified CMH (Cochran-Mentel-Haenszel) statistical comparison.

In the BTK, panelists were asked to keep a daily diary of skin problems experienced at the test sites, as previously described (22,23). Panelists were asked if they experienced one or more of eight specific sensations, including the sample rubbing against the skin, the sample sticking to the skin, chafing, burning, itching, pain, edema, or any other discomfort. Results were evaluated for a higher occurrence of each individual skin problem with one treatment versus another using McNemar's test.

For Table 1, mean irritation scores were evaluated versus reported sensory effects using logistic regression, i.e., the presence/absence of a particular skin complaint was regressed against the specific irritation scores.

The DAP uses 15 individuals trained to evaluate six different physical characteristics of the products: degree of plasticity, scratchiness, glide and cottony feel of the top sheet, and compression and flexibility of the pad. These individuals evaluate products using their fingertips and assess the intensity of the various product characteristics on the basis of an 8-point scale, with 0 = not at all, 4 = moderate, and 8 = extreme. Results were analyzed by ANOVA, and mean differences were tested for statistical significance using Duncan multiple comparison at the 90% confidence level.

SENSORY EFFECTS AND UNAIDED VISUAL SCORES

Sensory effects have been collected and analyzed statistically in nine BTK studies containing 16 comparisons between two products or sets of test conditions. In each experiment, the reported sensory effects for the test samples were compared to determine if the results were

Table 1 Correlation of Sensory Effects with Visual Irritation Scores in Behind-the-Knee Studies

Study number	Products tested	Correlation of sensory effects with irritation scores
02017	Products N and L	Reports of burning, pain, itching, and rubbing correlate to irritation score.
03005	Products A and B under 4 different test conditions	Reports of burning, pain, sticking, and "other" correlate to irritation score.
03005-3	Products S and R under 4 different test conditions	Reports of burning, sticking, and chafing correlate to irritation scores for the w/c [a] test sites only. No correlation for d/i [b] test sites.
03005-4a	Products GT and M	Reports of burning correlate to irritation score.
03005-4b	Products GT and E	Reports of burning, pain, and sticking correlate to irritation score.
03005-4c	Products GT and H	Reports of pain, itching, rubbing, and "other" correlate to irritation score.
03005-9	Products A and B	Reports of burning, edema, and itching correlate to irritation score.
02008	Products P and IL25	Reports of burning, pain, and chafing correlate to irritation score.
02036	Product C under 2 different test conditions	Reports of burning and sticking correlate to irritation score.
02008	Products P and IL2	Reports of burning, pain, and chafing correlate to irritation score.

[a](w/i) = wet sample on intact skin.
[b](w/c) = wet sample on skin compromised by tape stripping. Studies were conducted as described in the legend for Table 2. For each study, the irritation scores and reported sensory effects were considered for all test sites, regardless of the products or treatments being tested. Numerical irritation scores were then evaluated versus reported sensory effects using logistic regression.

similar to those obtained when the mean irritation scores were compared. Results are summarized in Table 2. In 8 of the 16 individual product comparisons, the results are consistent (shown at the top of the Table 2). In seven of these cases, there were no differences in either the mean irritation scores or in the percentage of subjects reporting various sensory effects. In the remaining case, significant differences in the mean irritation scores appeared for at least one scoring time point, and the sample giving the higher mean score (product A) produced significantly more reports of sensory effects. In 5 of the 16 comparisons, significant differences in the mean irritation scores appeared for at least one scoring time point, and no differences appeared for the reported sensory effects. In 2 of the 16 comparisons, differences appeared in the reports of sensory effects that were not evident in the mean irritation scores. In one case, the reported sensory effects were in conflict with the mean irritation results (Table 2, study #02008, product P vs. product IL2).

Products A and B have been compared seven times in the BTK test in addition to the three comparisons shown in Table 2. In 8 of the 10 total tests (including study #03005-T9 shown in Table 2), product A was significantly more irritating than product B, on the basis of mean irritation scores with visual grading. In two comparisons (study #03005, shown in Table 2, and one other study not shown), the differences did not achieve significance.

Another analysis was conducted to determine if reports of adverse sensory effects were likely to increase as the objective score for irritation increased. In other words, if a test site is scored as a "2.0" for erythema, is it more likely that that panelist will report a sensory effect than if the site were scored as a "1.0"? Table 1 shows the results of regression analyses conducted to determine if an increase in reported sensory effects correlated to an increase in the irritation scores recorded for the eight experiments (16 comparisons) shown in Table 2. In eight experiments, reports of one or more sensory effects were correlated to the degree of irritation. In one experiment (03005-3), there was a correlation between the panelists tested under one set of experimental conditions (wet samples on compromised skin), but not under the other set of conditions (dry samples on intact skin). Burning was the effect that was most commonly correlated to higher irritation scores (in seven cases), followed by pain (in five cases).

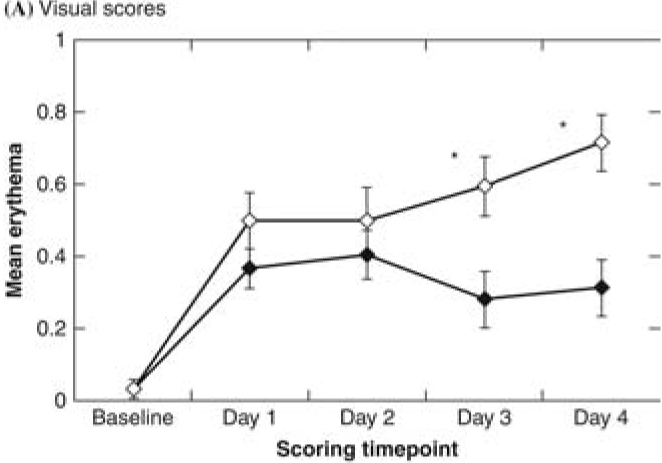
SENSORY EFFECTS AND POLARIZED LIGHT-ENHANCED VISUAL SCORES

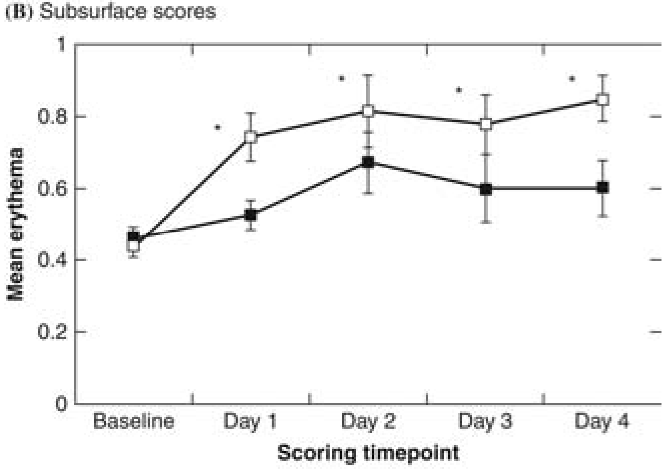
Two feminine protection products were evaluated in the BTK using unaided and enhanced visual grading. Scores were recorded after each product application (6-hour application

Table 2 Behind-the-Knee Studies with Comparison of Visual Mean Irritation Scores and Sensory Effects

Study number	Samples compared		Results: Comparison of mean irritation scores	Results: Comparison of adverse sensory effects
Consistent results from nonaided visual scoring and sensory effects				
02017	Product N	Product NL	Not significantly different	Not significantly different
03005	Product A (d/i)	Product A (w/c)	Not significantly different	Not significantly different
03005-3	Product S (w/c)	Product R (w/c)	Not significantly different	Not significantly different
03005-3	Product S (d/i)	Product R (d/i)	Not significantly different	Not significantly different
03005-4a	Product GT	Product M	Not significantly different	Not significantly different
03005-4b	Product GT	Product E	Not significantly different	Not significantly different
03005-4c	Product GT	Product H	Not significantly different	Not significantly different
03005-9	Product A	Product B	Significantly different on days 3 and 4. Product A > product B	Percentage of subjects experiencing burning is different on all days (1–4). Product A > product B
Inconsistent results from nonaided visual scoring and sensory effects				
02008	Product P	Product IL25	Significantly different only on day 5. Product P > product IL25	Not significantly different
02036	Product C (w/i)[a]	Product C (w/c)[b]	Significantly different only on day 1. Product C (w/c) > product C (w/i)	Not significantly different
03005	Product B (d/i)[c]	Product B (w/c)	Significantly different on days 1, 3, and 5. Product B (w/c) > product B (d/i)	Not significantly different
03005-3	Product S (w/c)	Product S (d/i)	Significantly different on days 2, 3, and 5. Product S (w/c) > product S (d/i)	Not significantly different
03005-3	Product R (w/c)	Product R (d/i)	Significantly different on days 2, 3, and 5. Product R (w/c) > product R (d/i)	Not significantly different
03005	Product A (d/i)	Product B (d/i)	Not significantly different	Percentage of subjects experiencing burning is different on day 1. Product A (d/i) > product B (d/i)
03005	Product A (w/c)	Product B (w/c)	Not significantly different	Percentage of subjects experiencing pain and sticking is different on days 2 and 3, respectively. Product A (w/c) > product B (w/c)
02008	Product P	Product IL2	Significantly different only on day 1. Product IL2 > product P	Percentage of subjects experiencing itching is different on day 3. Product P > product IL2

[a](w/i), wet sample on intact skin.
[b](w/c), wet sample on skin compromised by tape stripping.
[c](d/i), dry sample on intact skin (standard protocol).
In each study, panelists wore the test substances for 6-hr daily for 4 days in close contact with the skin behind the knee. The two test areas (left knee and right knee) were randomly assigned 1 of the 2 test materials or conditions being compared in that particular study. Test sites were scored 30–60 min after removal of each product application. The mean irritation score for each treatment at each afternoon scoring was determined. There were compared using ANOVA/ANCOVA or, where model assumptions failed, CMH/stratified CMH tests. In addition, subjects kept daily diaries of sensations associated with each test site throughout the course of treatment. Panelists were specifically asked about the sample and knee brace rubbing against the skin or sticking to the skin and about chafing, burning, itching, pain, or any other discomfort. Each individual complaint was tabulated, and the percentage of subjects who experienced that compliant was determined. The data were evaluated using McNemar's test.





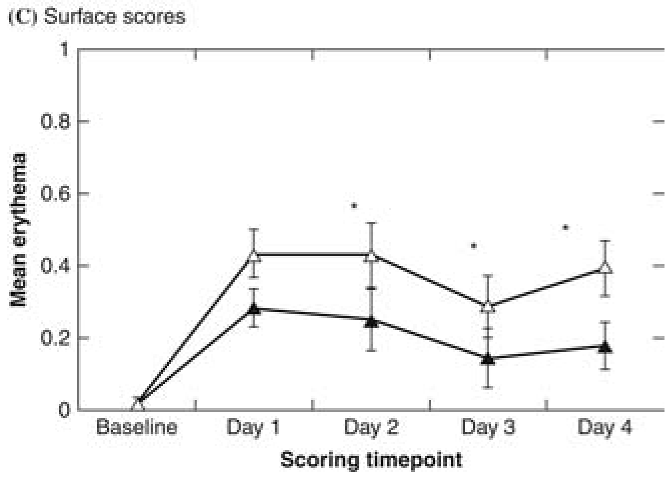


Figure 1 Standard visual and enhanced visual grading using two similar products in the BTK. Two feminine protection products (pad A and pad B) were evaluated in the BTK. Samples were applied for six hours per day for four consecutive days (14–16 panelists per group). Scoring was conducted at baseline and the morning following each patch removal (day 1–4). The graph plots mean erythema (±S.E.) at each scoring time point. Figure 1(**A**) shows visual scores; 1(**B**), subsurface scores; and 1(**C**), surface scores. Treatment comparisons were evaluated using the stratified CMH test. (*Significant difference between pad A and pad B, $p < 0.05$). Pad A = open symbols. Pad B = closed symbols. *Abbreviation*: BTK, behind the knee.

followed by 18-hour recovery period). As shown in Figure 1A, significant differences between the two products were apparent on day 3 (after three applications) using unaided visual scoring. Using polarized light-enhanced scoring, significant differences were observed after

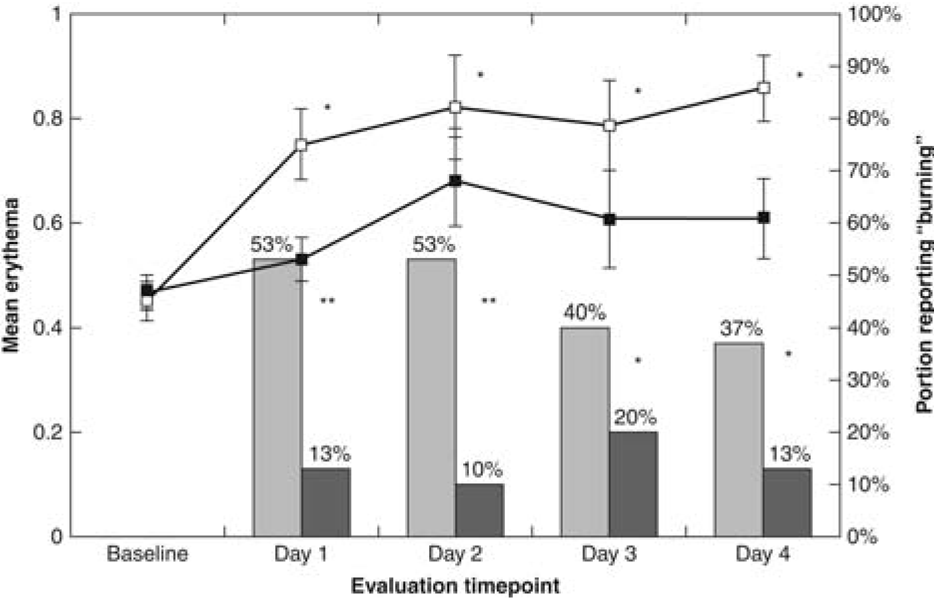


Figure 2 Reports of burning sensations in the BTK. In the BTK, each of the 30 panelists was asked to keep a daily diary of skin problems experienced at the test sites. Results were evaluated for a higher occurrence of each individual skin problem with one treatment versus another. The graph plots mean erythema (±S.E.) at each scoring time point (as shown in Fig. 1), and the portion of the subject population reporting sensations of burning at the test sites during each patch application. Treatment comparisons for the sensory effect was evaluated using McNemar's test. (**Significant difference between pad A and pad B, $p < 0.001$). (*Significant difference between pad A and pad B, $p < 0.05$). Pad A = open symbols. Pad B = closed symbols. *Abbreviation*: BTK, behind the knee.

the first and second application (for subsurface and surface scoring, Fig. 1B and 1C, respectively).

The number of panelists who experienced burning sensations at the test sites is shown in Figure 2. With every sample application, there was a significantly higher number of individuals reporting burning sensations with pad A compared with pad B. In addition, a significantly higher number of individuals reported pain with pad A during the third sample application and sensation of the sample sticking to the skin during the second and third application (data not shown).

SENSORY EFFECTS AND DESCRIPTIVE ANALYSIS PANELS

DAPs, conducted in the course of normal product development activities, evaluate certain physical characteristics of the products. Results from DAPs on products that have also been tested in the BTK are shown in Table 3. In the comparison of products A and B, product B scored lower in the negative attributes and higher in the positive attributes. Results on the comparison of products G and E were mixed, with product G scoring higher on the negative attribute of plastic feel, while product E scored higher on the negative attribute of scratchiness. Likewise, product E scored higher on the positive attribute of glide but lower on the positive attribute of cottony feel.

DISCUSSION

This study examined data collected in the course of the development and conduct of the BTK test for correlations between the objective scores of erythema and the sensory effects reported by the panelists. As shown in Table 2, in about half of the comparisons (7 of 16), the samples were not significantly different on the basis of either a difference in unaided visual scores for

Table 3 Descriptive Analysis Panel (DAP) Results for Products Tested in the BTK Test

	Mean scores			
	Product A	Product B	Product G[a]	Product E
Negative attributes:				
Plastic feel	6.8[b]	0.0	3.9[b]	0.3
Scratchiness	5.4[b]	1.5	3.5	5.6[b]
Positive attributes:				
Glide	4.8	6.7[b]	2.6	5.5[b]
Cottony feel	0.0	6.3[b]	4.2[b]	3.4

[a]Top sheet tested.
[b]Higher at the 90% confidence level.
In the DAP, individuals specifically trained to evaluate the positive and negative physical characteristics of products graded these attributes on an 8-point scale, with 0 = not at all, 4 = moderate, and 8 = extreme. Mean differences were tested for statistical significance using Duncan multiple comparison.

irritation or reported adverse sensory effects. For one sample, both the irritation scores and the sensory effects were significantly different. In 5 of the 16 comparisons, sample differences were detected in the objective scores that were not apparent in the reported sensory effects. In the remaining three sample comparisons, the reported sensory effects indicated sample differences that were either not detected in the objective scores, or in one case, were contrary to the objective scores.

In most of the studies in which sensory data were collected, the differences in mean irritation caused by the test samples were generally small, with either no significant differences or differences only at isolated time points. In the early development of the BTK, those control samples were tested that produced more pronounced differences in mean irritation, which persisted throughout the experiment. Unfortunately, sensory data were not collected at this early stage of the development of the test model. Tests on products that produce a more pronounced difference in mean irritation may improve the ability to detect product differences solely on the basis of the sensory effects and may enable more definitive conclusions about the correlation between observable irritation, as determined by objective scoring of erythema, and what the panelists feel. A logical next step is to collect and analyze sensory effects in studies where the test samples and/or test conditions would be expected to produce greater differences in the mean irritation.

Although we were not always able to reliably differentiate between the samples using solely sensory effects in these particular studies, we did observe that an increase in the irritation score is correlated with a higher percentage of panelists reporting adverse sensory effects, as shown in Table 1. This indicates that even among volunteer panelists who are not specifically trained in observing and reporting sensory effects, the increased reports of sensory effects reliably reflects an increase in irritation, as measured by objective observation. This correlation may be improved by modifying the manner in which data on sensory effects are collected. Currently, panelists are given eight different terms to describe an unpleasant sensation, including rubbing, sticking, chafing, burning, itching, pain, or "other". By providing eight different choices, and treating these as separate endpoints, we may have inadvertently designed the experiments in a way that undermines our ability to detect trends. Statistical differences for any one of the eight choices may be extremely difficult to achieve with a panel of only 15 to 18 individuals. Modifying the sensory diaries to reduce the number of choices that panelists can choose to describe any unpleasant sensation may improve our abilities to discriminate based on sensory effects.

Enhanced visual scoring enables detection of physiological changes that are not apparent using standard visual scoring, i.e., subclinical changes. Previously, we have reported that subjective consumer comments indicate that consumers can detect differences in skin effects caused by the use of two similar products. For example, consumers have consistently indicated that pad B is seen as less irritating than pad A in "real use" situations; however, most test protocols repeatedly fail to differentiate between these two products (7). Sensory effects in the BTK have been shown to be consistent with consumer comments and reliably differentiate between pad A and pad B (23). This current investigation confirms that sensory effects

Table 4 Qualitative Summary Comparison of Objective and Subjective Endpoints

Product comparisons	Comparison of mean irritation scores	Adverse sensory effects	Topsheet attributes in DAP			
			Negative attributes		Positive attributes	
			Plastic feel	Scratchy	Glide	Cottony feel
Product A vs. product B	Product A $>$ product B in 8 of 10 studies [a]	A $>$ B [b]	A $>$ B	A $>$ B	B $>$ A	B $>$ A
Product G vs. product E [c]	Product G = product E in 1 study	G = E	G $>$ E	E $>$ G	E $>$ G	G $>$ E

[a]Seven studies were conducted on complete products A and B, and 3 studies were conducted on the product top sheets.
[b]Sensory effects collected in one study on complete products.
[c]Top sheet for product G tested in DAP.
Mean irritation scores and adverse sensory effects data were taken from Table 2. In addition, several other studies were included in which products A and B were compared (data not shown). DAP results were taken from Table 3.

correlate with visual scoring in the BTK and confirms that sensory effects enable the differentiation between two very similar products (Figs. 1 and 2).

Unlike the BTK test, which uses healthy volunteers as panelists, the DAP is conducted using individuals who are specifically trained in detecting and grading physical attributes of products. As shown in Table 3, this trained panel was able to clearly differentiate between products A and B for four key product attributes (two positive and two negative). In contrast, products G and E produced mixed results, resulting in no clear conclusion on which product would be superior, overall.

While the endpoints in the DAP are completely different from the sensory effects reported by panelists in the BTK, the product attributes graded in the DAP are likely responsible for the adverse sensory effects the panelists reported in the BTK. This is illustrated in Table 4. This table provides a qualitative summary comparing the results on products or top sheets tested in the BTK and in the DAP. As mentioned above, in most BTK tests (8 of 10 studies), product A produced significantly higher mean irritation scores than product B. In the one BTK study where sensory effects were collected, adverse sensations were reported significantly more often with product A than product B. This is consistent with the positive and negative product attributes identified in the DAP where product A had significantly higher scores for the negative attributes and significantly lower scores for the positive attributes. When products G and E are compared in the BTK, they are similar with regard to both mean irritation scores and adverse sensory effects. Likewise, these products could not be clearly differentiated in the DAP, where each product scored higher in one of two positive attributes and in one of two negative attributes.

In future BTK studies, we plan to incorporate terms in the BTK test that are comparable to those used in the DAP. Currently, the descriptive terms are completely different for the two test methods, making it difficult to relate the results. To some degree, this is unavoidable, since the two tests are designed to measure different endpoints. However, some of the descriptors in the DAP may be meaningful for use in the BTK test. For example, a material described in the DAP as having a "plastic feel" may cause the sensation of "stickiness" or "chafing" in some individuals in the BTK. Therefore, a logical next step is to conduct a BTK test using volunteers trained for the DAP testing to determine how these individuals describe the sensations experienced in the BTK.

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37 Decorative Products

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INTRODUCTION

Makeup was created in the Middle East 5000 years ago. It was to give themselves a more attractive image, to protect their bodies from a dry and very sunny climate, and also as medical care that all the Egyptians of the Antiquity used makeup. For a long time being considered as an art, the makeup entered, with the development of the cinema and the television, in a phase or science and technique to help the esthetic preoccupations of the women. Over this century, the role of the makeup changes with the changing culture of the society. However, central themes are easily recognizable through each decade. The natural look of perfection continues in the year of today, as with the goal of restoring youth and looking younger. The cosmetics' industry has experienced some significant changes in the past five years. Marketing has evolved, the consumer has become more knowledgeable and demanding, and cosmetics themselves have become more sophisticated and innovative. Technological advances have allowed for the creation of multifunctional products that perform more than their basic role. For example, foundations with high sun protection factor (SPF) protect from ultraviolet (UV) rays, lipsticks moisturize, and mascaras lengthen, curve, and thicken. The biggest performance development in color cosmetics has been the creation of "stay-on" products, which are long lasting. These products first appeared in the foundations category, then transferred across product categories to include lipsticks, eye shadows, mascaras, and nail enamel. The concept has been extended with stay-on lipliners in addition to transfer-resistant lipsticks. All these evolutions could not have appeared without the progress of chemistry, which made pigments easier to formulate and brought polymers, which improves the sensory and physicochemical properties of makeup products. An improved incorporation of specific raw materials used in skin care products and pigments with visual effects allowed the creation of some specular effects. Makeup products contribute also in a significant way to the general health and well-being of women. They have a major role, which is to provide psychological stimulation to satisfy personal desires for self-improvement, self adornment, and good grooming for one's own sense of well-being and for the general attention or attraction of others. The "psychology of cosmetics" appears therefore as a new field concerning the characterization of the beneficial effects of cosmetic practices (1). This heightened technology of makeup products, the sensory interactions based on the skin-brain connection, and ever more stronger claims are today accompanied by the development of specific evaluation methods used to quantify these improvements and to prove the claimed efficacy.

MAKEUP FORMULATIONS

In makeup products, pigments play an essential role because they provide the chromatic modifications necessary for these qualities. Pigments used in current makeup applications can be classified as organic pigments, mineral pigments, and nacrous pigments (Table 1). Twenty years ago, the cosmetic industry introduced the treated pigments. The surface properties of pigments differ with the size and the shape of the pigment particles as produced by the various manufacturing methods. It is know that the behavior of pigments is closely related to surface properties, and can be classified into hydrophilic and lipophilic. It is very important to know these properties of powders when makeup products are formulated. Generally, pigments and substrates used (mica, talc, sericite, borum nitride, etc.) for makeup cosmetics are hydrophilic. The surface properties of these powders can be changed from hydrophilic to hydrophobic with

Table 1 Pigments Used in Current Makeup

Pigments	Description
Organic pigments	Selection of pigments for use in makeup is limited to those allowed by regulations of the Food and Drug Administration in the United States. The pigments are formed by precipitating the colorant onto a substrate, often aluminum or calcium hydroxide and thus forming an insoluble salt.
Mineral pigments	Titanium dioxide and iron oxides are the most commonly used materials
Nacrous pigments	Nacrous or pearlescent pigments are used to create frosted appearance, often with other special effects.
	There are 3 classes of nacrous pigments used in makeup:
	Natural pearl essence (for nail enamel), known as guanine (2 amino-6-hydroxy-purine), is derived from the scales of atlantic herring. It provides a soft luster. The pigment's density is lowest of the 3 types noted above, and it is therefore the easiest to suspend. Natural pearl essence exists in the form of platelets or needles.
	Bismuth oxychloride
	A commonly used synthetic nacrous pigment is bismuth oxychloride. This material's luster is more metallic than that of the other two types. The particles also have a higher density (7.7), making then much more difficult to suspend.
	Titanium dioxide—mica or mica coated by several different layers (silica, iron oxide, etc.).
	Another type of pearlescent material is mica coated with a thin layer of titanium dioxide. The interference effects change according to the thickness of the layer of titanium dioxide.

several of the aforementioned coatings depending upon the final products' function. There are many processes by which pigments may be surface treated. The most common methods used today are through chemical interactions, electron-charge reactions, mechanochemical processes, and mechanical processes. Other processes are essentially variants of these four basic methods, as witnessed by an introduction in surface treatment manufacturing called ultramicronization. Surface treatments impart easier dispersibility, better stability, and flocculation resistance to pigments. The second basic important advantage for the cosmetic chemist in using treated pigments is in development of hydrophobic systems (e.g., silicon media). Hydrophobic pigments of both types are available, including treated inorganic colors, DC colors, nylon, titanium dioxide, talc, kaolin, mica, and other minerals. These pigments, and the products made with them, show water-resistant properties, improved skin adhesion, improved color consistency, and better smooth skin feel than when uncoated materials were used in the same formulations.

Lisptick (2)

Lipsticks are mixtures of waxes, oils, and pigments in varying concentrations to yield the characteristics of the final product. Waxes will give rigidity and solidity to the stick. They can be of vegetable origin like candellila wax, which brings brightness or carnauba wax that gives hardness. They can also be of mineral origin like ozokerite wax for adhesion or of synthetic origin like polyethylene, which is compatible with silicones and avoids the exudation of oils. Usually, lipsticks contain a combination of these waxes (~20%) that are selected and blended carefully to achieve the desired melting point. Oils (40–50%) are used to give the lipstick its slippery and soft aspect when applied; for example, castor oil is used to disperse pigments and white mineral oil or oleyl alcohol is used to form a film suitable for application to the lips. Colouring agents are present between 2% and 10% and can be of several types. One mainly finds synthetic pigments, but minerals like iron oxides (red, yellow, or black) are also used to give the color and titanium dioxides bring coverage, opacity, and intensity. Pearl pigments can also be used to give color highlights. It should be noted that pigments of vegetable origin are rarely used in cosmetics because most of them are unstable to heat or light. Those of mineral origin also create a problem because they contain too many heavy metals according to the cosmetic legislation. In relation to the claims, additives can be added such as sun filters, which protect the formula or bring a sun protection index, antioxidizers, vitamins (E, C, and B5), moisturizing agents, or ceramides. A light fragrance (<1%) will be added to give the lipstick a pleasant taste on lips and mask possible smells of raw materials. Overall, a standard lipstick manufacture can be simplified in four stages: *mixing waxes, oils and extenders at high temperature; producting a concentrated dispersion of pigment (generally the pigments are grounded in an oily base*

such as castor oil); adding the colored paste at high temperature with the rest of the formula of the first stage; molding the colored paste. The lipstick chemist must develop a formula, which has a good cosmetic feel for the range of formulated shades. Therefore, the interest for him is to estimate the influence of the pigments on the texture of the stick. From rheological studies, Tranchant and Poulin (3) showed that one can monitor the manufacturing stages. Knowing the physicochemical properties of pigments and their rheological behaviors after grinding, they demonstrated and explained the influences on the texture and the mechanical properties of the stick. One of the disadvantages of conventional lipsticks, which almost all consumers point out, is the deterioration of its fresh appearance in a short period of time. To solve this problem and improve the long-lasting properties, one of the most efficient techniques has been the use of a film-forming polymer in combination with volatile oils (cyclomethicone) (4), which are evaporated on contact with lips (no transfer effect). However, incorporation of the volatile oil into the lipstick's composition is accompanied by a loss in the application gloss over time and comfort on lips. For a very glossy lipstick, the formulation is different from that of classical and no transfer lipsticks because the level of the waxy phase goes up to about 80%. The ratio of oil to wax is higher, whereas the rate of pigments is weaker (0–5%). For matte lipsticks, we use talcs, some nylon powder, silica or polymethylmethacrylate, which give a powdered, slippery, and very soft touch.

Nail Enamel

Nail enamel constituents can be grouped in six families. Lacquer, agent for adhesion and gloss, solvents, plasticizers, pigments, and thixotropic agents, which, of course, must be compatible. The heart of the nail enamel formula is the lacquer, which may be defined as a coating that hardens and dries by evaporation of the solvent. The lacquers determine application properties, gloss, wear properties such as adhesion, flexibility and abrasion resistance, water resistance, viscosity, and suspension ability. The polymers selected must be soluble in cosmetics solvents that dry rapidly, leaving a smooth, glossy film with excellent adhesion properties and have a good pigment-wetting ability. Nitrocellulose (10–20%), a polymer obtained by nitration (12% nitrogen) of the cellulose, is the filmogenic agent currently used in nail enamel. However, used alone, it produces films, which tend to shrink and become brittle, with only moderately good adhesion to nail surface. Different modifying resins (5–10%), such as toluene sulfonamide resins, are added to improve the properties of the lacquers such as to increase wear resistance and gloss. These effects are usually adjustable by selection of an appropriate plasticizer (5–10%), a film-forming agent, which acts on the hardness of the film giving it flexibility. Indeed, nail is deformable and elastic. A film adhering to the nail surface must therefore be able to withstand any motions. Typical plasticizers used include phtalates, citrates, camphor, etc. These polymers, resins, plasticizers, and pigments are mainly dispersed in organic acetates and aromatics solvents with amount of solvents in nail enamel roughly of 70%. For formulation reasons, the ratios of solvents can be modified because the choice of solvent can affect the drying time, flow characteristics of the film, and the flexibility of the film. Note that regulations such as the "Proposition 65, California's, Safe Drinking Water and Toxic Enforcement Standard" limits use of certain solvents, such as toluene, which is considered as a reproductive toxin. Hence, most of the new formulations are without aromatic solvents. The physicochemical factors that determine the suspension stability of nail enamel are complex, such as the particle size and distribution, density differences between particles and the continuous medium, viscosity of the continuous medium, particle concentration, and particle-particle interactions. However, several authors (5–7) showed that one can predict a dispersion stability through dynamic studies of rheology, by relating directly the visco-elastic parameters (viscosity, elastic modulus, and viscous modulus) to the dispersed system structure. Thus, different formulation systems need different viscosity profiles to achieve equivalent esthetic results. Indeed, the viscosity of nail enamel has to be sufficiently high to avoid the sedimentation phenomena of pigments in the bottle, remain on the brush before the application, and facilitate precise application, but the viscosity has to be low enough for the nail enamel to be taken out of the bottle. This can be done if the viscosity is not linear. This nonlinearity is obtained by using modified clays (thixotropic agents). To prevent settling, pigments and pearlescent pigments are suspended by a rheological agent, and the organic clays are the most commomly used material. Note that smectites are the mineral group of the clays, which swell and have high cation exchange properties and can be observed as thin

plates. The organic treatment of these clays consists of replacing the cations that are in the natural product (Na, Ca) by some quaternary ammonium salts. The rheological properties of the medium originate from the hydrogen bridges between the hydroxyl groups of the dispersed plates and can be summarized as undisturbed nail enamel [the plates of clay are packed and linked together by hydrogen bonds (high viscosity), when shearing, all these bonds are broken, plates are all oriented in the same direction (low viscosity), and after application, there are associations between the plates, and after application, there are associations between the plates, and thus the viscosity increases. In the early 1990s, environmental laws and regulations as well as the green movement in Europe were giving rise to new formulation and an interest in a water vehicle, because it is known that organic solvents can damage nails (e.g., moisturization and lipids].

Face Makeup

These products are used to make the skin look natural and beautiful for as long as possible; to achieve this purpose, they unify the color of the skin, improve a dull and tired complexion, give a matte finish, and mask possible imperfections like dark spots, small wrinkles, dark rings under the eyes, and the pores of the skin surface. Their application must be easy and give coverage for a natural complexion. They must have a pleasant texture, a good adhesive property, be comfortable, and have a consistent color and smooth finish. There are more and more no transfer and oil-free products. New pigments, such as pigments with an action on the light such as soft focus, photochromic (8) or auto-adaptive effects, and pigments coated with silicone or with fluoride oil giving a very specific sensory touch are also used. Foundations can contain ceramides, used to strengthen the skin barrier, moisturizing agents like glycerin and hyaluronic acid, vegetable oils, which improve the hydration and flexibility of the skin, and free radical scavengers (vitamin E) as well as UV filters (UVA-UVB) giving makeup products a sun protection index (SPF 5–30). Firming complexes to tone up and restructure the skin and seboregulating can also be added.

Face Foundations

The formulations vary according to the qualities required for the product. Foundations are available in various forms: liquids, gels, creams, solid creams, cakes (pancakes), mousse, or in sticks (pen stick). There are four basic facial foundation formulations, which are the most popular products for complexion: oil-in-water, water-in-oil, oil-free, and water-free or anhydrous forms. Oil (silicone)-in-water emulsions are better for normal- and oily-skin types, whereas water-in-oil (silicone) emulsions are better for normal- and dry-skin types. Oil-free formulations are used for women with oily complexions, and anhydrous forms are used by women with facial scarring who require camouflaging. Oil-based foundations are water-in-oil emulsions containing pigments suspended in oil, such as mineral oil. Vegetable oils (e.g., coconut and sesame) and synthetic esters (octyl palmitate and isopropyl myristate) may also be incorporated. Oil-based formulations also contain water (30–45%), siliconed tensio-actives (5%), and some specific actives (vitamins, UV filters, moisturizing agents, etc.). The water evaporation from the foundation just after application leaves the pigment in oil on the face. Water-based foundations are oil-in-water emulsions containing a small amount of oil in which pigments (10–15%) are emulsified with a relatively large quantity of water, which is the dominant substance (50–60%). Oil-free foundations contain vegetable or mineral oils, but also other oily substances, such as the silicones dimethicone or cyclomethicone, which leave the skin with a dry feeling. They come in three forms: alchohol based, glycerin based, and creams or lotions (ideal for oily or acne-prone skin). These foundations go on smoothly but dry fast, so they must be blended quickly for even coverage. Water-free or anhydrous foundations are waterproof, and high concentration of pigments can be incorporated. These several formulations and the effects of pigments can control the darkening phenomenon done from the sebum, hide the wrinkles, protect from the UV through the diffused reflection, give a smooth finish, and do a long-lasting and no transfer effect of makeup. No transfer formulations are an ingenious combination of volatile oils, which fix the pigments on the skin after evaporation giving permanent effects. The most-used ingredients in the face makeup were mica mat and micro titanium dioxide and silicon dioxide. Mica is transparent and easy to use; the titanium dioxide has good covering effects, and it is very efficient against the UV, but its

touch is hard; and the silica is a multi-porous ingredient, which absorbs the oil and sebum. Nowadays, the surface treatments are wonderful techniques to give some special functionalities to the raw material pigments and sensory properties (9). The smooth feeling of a foundation mainly depends on the physical properties of the raw material pigments, such as particle size, shape, and so on. Furthermore, by adding the moisturizing and water-absorpting effect to the raw material pigments with surface treatment, the much more elegant and smooth feeling in use can be completed, and these days, polysolixane (SI) treatment is very popular for makeup products. The long-lasting effect is also a very important functionality for makeup products and especially for face makeup. A lot of women use makeup products to maintain their soft and smooth skin for a long time. For women with oily complexions, lipids secreted from skin tissue collapse the makeup finishing and the surface of the finishing skin becomes glossy, since lipid from skin tissue is miscible with pigments and binder in makeup cosmetics. This disadvantage will not be solved with SI treatment, since polysiloxane is miscible with oil. To overcome these problems of raw material pigments for cosmetics, such as poor dispersibility, high activity against skin, and collapse of makeup finishing by lipid from skin tissue, there exists a surface treatment using perfluoroalkyl phosphate (10). Fluoro-compounds are used for several kinds of fields to avoid water and oil. This treatment showed an excellent water and oil repellency and also a good dispersibility to organic and inorganic pigments, and an ability to depress the activity of ultrafine pigments. Other surface treatments can be carried out to give, for example, much more UV cut effect to pigments. Several types of organic low–molecular weight UV absorbers are commonly used, such as benzophenone and p-amino benzoic acid. It is also known that ultrafine titanium dioxides and zinc oxides absorb UV light, especially zinc oxides, which absorbs UVA and UVB both. However, it is recognized that titanium dioxide is not so stable against UV rays. To overcome the disadvantage of the photostability, new technology to coat titanium dioxide in high uniformity was explored, and a novel silica-coated titanium dioxide with broad spectrum protection against UV rays was developed (11). Other surface treatments with polymer materials were carried out to give much more UV cut effect to pigments (12). This allows the formulator to avoid the main disadvantage of physical sunscreens, namely, the visible whitening that occurs when titanium dioxide and zinc oxide are used in high concentrations to obtain a high SPF. These novel polymer surface treatments, such as organic polymers (teflon and silicones) or active ingredients (collagen, elastin, and vitamine E), give better properties of cosmetic makeups, and use of polymer or polymer matrix system are very safe compared with use of ultrafine titanium dioxides or zinc oxides.

Face Powders

Face powders provide coverage of complexion imperfections, oil control, a matte finish, and tactile smoothness to the skin. Powders give a good lasting effect to foundation makeup and possess oil-absorbing properties that are very useful for oily-skin types. Free powders are used to fix the foundation and compact powders to retouch one’s face during day. Face powder is more complicated and made by a mixture of products: Talc and serecite (to help to spread), chalk or kaolin (to give moisture-absorbing qualities), magnesium stearate (gives adherence), zinc oxide and titanium oxide (to help cover the skin thorougly), and pigments (for color). The use of mica in powder formulations improves skin feel, product application, and skin adhesion. The favourable effects are obtained with wet ground micas that have a particle size of <15 μm. Mica can also be modified by coating with inorganic or organic materials to produce another large group of fillers (spherical, special, and surface modified). Spherical fillers are widely used to improve skin feel. There are a variety of materials available, the organic types consisting mainly of polyamides, nylon spheres and inorganic types consisting mainly of silica, both as solid or hollow spheres. The improvement of skin feel is attributed to the ball bearing–like action of the spheres between other powder ingredients in the formulation and the spherical filler. When spherical materials are used, there is also an increase in the viscosity of the emulsion, allowing for a reduction of viscosity modifiers in the final formulation. Bismuth oxychloride (BiOCl), a fine white powder with a high bulk density, is well known as a pearlescent pigment, but it can also be used as a filler with no luster when in a particular crystal form. It has relatively low oil absorption characteristics and also gives rise to excellent compressibility when used in pressed powder formulations. Its hydrophobic character and good affinity with the skin also improves the skin adhesion and wear properties

of powder and makeup products. However, one disadvantage of BiOCl has been a low-light stability. Nowadays, BiOCl is much more stable to UV light. Special fillers are a group of fillers that are made up of several components, which combine their individual advantages when they are processed together into a composite material. Coated mica pigments, for example, are often found as light-diffusing pigments' agents in color cosmetics, where fine particle–size micas enhance the light diffusion properties of the material coated on the surface of the mica. Mica can also be coated with very small particles of metal oxides, allowing ease of incorporation into liquid formulations. Examples of coating materials for micas are titanium dioxide (e.g., low loster pigment), barium sulfate, and BiOCl, as well as organic compounds. For surface-modified fillers, most coating materials are organic polymers (collagen, elastin, and vitamine E). Powders also contain between 10% and 20% of organic texture agents (polymers) or mineral agents (boron nitride and silica), preservatives, anti-oxidants, and perfumes (neutral or more sophisticated) can also be added there. Table 2 collects some possible raw materials for foundations and powders.

Eye Makeup

Eye makeup consists of three major categories: mascaras, eyeshadows, and eyeliners. Mascaras thicken, sheathe, separate, and lengthen eyelashes to obtain an intense look. Eyeliners help draw a precise line at the base of eyelashes, and eyeshadows bring light to the look and highlight the color of the iris.

Mascaras

Among all the makeup products, mascara formula requires a particular development. The choice of mascara depends on the type of eyelashes (short or long, stiff or curved, poor or bushy, and fair or brown) and of the required effect (lengthened, curved, and\or thicker eyelashes). Liquid mascaras are the most popular modern formulation, and they can be divided into water-based, solvent-based and water/solvent hybrid varieties. Water-based mascaras are formulated from waxes (e.g., beeswax, carnauba wax, and synthetic wax), water, pigments, which are often iron oxides, and resins dissolved in water. The water evaporates readily, creating a fast-drying product, which thickens and darkness the eyelashes. Some water-based mascaras, very rich in wax (30%), are labelled waterproof or water resistant. To color eyelashes, inorganic pigments are the most commonly used because the vast majority of mascaras are black. Note that certain pigments, like cochineal carmine, may generate some problems such as the coloration of contact lenses. The formulae also contain antioxidizers to avoid the rancid smell of fatty substances and preservatives, which protect the eye from any risk of infection. Vitamins and hydrocarbon volatile or silicon solvents can also be used to improve the performance of the makeup. Concering the solvent-based mascaras, they are formulated with petroleum distillates to which pigments (e.g., iron dioxides, ultramarine blue, etc.) and waxes (candellila wax, ozokerite, and hydrogenated castor oil) are added, making them waterproof. If it is clear that the makeup effect depends on the formula, it is also important to consider the type of brush and the diameter of the aperture of the mascara tube. Indeed, it must automatically adjust the quantity of product on the brush to avoid loads on the eyelashes during application. The packaging also must be totally airtight to avoid the degradation and oxydation of the formulation. Thus, to obtain a good application on the eyelashes, it is necessary to develop a compromise between the mascara formula viscosity and the brush type. A rheological approach can be made through the rheological characterization in situ of mascara pastes with the brushes (13). This procedure used to quantify the take up of mascara brush in the container allows to visualize the influences of the shaft, the bristle length, and the hardness and pattern on the take up, therefore to characterize the product transfer.

Eyeshadows and Eyeliners

Eyeshadows are a cosmetic designed to impart color, primarily to the upper eyelid. The formulations of eyeshadows are identical to those of compact powders for the face, but the color range is wider. Application is helped by tiny particle size, flattering the eyelid by giving a smooth rather than crepey appearance. Eyeliners are mainly liquid formulations using ultrafine pigments.

Table 2 Possible Raw Materials for Foundations and Powders

	Pigments and mineral charges				
White	Colored	Unifying charges	Mineral charges light	Silicones	Active
- Titanium dioxide - Talcs - Pearl pigments (*mica, titanium dioxide–coated mica, bismuth oxychloride*)	- Iron oxides (*Yellow, red, black*) - Ultramarine blue - Colored pigments and *pearls* (*mica* + *iron oxides, mica-titanium dioxides* + *iron oxides*)	- Nylon (*orgasol*) - Polymethylmetacrylates - Silica - Boron nitride	- Soft focus (*light diffusing*) - Photochromic - Light correcting (*auto-adaptative*)	- Dimethicone - Volatile cyclomethicone - Silicone gum blends Emulsifying - Cyclomethicone and dimethicone copolyol	- Vitamin C, A, and E. - UV filters - Enzymes - Phospholipids - Moisturizing agents (*Glycerin*)

INTERACTIONS BETWEEN SKIN AND MAKEUP PRODUCTS

Physical appearance, and more particularly physical attractiveness, is one of the most important determinants of interpersonal attraction in the early stages of many relationships (14,15), and it is obvious that the face is the part of the human body, which attracts the most attention (16,17). Thanks to makeup products, it is quite possible to bring out certain characteristics of the face or, on the contrary, to lighten them to achieve a degree of attraction for all types of face (18–20). In all cases, the perception of a makeup is an interaction between the light, the makeup product or raw-materials, which compose it, and the human skin. Two main types of interaction can be observed: visual interactions, where the optical way of light is modified by the optical properties of cosmetic ingredients and the relief of the skin surface and biophysical interactions, where the finished makeup is perturbed by the biochemical and mechanical functions of the skin surface. Concerning the visual interactions, they are mainly generated by pigments with specific effects (21): perlescents, iridescent effects (color changes according to the angle of observation), thermochromic (color changes according to the temperature), photoadaptive (preserve a radiant complexion following the lighting conditions), or soft focus effects (22–24). These soft focus pigments, mainly composed of polymers, micas, and talcs covered with rough or spherical particles of small diameters, such as silica or titanium dioxide, are used to optically reduce the appearance of wrinkles. These effects are obtained by optimizing outlines of wrinkles and reducing the difference of brightness due to diffuse reflection. Concerning the biophysical interactions, it is known that behavior of a makeup product can be modified by the biochemical and biophysical properties of the skin. For example, a woman's skin type and her facial movements throughout the day actually remove the foundation from the face. Foundation tends to shift and wear off during the day and migrate into the fine lines of the face. The outside environment such as air pollution, smoke, sun, and stress that we feel in the current world are also factors, which can disrupt the normal balance of the skin (e.g., skin discoloration or extreme dehydration) and therefore modify the behavior of makeup products. The perceptions of a makeup can create other interactions based on the relation existing between the pleasure felt after application of a makeup product and psychological and social parameters (25). These interactions are difficult to define because they often include physiological and psychological attributes. Indeed, it is difficult to quantify precisely how much pleasure a person derives from using a makeup. Overall, makeup acts and stimulates three of our senses: the touch (which encompasses all sensations from the body surface), the smell (fragrance), and the sight (the process of becoming and looking beautiful). The positive stimulation of these senses by makeup can induce sensory as well as psychological pleasure. Indeed, in a previous study whose subject was to explain the reasons that motivate women to make up, we observed clearly a high implication of psychological traits in the makeup functions (26,27). This positive stimulation can also contribute to a healthy mind and body through enhanced functioning of the body's immune system (28). An excellent article by Lévêque (29) on the relationship between appearance and health underlines the effects of makeup product on the mental health of a women who is badly affected by a serious illness or who is still in the process of creating and presenting her own image and personality. With facial makeup, women become more confident, physically attractive, enhance their well-being, and act more positively toward the external world. The implications of facial attractiveness are equally important across all of the stages of life, associated with greater or lesser sensitivities to the social interactions. For example, with changes in age, the psychological effects change (30). The young woman will use makeup to capture the eye of others and is perceived as having a more favorable personality, whereas a mature woman will use makeup rather to enhance her feelings of self-satisfaction. Beyond the simple application of colorful products on the face, makeup appears as a holistic technique that modifies not only one's appearance but also helps to cope with one's self-image, emotions, and mood.

QUANTITATIVE ASSESSMENT OF PROPERTIES OF MAKEUP PRODUCTS

Over the past few years, cosmetic research has enabled the makeup product to become a high-technology product, claiming some similar cosmetic properties to those of skin care products (hydration, protection against free radicals, etc.) as well as new physical properties (e.g., long

lasting or no transfer effects). Although qualitative evaluation (mainly obtained through consumers' perception and experts' evaluation) is commonly used and remains indispensable to prove the effects of makeup products (e.g., color, comfort, or lasting effect), it is more and more necessary to associate it with instrumental data. Some may be obtained through devices already used for the evaluation of skin care products, such as the Corneometer®, which can demonstrate a moisturizing effect (31). However, the most representative effects are those, which are associated with a visual effect, which is the main property of a makeup product. Video imaging appears as a good method to prove the efficacy of makeup products. Indeed, thanks to high-resolution video cameras and sophisticated image analysis sofwares, we can visualize what the consumer perceives of the product (concerning either the whole face or smaller areas) and at the same time to associate some quantitative information. However, video imaging has its limits, and in this case, biophysical methods can be very useful to quantify certain properties.

Lip Makeup

Quantification of the Color, Brightness, and Streakiness

The color parameters can be achieved either by reflectance spectrophotometry of the optical spectrum of visible light reflected (Spectrophometer CM-508i®, CM-2002®, Konica Minolta Business Solutions Europe GmbH, Germany) or by reflectance tristimulus colorimetry (Minolta Chromameter CR-200®, CR-300®, Minolta, Osaka, Japan) following the CIE (Commission Internationale de l'Eclairage) recommendations (32). These methods give quantitative information on the color but are limited to a small analysis surface, from 7 mm^2 to a few square centimeters according to the device used. And these techniques require direct contact between the made-up skin surface and the device, which generates shrinking at every measurement and therefore modified the aspect and the long-term behavior of the product. To avoid this phenomenon, color can be evaluated by image analysis. Thanks to this technique, it is possible to study skin areas of various sizes, take measurements without any contact with the skin, and analyze the image pixel by pixel (a word invented from "picture element," which is the basic unit of programmable color), providing quantitative information according to localization. In the case of lipstick, the global color of the made-up area and the shades most represented in the global color can be quantified. Thanks to the development of specific data-processing programs (33). For example, a segmentation method by maximization of the entropy and an algorithm based on the principle of the probabilistic segmentation were used to separate the color information sent back by the skin to the makeup area. It is also possible to evaluate the streakiness, which represents the amount of lipstick in the small lines surrounding the lips, and the brightness (Fig. 1). Concerning the streakiness, the principle consists in extracting the surface of the lipstick that has migrated (lips + streakiness), smooth out the outline, and calculate the difference between that and the surface of the unsmoothed lips. All that remains is to divide the calculated surface by the perimeter of the smoothed lips to obtain a streakiness index, independent of the morphology. The brightness assessment is more complex. Indeed, the bright zones are not necessarily the part of the image where the pixels get closer to white. A darker lipstick having the same brightness surface in pixels as a clear lipstick will be perceived by the human eye as more glossy. Then, the brightness assessment is realized by calculating the difference of contrast between two adjacent pixels. From these different parameters (color, brightness, and streakiness), the long-lasting properties of a lipstick can be evaluated.

Quantification of the Moisturizing Effect

The hydration state of the skin surface is commonly evaluated by an indirect method based on the measurement of electrical conductivity (34). Various experimental instruments are commercially available such as the Corneometer (CM-820, CM-825, Courage and Khazaka, Germany) based on the measurement of the capacitance and the Nova Derm Phase Meter DPM 9003 (Nova® Technology Corporation, Portsmouth, New Hampshire, U.S.) based on the meaurement of skin impedance. Thanks to this small diameter probe (5 mm), the Nova DPM 9003 is perfectly adapted to measure the electrical properties of lip surfaces (35). However, this device requires a sufficient hydration level of lips to show differences after application of moisturizing lipstick. In all cases, the measurement protocol used to quantify the hydration

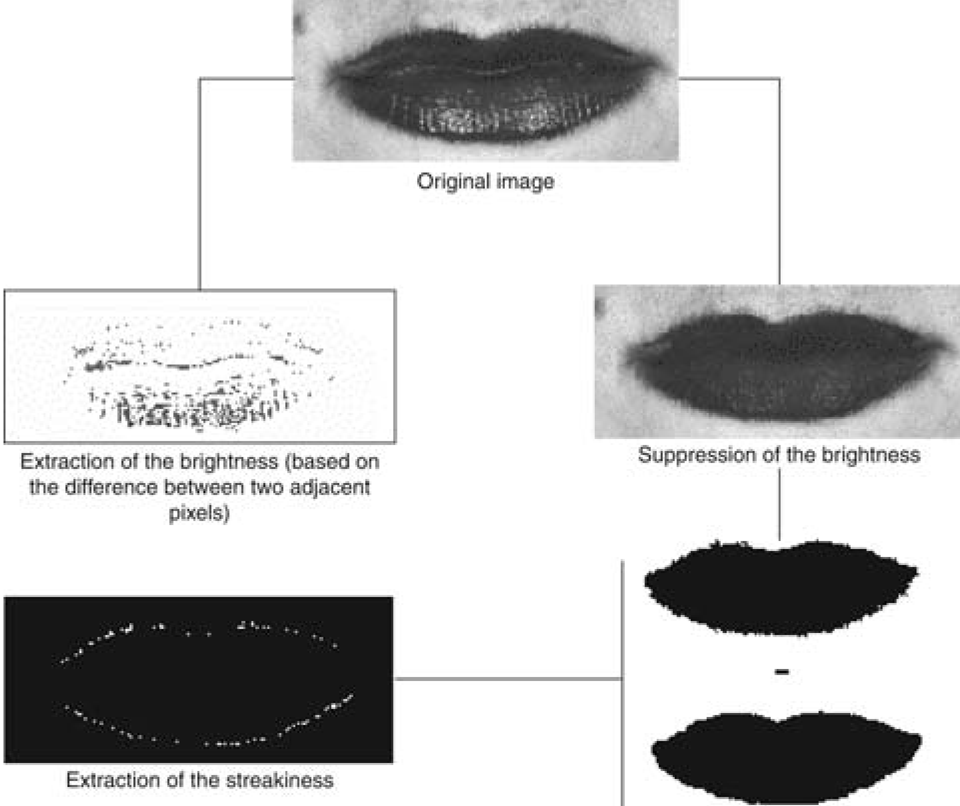

Figure 1 Extraction of the brightness and streakiness.

state of the lips is complex, especially for long-term studies and comparisons between lipsticks. Indeed, one week of "wash out" is necessary to obtain a constant physiological state of lips, and only one lipstick can be studied at the same time on the same volunteer. To screen many lipstick formulae, an original method consists in applying lipstick on the forearm zone after delipidation of the skin surface with an acetone/ether (1:1) mixture during 30 minutes (36). Once the lipstick is applied (the same quantity, which is applied on lips), it stays in contact with the skin for one day. Then, the hydration of the skin surface is measured with a Corneometer the next day after a standardized wash. The results after one week showed that a lipstick containing a glycerin/water mixture significantly increases the hydration of the skin compared with the same base of lipstick without glycerin. Note that before testing the efficacy of a moisturizing lipstick, it is recommended to check the physicochemical properties of the pigments and raw materials contained in the formulation, and more particularly those properties, which possess an electrical conductivity.

Eye Makeup

Evaluation of the Curving and Lengthening Power of a Mascara

The image analysis can also be used to evaluate the curving power of a mascara. The principle consists in visualizing the profile of the eyelashes by a video camera placed perpendicularly to the eye and calculating from the image obtained the curving power with regard to two axes situated at 90° from each other. The 0° to 180° axis corresponds to the eyelid and the 90° to 270° axis to the ciliary edge. The angles created by the highest and farthest eyelash from the eyelid are measured, and the average angle calculated. The curving power of mascara is then determined by calculating the difference between the two average angles: angle 2 (after makeup) – angle 1 (before makeup) (Fig. 2). The quantification of the lengthening power is also possible by measuring the length (in pixels) of the farthest eyelash before and after application of the mascara.

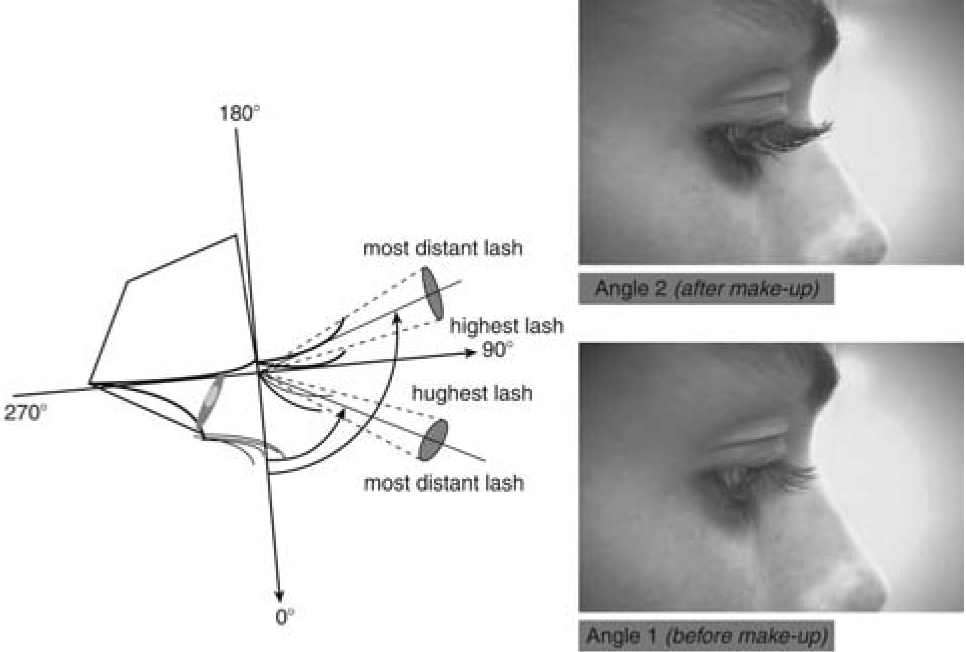

Figure 2 Measurement of the curving power of a mascara.

Face Makeup

Certain makeup products such as foundations and tinted day creams (which are a colored extension of skin care products) contain some raw materials in their formulation that we usually find in skin care products. Although their efficacy on the skin has already been demonstrated by several noninvasive methods, such as the measurement of epidermal capacitance, transepidermal water loss, biomechanical properties, UV protection, or free radical scavengers, their incorporation in makeup formulations can generate several problems of compatibility with pigments and some difficulties in the efficacy assessment. The best known and most difficult claims to quantify are those in relation to the lasting properties (color, degradation of the makeup surface film, migration of pigments in fine wrinkles, and modification of the shade), the intransferability on surfaces like the skin or clothes, and skin radiance.

Quantification of the No Transfer Properties of a Foundation

Foundation is considered no transfer only if it is insensitive to two types of constraints: a transfer by contact, called a "static transfer," and a transfer by friction, called a "dynamic transfer." To reproduce the effect observed by the consumers (e.g., transfer on the collar of blouses or pullovers), a device to simulate these two types of transfer has been developed in our laboratory. It is composed of a probe used to fix the chosen support and to control the contact pressure between the support and made-up surface, and a motorized arm allowing it to move laterally, over a known distance and at a constant speed. Once the transfer is made, the foundation print is digitalized by a color video camera. The image is then treated to extract and count the pixels associated with the colored print (Fig. 3). A foundation will be considered no transfer if no pixels are detected. For very short-term effects (e.g., 5 minutes after makeup), the no transfer properties of a foundation can be evaluated on the forearm. For long-term no transfer effects, it is better to carry out the study on the forehead because the sebaceous glands' activity can modify the behavior of the makeup in time.

Quantification of the Long-Lasting Properties of a Foundation

The appearance of the made-up skin is dependent on many optical and physiological properties of the skin. The appearance is mainly determined by the factors of color and texture. Color perception is associated with light and the way it is reflected. The texture depends on the

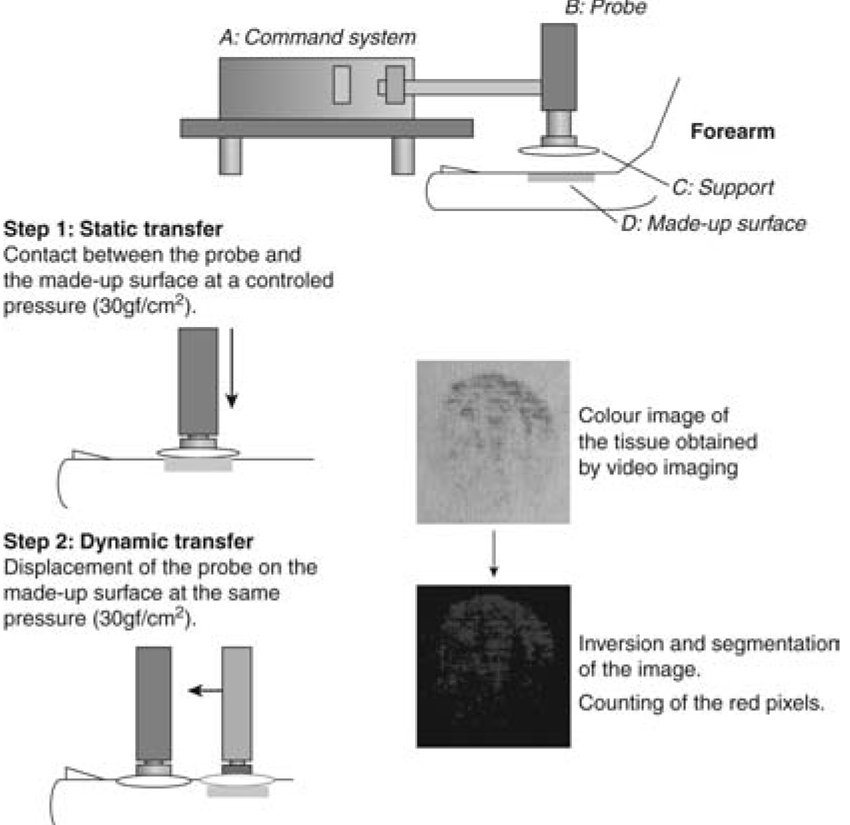

Figure 3 System allowing to simulate a static and dynamic transfer. Image analysis and extraction of pixels containing the transferred pigments.

brightness and the relief, respectively, attributable to light diffusion on the skin surface and the distribution of foundation layers. A foundation will be considered long lasting if the made-up skin preserves these properties in time. Whereas the color is the most studied and explored field, few studies have been carried out on the behavior of the skin texture in time after makeup (37–39). To follow the behavior of a makeup film, a noninvasive method based on the determination of changes in the intensity of dermis autofluorescence excited by UV irradiation (40,41) can be used. The instrumental setup for fluorescence measurements consists of a commercially available luminescence spectrometer (LS-50B Fluorescence Spectrometer®, Perkin Elmer, U.K.) equipped with flexible fiber optics surrounded by a metallic cylinder of about 1.5 cm in diameter for in vivo measurements. Skin fluorescence is induced by a radiation at 325 nm (UV light in UVB range). The excitation light was transmitted perpendicularly to the skin area and the emitted light was collected and transported at the emission slit. The emission spectra of the skin site (e.g., the forehead) present a peak at 390 nm and additional shoulder at 440 nm (42). These two wavelengths are associated with the fluorophores of the skin, such as collagen and elastin, both major compounds of human dermis, and depend on the skin's chromophores, which absorb UV radiation. The application of a makeup product creates a physical and chemical barrier between the excitation light and the skin, which decreases the skin's autofluorescence response (Fig. 4). The makeup film created by foundation layers will be considered long lasting if the emission spectrum carried out just after makeup is similar to the following ones, considering that raw materials used in formulations do not absorb UV radiation. The long-lasting effect can be studied on the first emission peak ($\lambda = 390$ nm) as well as on the second one ($\lambda = 440$ nm). The other factor, which defines the appearance of the made-up skin texture is the brightness. In relation to the degree of brightness, the skin may appear bright and healthy or dull and unhealthy. Brightness can be brought by the foundation itself or by the sebaceous gland's activity, which gives the skin an oily appearance (43). There exist a

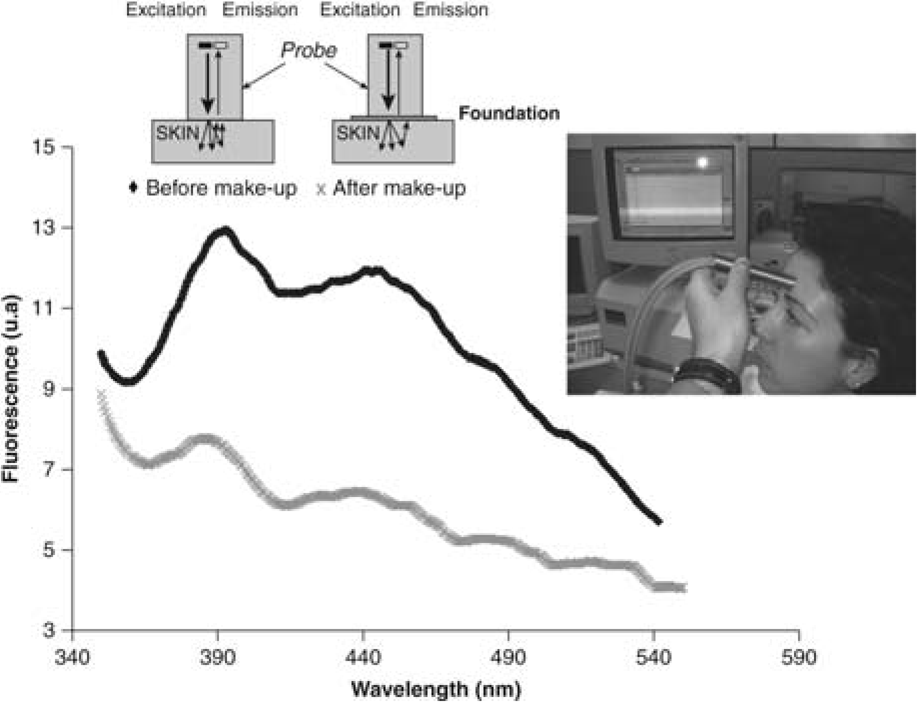

Figure 4 Measurement of the skin's autofluorescence before and after makeup. Study on forehead.

variety of established methods to estimate the oily skin (44,45). For measuring brightness, methods are mainly based on the evaluation of the intensity reflected in the specular direction, when the surface to be analyzed is illuminated by a beam of light with a fixed angle of incidence. However, these methods are not easy to use on the skin surface because they require an analysis surface as flat as possible. To avoid these constraints, brightness assessment can be performed using the same instrumental setup for in vivo fluorescence measurements. To record only reflection and eliminate fluorescence of different wavelength, the fluorescence spectrometer (LS-50B) is used and operated in the so-called synchronous mode, i.e., both monochromators (excitation and emission) are moved synchronously through the whole spectrum (250–700 nm) with a wavelength difference of zero ($\Delta\lambda = 0$ nm). Certain chromophores of the skin (melanin, etc.) and the color of makeup can influence the visible reflection spectra of human skin. For this reason, the spectral differences observed before and after makeup will be preferentially studied and compared in the UV range (250–400 nm), where the wavelength giving the maximum of difference will be extracted. As for the study of the makeup film long lasting, the matte effect will be considered long lasting if the reflectance value, recorder just after makeup, is similar to the following ones.

Quantification of Skin Radiance

There is no universal definition of the skin radiance. Each individual perceives it in a different way because it often includes physiological and psychological attributes. If there are differences in the perception of the skin radiance, dermatologists and experts in cosmetology consider that the complexion must reflect the general health of each human. The main factors that affect the skin complexion are the skin color, surface texture, luminosity, and mainly microcirculation, which gives the skin a rosy appearance. The skin radiance being a global phenomenon, it is very important to study the interaction between all these visual effects before any quantitative and qualitative interpretation. Although the qualitative evaluation (besides the consumers' perception obtained by questionnaire and experts' evaluation) (46) is commonly used and remains indispensable to prove the effects of cosmetic products used to improve the skin radiance, we are searching for more instrumental data. To approach the skin radiance in a scientific way, there are very few instrumental techniques. Color and

microcirculation are the most studied and explored fields, but these methods only give quantitative information limited to a small analysis surface according to the device used. To obtain what is closer to the consumers' perception, the existing conditions of the skin radiance can be performed via pluridisciplinary approaches combining digital image analysis, consumers' perception, and experts' evaluation. From a round table meeting, several items linked to skin radiance but also taking into account emotional, psychological, and behavioral factors are defined. Then, a self-assessment questionnaire built according to the information obtained is proposed to experts to evaluate the skin radiance perceived. In parallel, facial images are carried out using a video imaging, and algorithms are required to extract skin parameters such as the brightness, the grain of the skin, skin color, and the homogeneity of the color. The comparison between image analysis and expertise allows evaluation of the degree to which each of the independent parameters contributes to the skin radiance perception, and suggests a mathematical model to quantify the global radiance phenomenon (47).

Nail Makeup

Few studies have been carried out on the behavior of nail enamel. The brightness of nail enamel can be quantified by the luminescence spectrometer (LS-50B) for in vivo measurements used in synchronous mode. However, the irregular shape of the nail requires that the diameter of the optical probe be reduced (3 mm) to adapt to the surface of the nail.

CONCLUSION

The heightened technology of makeup products (pigments, formulations, etc.), the sensory interactions based on the skin-brain connection, and more stronger claims are accompanied by the development of specific evaluation methods used to quantify these improvements and to prove the claimed efficacy. In cosmetology, the quantitative assessment of the visual effects of a makeup product becomes more and more complementary to a qualitative evaluation (questionnaires according to consumers' perception and expert evaluation). Certain quantitative studies are restricted to a small surface of measurement and require a direct contact with the skin. Video imaging appears as a method of the future to prove the efficacy of makeup products. Today, this technology allows us to study precisely the color, brightness, or streakiness of a lipstick, but also some physical properties, such as the curving and lengthening power of mascara or the no transfer properties of a foundation or a lipstick. Technological progress, combined with video imaging and data processing, allows us to imagine, in a near future, the appearance of new and more sophisticated visualization tools that will permit us to study some visual effects, which are still difficult to quantify such as certain optical effects (soft focus, photochromic effects, etc.), interactions between the product when you make up your face (distribution and migration of the pigments during the makeup process, etc.), or again, emotions generated during the makeup process. Note that biophysical methods can also be very useful when they are used differently from their main function.

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38 Skin Radiance Measurement

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INTRODUCTION

The term "complexion" has always attracted attention; it had to be white from the antiquity to contemporary times, and then tanned from the 1920s. Nevertheless, we are at the beginning of a new era, where a tanned complexion is no longer fashionable; a young homogeneous complexion without imperfection is now the preferred option. Today, the essential preoccupation is not so much the color of the complexion but its texture, which should evoke youth, health, and happiness (1).

The term "radiance" refers to shine and light. A vivid red is a red that shines. Radiance also defines what arouses admiration, attention, glitter, and magnificence.

In fact, the radiance/complexion is the mirror of general health, both physical and psychological. A gray skin, a "blotchy complexion" may reflect bad looks because of great fatigue, weariness, hormonal status, emotional states (sadness, stress, etc.), illness (dysfunction of vital organs such as the liver etc.), nutrition deficiency or excess, tobacco, alcohol, pollution, seasons (2–6). Inversely "peach-colored skin" reflects a smooth, pink and velvety skin, and good health. The complexion plays essential psychological and social roles (1).

Skin radiance is referred to without any precise definition and quantifiable appreciation data. All the parameters that constitute it and their relative proportions are difficult to list. The skin radiance seems to be a balanced mixture of color, itself mostly influenced by the skin microcirculation, of light reflection, and more globally of texture of the skin surface (1). Its study therefore implies the quantification of each of these components.

METHODS

When the light meets the skin surface, a small quantity [about 5% (7)] is reflected directly while the major part penetrates the different layers of the skin. The specular light gives its luminous aspect whereas the back scattered light (Fig. 1) gives the complexion (peachy-color). In other words, specular reflection (depending on the nature and state of the surface of the skin) explains the glow, absorption phenomena (depending on the skin's chromophore content) explain its hue, whereas diffusion phenomena (depending mostly on the collagen content) define its saturation.

The skin color depends mainly on the melanin as well as the hemoglobin concentrations and distributions. The melanin (brown) absorbs all wavelengths, but this absorption decreases considerably from purple to red, which makes the melanin look like a mixture of gray (global absorption) and yellow (significant absorption of blue). Hemoglobin looks red because it absorbs selectively the green and therefore retro-diffuses a light where this complementary color is missing.

The hemoglobin plays an important part in the skin radiance, which is usually perceived as "pink" skin. An indirect way to assess the radiance is consequently to study the skin microcirculation.

Assessment of Skin Microcirculation

The hemoglobin transports the oxygen inside the red globules: if there is not enough oxygen, the face will be gray and dull. If the microcirculation is effectively stimulated, the light will more easily reflect off the red blood cells at the source of the skin color.

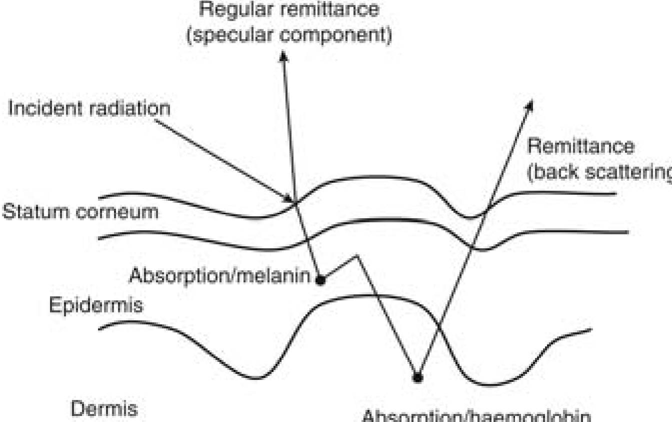


Figure 1 Optical pathways in the skin (the layer is so thin in the stratum corneum and the epidermis that its contribution to remittance other than specular is minimal over the entire visible and near infrared regions). *Source*: From Ref. 8.

The skin microcirculation is therefore an important factor of the dull complexion:

- Under stress, catecholamines are delivered in the skin, inducing vasoconstriction (and thus skin pallor) (2,9,10),
- With tiredness (lack of sleep and/or intensive activity), the body reacts by sending more blood to vital organs, and the cutaneous microcirculation is "sluggish" (6).
- With pollution and smoking, the microvessels lose their colors (grayish aspect of the skin) (4–6,11,12).
- With aging, papillar capillary loops disappear, inducing a loss in dermal nutritional vessel density and surface area for exchange (13).

Among the existing available techniques, capillaroscopy and videocapillaroscopy allow direct visualization of the capillary network in vivo. After depositing a drop of oil to enhance skin transparency, an optical magnifying system is used to visualize its vascular network. Different levels of magnification can be used to assess the surface of the microvasculature structure as well as its color (Fig. 2) (14,15).

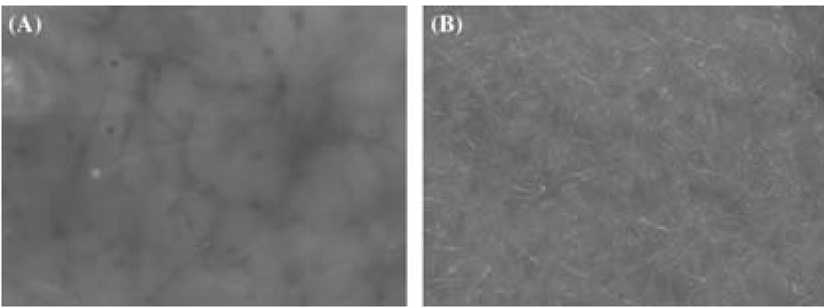


Figure 2 (**A**) ($\approx$7 mm^2) A magnification of 100$\times$ allows to quantify the vascularization network (arrows show the capillary loops and the vessels) of the skin, whereas (**B**) ($\approx$28 mm^2) a magnification of 50$\times$ allows to quantify the skin redness.

Colorimeters

The most logical way to assess skin complexion is to study its color directly. Taylor et al. described in 2006 the different techniques available (16). Among them, reflectance spectroscopy, chromameters, and narrowband reflectometers should be the most frequently used. The latter are dedicated to the assessment of erythema or pigmentation (by measuring more specifically the skin chromophores) (17,18).

The color variations of the skin surface can be measured by reflectance spectroscopy, spectrocolorimeter (CM2600D, Konica Minolta Sensing) or chromameter (CR400, Konica Minolta Sensing). These devices have sensitivities corresponding to those of the human eyes, but the measurements are performed in standardized conditions using the same light source.

The Commission Internationale de l'Eclairage (CIE) has defined the spectral characteristics of several types of typical illuminants (the D65 corresponds to average daylight and is usually used as the reference) (19).

The results are expressed in the standard L*a*b* system [CIE LAB 1976 (20)]. The L* in this space corresponds to the luminance axis (0 for the black and 100 for the white) characterizing the separation between bright and dark; a* and b* form the other two color orthogonal axes, with a* evolving from green to red (usually selected for the study of redness) and b* from blue to yellow (study of pigmentation).

The skin radiance can therefore be described by a*, which corresponds to the redness and L*, which defines the luminosity. However, additional information can be extracted, in order to qualify the skin color heterogeneity [color difference metric DeltaE (21)] and vividness [chroma = $([a^*]^2 + [b^*]^2)^{1/2}$) (19)], which are key factors in the problematics of dull complexion.

To conclude, videocapillaroscopy and colorimeters assess color hue, saturation, and heterogeneity directly or indirectly. They can be usefully combined with standardized photography, which allows to illustrate and even quantify these factors.

Photography

Once calibrated and standardized, photography offers many applications to study skin radiance/complexion. It appears more appropriate to study texture heterogeneity than glowing aspect because of the influence of the light. The image can be easily disturbed by brightness induced by the flashlight or by the environment. The nature as well as the positioning of the light source and the posture of the subject are of major importance and must be taken into consideration to avoid visual artifacts (Fig. 3).

Polarized light photography provides more information than usual in terms of heterogeneity of structure. Its principle consists in differentiating the regular reflectance (glare) from the light "back-scattered" (complexion) from within the skin. The regular reflectance contains information related to the surface relief/shininess and the other one to the color. A filter is placed in front of the camera and another one in front of the flashlight. When the orientation of the filters is perpendicular (cross polarization), the regular reflectance is blocked and only the back-scattered light from the tissues reaches the lens [revealing the complexion (skin redness or paleness, color, heterogeneity, etc.)] (Fig. 4), whereas when they are parallel (parallel polarization), only the reflected polarized light passes through the lens filter, and details of the surface are enhanced (wrinkles, shininess, scaling) (12,21–23).

Figure 3 Photography taken in an integration sphere, with a homogenous and symmetrical light.

Assessment of Skin Relief

Radiant skin is usually considered as smooth, homogeneous, without imperfections. Although innate, the microtopography of the skin is affected by the environment as well as aging, and undergoes changes in quantity, depth, and direction (24,25).

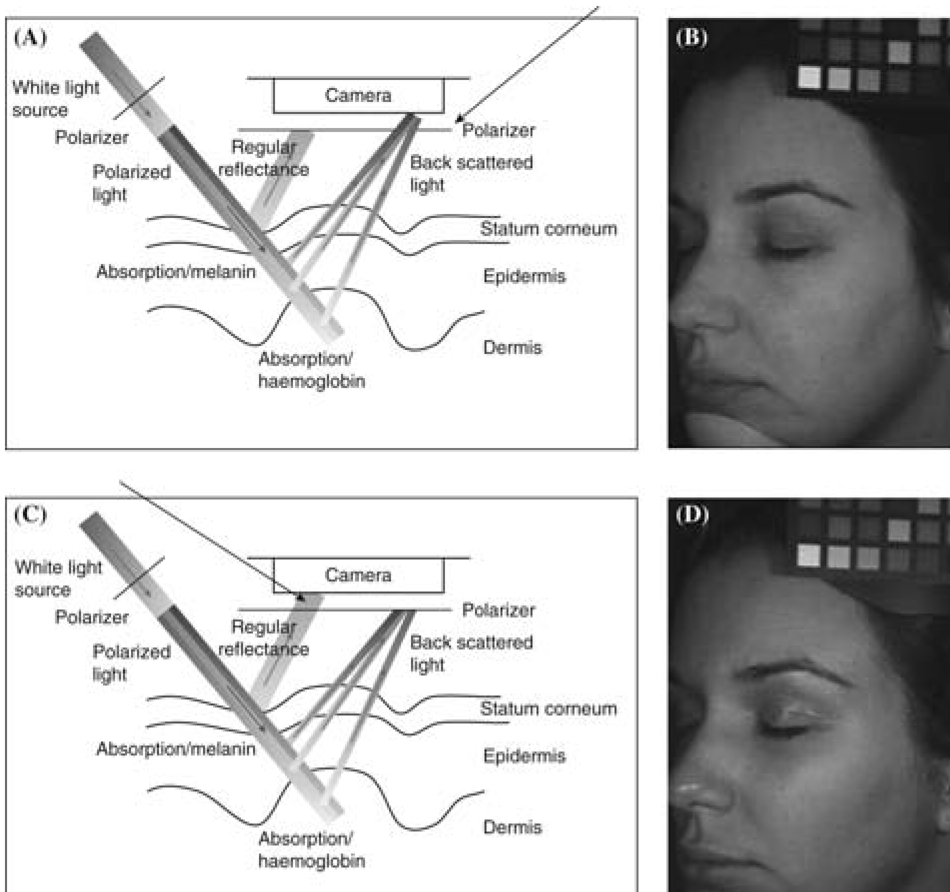
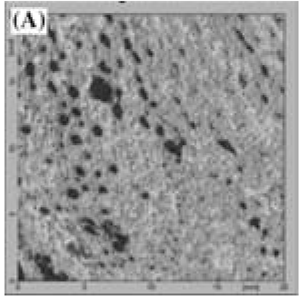


Figure 4 Simplified schema of the principle of polarized light photography. (**A**) With cross-polarization, only the back-scattered light reached the lens [revealing the complexion (photograph **B**)]. (**C**) With parallel polarization, only the reflected polarized lights pass [revealing the shininess and relief of the skin (photograph **D**)].

Profilometry (mechanical, transparency, or optical), initially developed for microtechnics, has been applied for many years to study the skin relief (26–29). From 2000, devices using fringe projections specifically dedicated to the skin have been created (DermaTop, Eotech, France and Primos, GFM, Germany). Interestingly, this in vivo technique allows to study the skin imperfections via the assessment of the volume and depth of the wrinkles and in particular the roughness and heterogeneity of the microrelief (dilated pores, comedones, fine wrinkles, etc.) (Fig. 5).



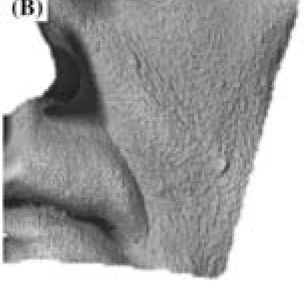


Figure 5 3-D reconstructions obtained (**A**) from the cheek (20 × 20 mm area), allowing to detect dilated pores, or (**B**) from hemi-face (60 × 80 mm area), allowing to quantify the roughness/heterogeneity of its relief.

Assessment of the Skin Ability to Reflect the Light

The relief of the skin produces different image contrasts depending on the brightness level of the surface (the same relief appears more easily in the case of a bright surface). When the skin surface is irregular, it absorbs light with difficulty and reflects it in many directions. This is particularly typical of dry and scaly skin. The assessment of the specular component provides direct information on the glare of the skin. Sandoz et al. have underlined the influence of the surface bidirectional reflection distribution function (BRDF) measurements for the visibility of the skin surface (30,31). The light intensity directed toward the observer's eye is proportional to the coefficient of reflection of the surface. The behavior of bright and matt surfaces is different since the BRDF is different for the direction of observation (Fig. 6).

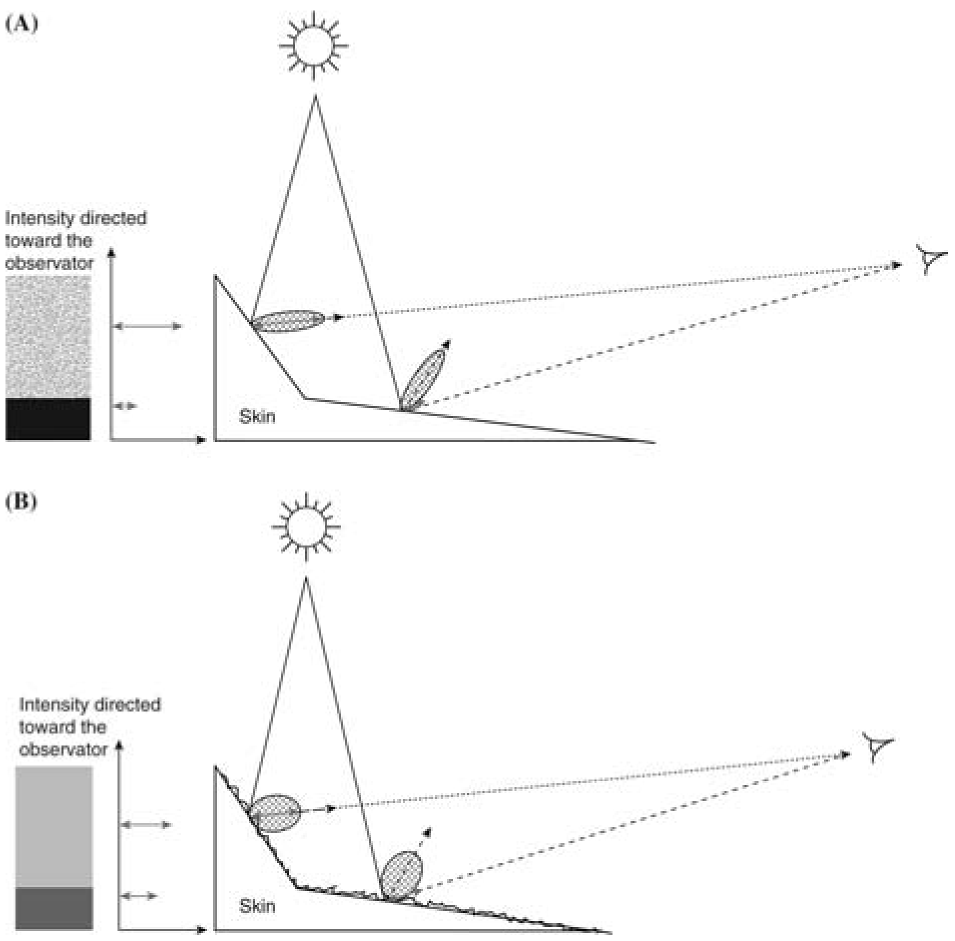


Figure 6 Simplified schema of the visibility of the skin relief: the contrast is given by the amount of light reflected in the direction of the observer. The perception of a wrinkle will be modified by the quality of the skin surface. If the skin is bright (**A**), the wrinkle will be more contrasted and more visible. If the surface is matt (**B**) it will be more blurred. ⬭, wider reflection cone ↑, specular component of the light ↔ intensity of the light perceived = simulation of the contrast of the skin perceived by the observer.

From this principle, a specific device has been developed in Besançon (31,32). Its aim is to assess the quantity of light reflected that depends on the brightness (Fig. 7). It consists of a system, which illuminates an area with white light through an objective at one end of an optic fiber. The beam is directed to the subject at a 25° angle. The light reflected is measured by inputting a profile of intensity of light for various angles values. It is generally admitted that radiant skin tends to act as a mirror, i.e., to reflect rays in a specular manner (with the same 25° angle) and dull skin tends to diffuse light more. This instrument has shown its good sensitivity and specificity to characterize the dull to glowing radiance of the skin (32). This technique allows to access to the breadth of the gaussian curve, defining the diffusion of light rays, as well as its maximal intensity, defining the skin brightness (reflection).

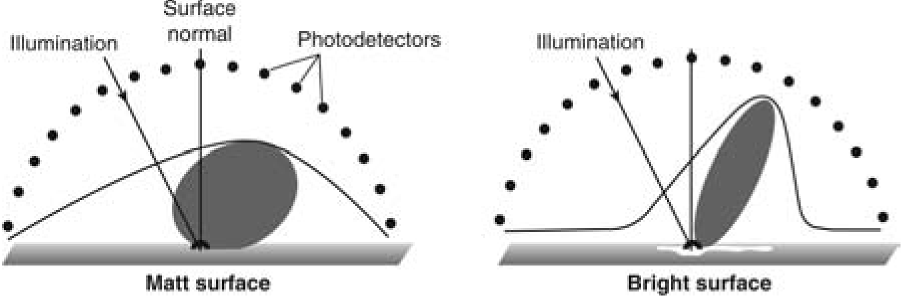


Figure 7 Principle of the instrument developed for BRDF measurement on the skin. Photodetectors distributed in the place of incidence sample the angular distribution of the reflected light. BRDF of a matt surface distributes the reflected light in a wider cone of diffusion (dull complexion). A bright surface concentrates the reflected light in a narrow cone of reflection (glowing complexion).

Gillon et al. have also developed a specific contactless device (brillanometry) (33). Its principle consists in directing a polarized light beam perpendicularly to the skin surface and to assess the reflected beam in the same direction.

The application of systems of polarization of the light currently used in photographs has given the opportunity to Bossa Nova Technologies to develop a system called SAMBA® a few years ago. It evaluates the gloss by assessing the scattering properties of the skin or the hair (34).

Finally, no instrument can quantify today the skin radiance globally, but there are devices that allow to assess its different components.

With contrast to the color, the glow remains difficult to quantify. The development of simulation systems would perhaps be one solution (35). Many terms are usually employed to characterize the skin radiance: shininess, brightness, glow, glare, gloss, etc. Shininess, brightness, and gloss refer to the reflection of light to something, and are more frequently used to characterize seborrheic skin or to assess the effect of make-up on lips. Glare seems to evoke a shine with a dazzling light, whereas the glow associates an intense color with a slight shine. The glowing aspect of the skin has, thus, to be differentiated from its brightness, which is the characteristic of greasy skin and is unaesthetic. If gloss is what women expect for their hair (34), glow is a better representation of radiant skin. All these notions show a real need to define the skin radiance more accurately.

Clinical Scoring

In 2006, Baret et al. organized a round table meeting with women (23). As a result of these exchanges, many items (including the skin grain, brightness, and color) were selected and studied in volunteers of different age groups from image analysis of video data of their face. Experts performed clinical scoring and then divided the volunteers in two groups: the young one, whose radiance was considered more dependent on skin luminosity and color (rosy color) and the old one, who was mainly characterized by irregular skin surface aspects and color variations.

These definitions corroborate the idea that radiance is multifactorial and naturally include an important subjective part.

As previously mentioned for photography, it is of great importance to perform the scoring in controlled conditions. The subjects have to be seated between "daylight" lamps and to wear white clothes as well as a white paper cap to avoid any influence from extrinsic colors, and to stay at rest in a temperature-controlled room (36).

In 2004, Musnier et al. organized a brainstorming session with several beauticians to develop a model of sensory evaluation of the skin radiance (36). One hundred volunteers were also interviewed to self-appraise their complexion. The synthesis of the data allowed to determine four skin-coloring descriptors and to construct a model "C.L.B.T.," on the basis of the visual perception of skin color (C), luminosity (L), brightness (B), and transparency (T).

CONCLUSION

Facing the increasing importance of appearance, consumers wish to reflect the image of their inner well-being represented mainly by a beautiful skin and healthy looks (34). The cosmetic industry has perfectly understood this concept and developed specific products designed to enhance the complexion, illuminate the skin, or fight off the detrimental effects of the pollution.

Several options are available to improve dull complexion. Most of the products currently on the market claim an action on the skin texture. They act either by optical effects with the help of pigments, or by a vitamin contribution to the antioxidant properties, or with exfoliating active principles that attenuate the imperfections, smooth the skin, making the complexion more glowing. Others claim an action on the microcirculation. The products often associate several of these effects.

All these data confirm that the assessment of skin radiance implies the study of the geometry of the light reflection as well as the interaction between the light (interaction with surface/glow) and the color (interaction within the skin/complexion). However, the skin relief seems to be therefore one of the key factors of the skin radiance (1).

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39 Tribological Studies on Skin: Measurement of the Coefficient of Friction

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INTRODUCTION

Because the skin is a surface, it is convenient to analyze and describe it in terms of its surface characteristics such as friction. Tribological studies of skin provide valuable insight into how the skin interacts with other surfaces and how it can change under various conditions, like age and health, and chemical treatments, such as lotions and moisturizers (1). Because the skin is the first-line of defense against the environment, it is repeatedly subjected to physical and chemical damage and alterations. For example, Naylor (2) showed that the moistened skin has an elevated friction response, and El-Shimi (3) demonstrated that the drier skin has a lowered friction response. Tribological studies offer convenient, noninvasive techniques to quantitatively assess skin health and hydration.

The tribological parameter generally measured is the coefficient of friction by bringing a surface into contact with the skin and moving it relative to the surface. When the two surfaces are brought in contact, the perpendicular force is defined as the normal force (N). The friction force (F_f) is the force that opposes the relative movement between the two surfaces. According to Amontons' law, the coefficient of friction (μ) is defined as the ratio of the friction force to the normal force:

$$\mu = \frac{F_f}{N}$$

Friction can be described in two different manners: the static friction coefficient (μ_s) and the dynamic or kinetic friction coefficient (μ_k). The static friction coefficient refers to the friction that builds up just before the initiation of relative movement; the dynamic or kinetic friction coefficient refers to the friction that exists as the two surfaces are moving relative to each other. Although most of the skin research has focused on the dynamic friction coefficients, with the two surfaces moving at a relative constant velocity, some work has focused on static friction coefficients.

According to Amontons' law, the dynamic friction coefficient remains unchanged regardless of the probe velocity or the applied normal load in making the measurement. Amontons' laws hold true for dry sliding contacts between solid bodies with limited elastic properties. Although Naylor (2) concluded that Amontons' law is true for skin, later studies (3–6) determined that skin deviates from Amontons' law. El-Shimi (3) and Comaish and Bottoms (4) reasoned that the increase in friction coefficient resulted from the viscoelastic nature of the skin allowing for a nonlinear deformation of the skin with reduced normal loads. Also, with the presence of liquids, as is the case for the surface of human skin, the friction coefficient can change with velocity too, because the contact moves into an elastohydrodynamic lubrication regimen.

Various experimental designs have been devised to measure the friction on skin. They focus on measuring friction by pressing a probe onto the skin with a known normal force, and then detecting the skin's frictional resistance to movement of the probe. The designs fall into two categories (Table 1):

1. A probe moved across the skin in a linear fashion.
2. A rotating probe in contact with the skin surface.

Adapted from Proceedings of the Institution of Mechanical Engineers Part J. J Eng Tribol, 2006; 220(J8): 729–737.

Table 1 Probes and Apparatus used to Measure the Dynamic or Static (When Noted) Friction Coefficient (μ) of Skin In Vivo

Author	Probe size and shape	Probe material	Motion of test apparatus	Maintenance of normal load
Naylor (2)	8 mm diameter sphere	Polyethylene	Linear, reciprocating	Static weights
El-Shimi (3)	12 mm diameter hemisphere	Stainless steel (rough), stainless steel (smooth)	Rotational	Static weights
Comaish and Bottoms (4)	15 mm diameter annular ring	Teflon, nylon, polyethylene, wool	Linear	Static weights
Koudine et al. (5)	Hemisphere lens	Glass	Linear	Static weights; balance beam
Highley et al. (7)	Disc	Nylon	Rotational	Spring load
Prall (8)	Disc	Glass	Rotational	Spring load
Cua et al. (9)	15 mm diameter disc	Teflon	Rotational	Spring load
Johnson et al. (10)	8 mm (radius of curvature) lens	Glass	Linear, reciprocating	Static weights
Asserin et al. (11)	3 mm diameter sphere	Ruby	Linear	Balloon; static weights
Elsner et al. (12)	15 mm diameter disc	Teflon	Rotational	Spring load
Sivamani et al. (6)	10 mm diameter sphere	Stainless steel	Linear	Computer controlled
Sivamani et al. (13)	13 mm diameter cylinder	Copper	Linear	Computer controlled
Egawa et al. (14)	100 mm^2 square	Piano wire	Linear	Computer controlled
Zhang and Mak (15)	Annular ring	Teflon	Rotational	Spring balance
Li et al. (16)	125 $\times$ 145 mm planar surface	Sandstone, slate, granite	Linear	Supplied by the participant
O'Meara and Smith (17) and O'Meara and Smith (18)	Cylindrical "grabrail"	Chrome, stainless steel, powder-coated steel, textured aluminum, knurled steel	Linear	Supplied by the participant

Source: From Ref. 1.

In the linear designs, the probe movement is accomplished in several ways. Comaish and Bottoms (4) utilized one of the simplest linear designs: They moved the probe across the skin by attaching it to a pan of weights by means of a pulley. More sophisticated linear designs followed the simple design used by Comaish and Bottoms (4) but provided motorized unidirectional movement of the probe or the use of a reciprocating motor to move the probe back and forth. In both designs, the motorization affords greater control in maintaining the velocity of the probe and the normal load applied to the skin. Strain gauges measure the friction force as the probe moves along the skin surface.

The second design category measures friction with a rotating wheel pressed onto the surface of the skin with a known normal force. Highley et al. (7) measured the frictional resistance by determining the angular recoil of the instrument as the wheel contacted the skin. Comaish et al. (19) developed a portable, hand held device (Newcastle Friction Meter®) that relied on a torsion spring to measure the skin's frictional resistance.

An important part of designing a friction measurement apparatus is choosing the probe size, shape, and material. Because friction is an interaction between two surfaces, the probe geometry and material will affect the values calculated for the friction coefficient of the other surface. Several shapes and material have been used as outlined in Table 1. Also, results will be more accurate when the probe's normal force is maintained at a constant value or continuously monitored; different methods have been used to maintain the normal force including spring mechanisms, static weights, or computer-controlled feedback (Table 1). Probe parameters are revisited critically later.

Previously measured values for the fiction coefficient are outlined in Table 2. Special mention should be made for friction measurements of the fingers, palms, and soles because these parts of the body are more often in contact with another surface. It may be more relevant to measure static friction coefficients at these sites, since common activities such as gripping handrails, gripping car steering wheels, or holding a cup involve the static friction coefficient more than the dynamic friction coefficient. Some authors have studied the dynamic friction coefficient of the palm (9,15), and other authors have studied the static friction coefficient of the palms and fingers (16–18). Increased sweating in the hands and feet (4) and increased roughness, because of the presence of epidermal ridges (15), may contribute to increased friction coefficients.

FACTORS

Normal Load

The control of the load is important because the friction coefficient does not adhere to Amontons' law. Wolfram provided a theoretical analysis where he shows that the dynamic friction of the skin should relate to the normal load as:

$$\mu \propto N^{-(1/3)}$$

where N is the applied load normal to the skin. Koudine et al. (5) found this dependence to hold experimentally:

$$\mu \propto N^{-0.28}$$

and this was confirmed by the analysis of Sivamani et al. (6):

$$\mu \propto N^{-0.32}$$

Hydration

Hydration is a multifaceted phenomenon influenced by intrinsic (i.e., age, anatomical site) and extrinsic (i.e., ambient humidity, chemical exposure) factors. Hydration studies have correlated increases and decreases in skin hydration with the changes in the friction coefficient. These studies reveal that drier skin has lowered friction while hydrated skin has an increased amount of friction (2–4,6–8,10,13,20–23). However, the skin response is more complex, because very wet skin also has a lowered friction coefficient much like the characteristics of dry skin (24). Most studies focus on an intermediate zone of hydration where the skin has been moistened without

Table 2 Reported Values of the Dynamic Friction Coefficient (μ) for Untreated "Normal" Skin In Vivo

Author	μ
Naylor (2)	0.5–0.6
El-Shimi (3)	0.2–0.4 (stainless steel, rough)
	0.3–0.6 (stainless steel, smooth)
Comaish and Bottoms (4)	0.2 (Teflon)
	0.45 (nylon)
	0.3 (polyethylene)
	0.4 (wool)
Koudine et al. (5)	0.24 (dorsal forearm)
	0.64 (ventral forearm)
Highley et al. (7)	0.2–0.3
Prall (8)	0.4
Cua et al. (9)	0.34 (forehead)
	0.26 (ventral forearm)
	0.21 (palm)
	0.12 (abdomen)
	0.25 (upper back)
Johnson et al. (10)	0.3–0.4
Asserin et al. (11)	0.7
Elsner et al. (12)	0.48 (forearm)
	0.66 (vulva)
Sivamani et al. (6)	0.33–0.55
Sivamani et al. (13)	0.45–0.65 (untreated)
	0.81–1.17 (occlusion)
	1.19–1.71 (petrolatum)
	1.25–1.81 (glycerin)
Egawa et al. (14)	0.2–0.3
Zhang and Mak (15)	0.40–0.62 (anatomical site)
	0.37–0.61 (probe material)
Li et al. (16)	2.48–3.25 (rock types)
	3.00 (no chalk on hands)
	2.47 (chalk on hands)
O'Meara and Smith (17)	0.78–1.39 (dry palm-no grip)
	0.90–1.09 (wet palm-no grip)
	0.14–0.34 (soapy palm-no grip)
O'Meara and Smith (18)	1.44–1.91 (dry palm-active grip)
	1.10–1.92 (wet palm-active grip)
	0.34–0.64 (soapy palm-active grip)

Authors that studied the static coefficient of friction have been noted in the Table 2, and their data for certain treatments conditions have been included.
Source: From Ref. 1.

an appreciable "slippery" layer of water on the skin. Studies investigating the hydration-induced increases in friction were varied and this possibly results from the various probes used. Although the addition of water increases the friction coefficient, this effect lasts only for minutes before the skin returns to its "normal" state (3,6,7,23). The water softens the skin and this in turn allows for greater contact area between the probe and the skin. Also, water results in adhesive forces between the water and the probe. Thus, there is more frictional resistance between the skin and the probe and results in a higher friction coefficient (20). Since the water evaporates in minutes, the skin returns to its normal state in the same time frame. For dry skin, the skin becomes less supple and the probe does not achieve as much contact area, and this allows the probe to move more easily across the skin surface. Sivamani et al. (6) showed that administering isopropyl alcohol, a dehydrating chemical, lowered the friction coefficient, and a lowered friction coefficient was also found in studies involving subjects with clinically dry skin (3,22). The agreement between the experimentally induced dry skin and clinical dry skin is expected (25).

Electrical changes in the skin have also been utilized to measure hydration. Although discussions here will focus on skin friction, a review of electrical measurements is available from Gitis and Sivamani (26). Briefly, electrical changes in the skin have been used to measure skin hydration. Skin with more hydration is more permissive of electrical flow and has a

lowered resistance when compared with dry skin. Decreases in hydration have been detected with electrical measurement in clinical conditions that have a loss of hydration, like psoriasis (27) or atopic dermatitis (22).

Anatomic Region, Age, Gender, and Race

So far no significant differences have been found with regard to gender (9,13,21) or race (13,28). Age-related studies have been debatable and may depend on the anatomical site that is used for comparisons.

The friction coefficient varies with anatomical site. Cua et al. (9,21) found that friction coefficients varied from 0.12 on the abdomen to 0.34 on the forehead. They attributed this difference in the friction coefficient to differing hydration, nonapparent sweating, and sebum secretions in skin of different areas of the body. Zhang and Mak (15) found friction coefficients to differ from 0.40 on the leg to 0.62 on the palm. They too suggested that variation in hydration may be accountable for the differences among anatomical sites. Elsner et al. (12) measured the vulvar friction coefficient at 0.66, whereas the forearm friction coefficient was 0.48. Manuskiatti et al. (28) studied skin roughness and found significant differences in skin roughness at various anatomical sites. Differences in environmental influences (i.e., sun exposure) and hydration may account for this. Elsner et al. (12) showed that the more-hydrated vulvar skin had a 35% higher friction coefficient than the forearm, and this is in agreement with the hydration studies that contend that skin has an increased friction coefficient under increased hydration. Sivamani et al. (13) found the friction coefficient to differ along the volar forearm with the proximal forearm to be more hydrated and therefore have a higher friction coefficient than the distal forearm. Normally, the proximal volar forearm tends to be covered by the arm when the elbow is flexed and this can lead to less water loss and increased hydration than the distal volar forearm. However, when using an amplitude/mean measurement that serves to normalize the friction coefficient against the volar forearm "stickiness" or stick-slip friction, Sivamani et al. (13) found no differences along the volar forearm (Fig. 1). In other words, the amplitude/mean represents the ratio of the difference between the static and dynamic coefficients of friction to the dynamic coefficient of friction:

$$\frac{\text{Amplitude}}{\text{Mean}} = \frac{\mu_{\text{static}} - \mu_{\text{dynamic}}}{\mu_{\text{dynamic}}} = \frac{\mu_{\text{static}}}{\mu_{\text{dynamic}}} - 1$$

With respect to age, increased sunlight exposure can increase collagen cross-linking and therefore alter the friction properties of skin. Although studies have been conflicting,

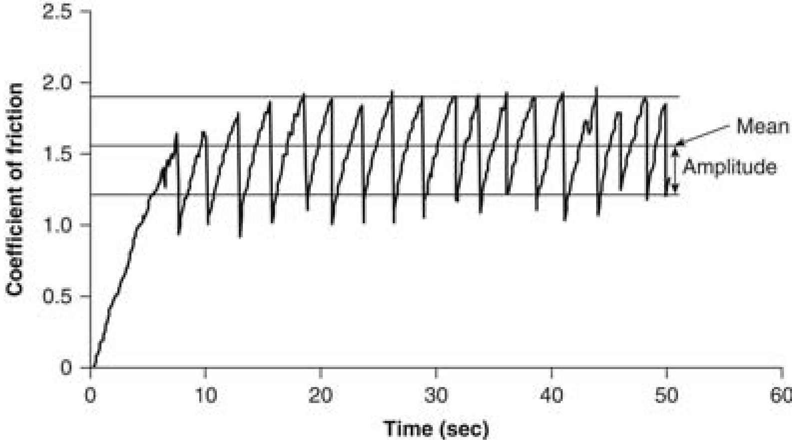

Figure 1 Calculation of the amplitude/mean measurement. The mean refers to the mean value of the measured friction coefficient as indicated on the graph. The amplitude refers to the deviation from the mean seen during the friction coefficient measurement as indicated on the graph, and is measure of the stick-slip friction along the surface. Then, the amplitude is divided by the mean to calculate the amplitude/mean. This is a measure of the skin's "stickiness." *Source*: From Ref. 13.

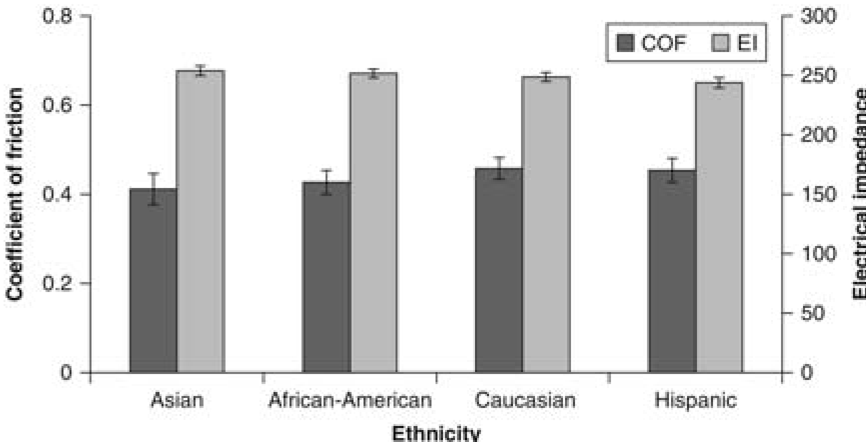

Figure 2 Coefficient of friction and electrical impedance across ethnicity. No significant differences were found among the ethnic groups. Error bars represent standard error.

recent studies have shown that the skin does not undergo age-related friction changes in areas protected from sun exposure. Cua et al. (21) found no significant age-related changes at various parts of the body, including areas that were exposed to the sun. Elsner et al. (12) conducted age-related tests and found no differences in the vulvar friction coefficient, but found a higher volar forearm friction coefficient in younger subjects. They postulate that the skin on parts of the body that become exposed to sunlight can undergo photoaging, and thus, volar forearm skin shows evidence of age-related differences, while the light-protected vulvar skin does not (12). However, Sivamani et al. (13) and Egawa et al. (14) found that the volar forearm did not change with age. This may be a reflection of variation in sun exposure among study populations.

Gender and ethnicity-related friction studies have shown no differences in the friction coefficient. Cua et al. (9,21) and Sivamani et al. (13) found no significant friction differences between the genders. Manuskiatti et al. (28) found no differences in roughness and scaliness between black and white skin; and Sivamani et al. (13) found no differences in the friction coefficient or electrical impedance among subjects of Caucasian, African-American, Asian, or Hispanic/Latino descent (Fig. 2) or in their responses to treatments (Figs. 3 and 4).

Lubricants/Emollients/Moisturizers

Much research has been devoted in determining how the application of certain ingredients influences the skin surface to appear and feel healthy, which is of interest to the cosmetic/moisturizer and lubricant industries. The studies focused on the effects of powder (3,4,16), oils (3,4,7,23), and skin creams/moisturizers (9,23). Hills et al. (29) analyzed how changes in the friction coefficient, following emollient application, differed with temperature.

Important qualitative characteristics in skin topical agents include skin smoothness, greasiness, and moisturization (20,30). Previous reports have described these subjective, qualitative descriptions in a quantitative fashion by correlating them against parameters considering the friction of the skin. Nacht et al. (23) found a linear correlation between perceived greasiness and the friction coefficient (Fig. 5). Prall (8) found a quantitative correlation for skin smoothness by taking into account the friction coefficient, skin topography, and hardness into the analysis. Sivamani et al. (13) found that an amplitude/mean measurement (Fig. 1) could be used as a marker of greasiness between different hydrative agents. When this marker is used in conjunction with electrical impedance, the quantitative effects occlusive versus directly hydrating agents could be differentiated. Occlusive agents, such as petrolatum, lowered amplitude/mean measurements and increased the electrical impedance. Directly hydrating agents, such as water or glycerin, increased amplitude/mean and increased the electrical impedance (13), and these effects are shown in Figures 3, 4, and 6. These two parameters may prove useful in studying and comparing lubricants, emollients, and moisturizers against one another.

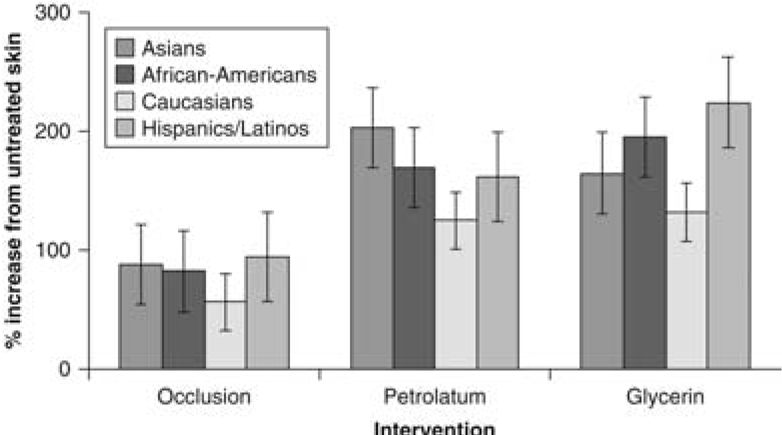

Figure 3 Coefficient of friction across ethnicity. Data represent increases in friction when compared with untreated skin of the volar forearm. No significant differences were found between the different ethnic groups. Petrolatum and glycerin increased the friction coefficient significantly more than polyvinylidene cholride (PVDC) plastic occlusion ($p < 0.01$). The increase in the friction coefficient was due to petrolatum and was not significantly different from the effect of glycerin. Error bars represent standard error. *Source*: From Ref. 13.

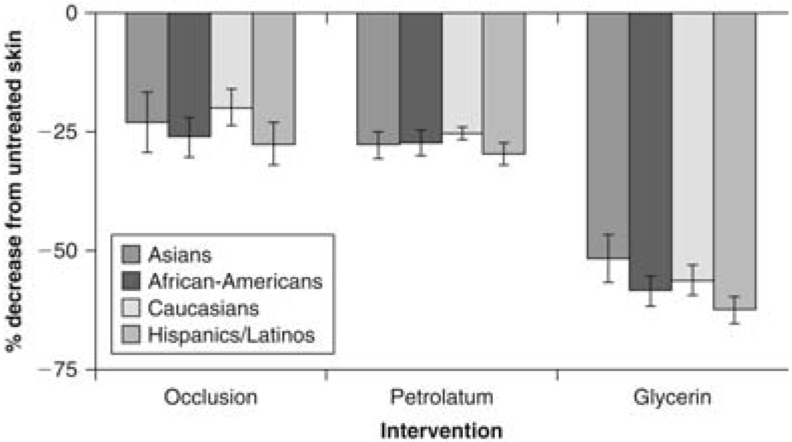

Figure 4 Change in electrical impedance across ethnicity. Data represent decreases in electrical impedance when compared with untreated skin of the volar forearm. No significant differences were found between the different ethnic groups. Glycerin lowered the electrical impedance significantly more than PVDC occlusion or petrolatum ($p < 0.01$). The decrease in the electrical impedance was due to PVDC occlusion and was not significantly different from the effect of petrolatum. Error bars represent standard error. *Source*: From Ref. 13.

Powder

El-Shimi (3) and Comaish and Bottoms (4) showed that the friction coefficient decreased after the application of powder. El-Shimi (3) found the friction coefficient to decrease by 50% after application; Comaish and Bottoms (4), in analyzing the static friction coefficient, observed an insignificant change for a wool probe and a 30% decrease in friction with a polyethylene probe. However, they also found that wetting the talc powder caused an increase in the measured friction.

Li et al. (16) also studied the effect of magnesium carbonate, or "chalk," in the static friction coefficient regimen and found that the application of chalk decreased the static friction coefficient. This contradicts with the popular use of chalk to increase friction in rock climbing. Li et al. (16) suggested that the chalk can be useful in drying the climber's hands before the climb, but suggested that all traces of the chalk be removed before the actual climb.

Figure 5 Correlation between changes in the friction coefficient and the sensory perception of greasiness. **A**, **B**, **C**, **D**, **E**, and **F** represent different creams that were applied to the skin. The reported percentage change in the friction coefficient is immediately after application, and the greasiness scores were subjective evaluations. *Source*: From Ref. 16.

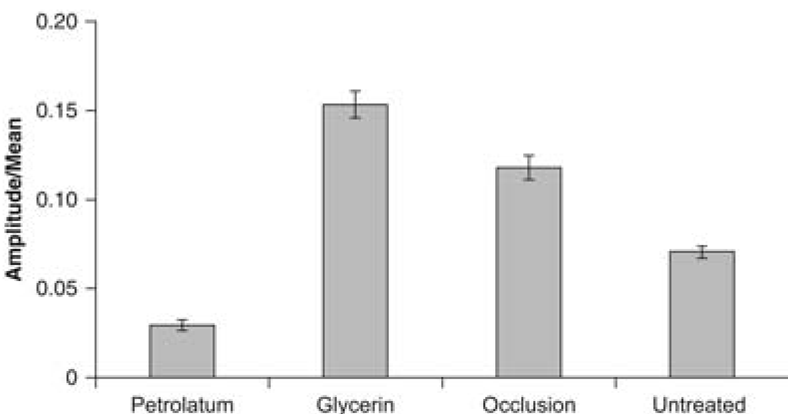

Figure 6 Amplitude/mean measurements for interventions. The application of glycerin and the PVDC occlusion increased the amplitude/mean of the volar forearm, and glycerin raised the amplitude/mean significantly more than PVDC occlusion ($p < 0.001$). Petrolatum significantly decreased the amplitude/mean, and this is quantitative evidence of petrolatum's greasiness ($p < 0.001$ when comparing petrolatum against glycerin or occlusion). Error bars represent standard error. *Source*: From Ref. 13.

Lubricant Oils

Initially the friction coefficient decreases after the application of oils and oil-based lubricants (3,7,23). Nacht et al. (23) and Highley et al. (7) also showed that after the initial decrease in friction, the oils eventually elevate the skin's friction coefficient (Fig. 7).

Emollients and Moisturizers

Prall (8) and Nacht et al. (23) found that the friction coefficient rises with the addition of emollients and creams in a similar fashion to water. However, the effects of the creams lasted for hours while the water effects lasted for about 5 to 20 minutes (6,8,23). Hills et al. (29) also studied emollients, but they examined how different emollients compared against one another and how changes in temperature changed the friction coefficient. At a higher temperature (45°C), most emollients lowered the friction coefficient to a greater degree than at a lower temperature (18°C).

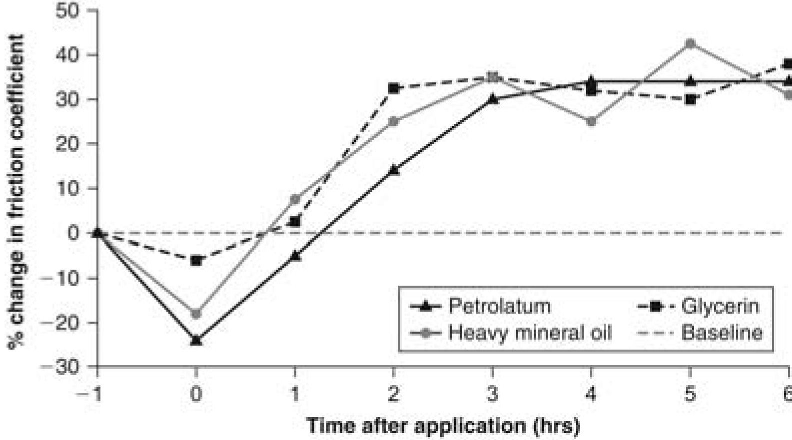

Figure 7 Effect of lubricant cosmetic ingredient on skin friction coefficient. Amount applied of each material: approx. 2mg/cm^2. Time = −1 is immediately prior to application; time = 0 is immediately after application. *Source*: From Ref. 16.

When lubricant/moisturizers are applied to the skin, the skin friction is affected in three general ways (20,23).

1. A large, immediate increase in the friction coefficient, similar to water application that follows with a slow decrease in the friction coefficient. These agents act by immediately hydrating the skin through some aqueous means to give an immediate increase in friction. In Figure 5, this is seen in creams A, B, and C.
2. An initial decrease in the friction coefficient that is followed by an overall increase in the friction coefficient. These agents are fairly greasy products (Fig. 7), and this greasiness causes the immediate decrease in the friction coefficient. The eventual rise in the friction coefficient is probably because of an occlusive effect that prevents water loss from the skin, thereby increasing the hydration of the skin. Representations of a few ingredients that elicit this response are in Figure 7 and in Figure 5 as cream F.
3. A small, immediate increase in the friction coefficient then continues to increase slowly. These agents act through a combination of mechanisms seen in the previous two cases. These lubricants/moisturizers have ingredients and agents that both hydrate through aqueous mechanisms and prevent water loss through occlusive mechanisms. Because of the presence of the these occlusive agents, which tend to be more slippery, the immediate rise in the friction coefficient is lower than in products that fall into the first category listed above. In Figure 5, this is seen in creams D and E.

Soap

O'Meara and Smith (17,18) showed that the application of soapy water to the palmar surfaces decreased the friction coefficient by 2 to 9-fold from the dry palm static friction coefficients. They also showed that textured surfaces (e.g., knurled steel) produce higher friction coefficients against soapy skin than smooth surfaces (e.g., chrome) and suggested that textured materials may be more appropriate as grabrails in showering areas (18).

Probes

As mentioned earlier, the probe geometry and material influence the measured value of the friction coefficient because friction is a probe-skin interaction phenomenon. Besides natural variations in skin, the wide range in results may be due to differences in probe movement, geometry, controlled monitoring of the normal force, and material chosen to make the friction measurement. In designing the friction measurement apparatuses, the two types of probe

movements utilized were rotational probe movement and linear probe movement (Table 1). As a result, the linear probe constantly moves over "untested" skin and the rotational probe spins over "tested" skin, leading to variations in the reported values for the skin friction coefficient. Few studies have examined probe effects: El-Shimi (3) studied probe roughness, Comaish and Bottoms (4) studied probe roughness and material, Zhang and Mak (15) studied probe material, Li et al. (16) studied various rock surfaces under static friction coefficient conditions, and O'Meara and Smith (17,18) studied static friction coefficient conditions for handrails of various materials.

El-Shimi (3) and Comaish and Bottoms (4) compared probes (Tables 1 and 2) and found that smoother probes resulted in higher friction coefficient measurements. El-Shimi (3) noted that higher friction coefficient measurements were made with a smoother stainless steel probe as opposed to a roughened stainless steel probe. Comaish and Bottoms (4) found a similar result with two types of nylon probes: a sheet probe and a knitted probe. The sheet probe (the smoother of the two) gave a higher friction coefficient measurement. El-Shimi (3) postulated that the smoother probe formed more contact points with the skin and has a greater skin contact area than the rougher probe, resulting in more resistance from the skin and a larger measurement for the friction coefficient. Zhang and Mak (15) postulated that friction depends on the contribution of two actions: the "ploughing" action and the force required to overcome adhesion. Of the five probes they tested, silicone probes resulted in the highest friction measurement. They proposed that comparable probes that have higher contribution from the adhesion force, such as silicone, will have higher friction coefficients. Li et al. (16) found that sandstone produced a larger static friction coefficient than granite and slate. O'Meara and Smith (17) found in their testing of grabrail materials that smoother surfaces, like chrome or powder-coated steel, produced higher friction coefficients for dry or watery (nonsoapy) palm skin, whereas textured surfaces, like knurled steel or textured aluminum, produced higher friction coefficients against soapy palm skin.

CONCLUSION

The reviewed studies show that differences in skin, because of various factors like age and hydration, can be correlated with the friction coefficient. It can serve as a quantitative measure of how skin differs on various anatomical sites, between different clinical conditions, and how it differs under various environmental and chemical interactions. It is also a useful method for tracking mechanical changes in the skin after the application of chemicals, like skin creams or irritants and this has important implications as a quantitative measure of the cosmetic quality of skin. The design of the test apparatus is an important factor in the measurement of tribological properties, and a better appreciation of the cosmetic understanding of skin will continue to evolve with further study of skin tribology.

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40 Skin Wettability and Friction

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INTRODUCTION

> "*... Since many of the functional and esthetic qualities of human skin are attributed to the skin moisture balance...*" (1).

The skin surface is an ecosystem, stable for a given body region, within which interfacial tension and friction phenomena play a major role because of the important forces they bring into action, especially in ruling the physical interactions between hydrophilic and hydrophobic substances. Examples of these interactions are the sorption of water by stratum corneum and the spreading of water and lipidic liquids, which influences skin absorption, thermal loss, as well as transcutaneous pressure of water vapor, carbon dioxide, and oxygen. The production of a sweat/sebum emulsion as well as the behavior and effect of topical drugs or cosmetics are also under the influence of these interactions (2).

In this chapter, we first cover the human skin wettability by showing the effects of some treatments and applications on the wettability parameters. Secondly, the skin friction coefficient μ is studied in function of the surface hydrophobic/hydrophilic balance (Ho/Hi).

HUMAN SKIN WETTABILITY

The wettability—or wetting—results from the interactions between a fluid and a surface in the presence of vapor or another fluid. The wettability intervenes in many technological processes: the liquid could be a paint, a dye, an ink, water, etc. The solid could have a regular and simple surface, but could be more complex (i.e., a fiber, a porous environment, the skin, etc.).

The skin (or solids) wettability by a liquid is the fluid's aptitude to occupy a large surface. This is quantifiable by measuring the contact angle (θ) formed between these surfaces. When $\theta = 0^\circ$, the surface wets completely, while the opposite corresponds to $\theta = 180^\circ$ (dewetting), and the partial wetting refers to θ ranging between 0° and 180° (Fig. 1).

Contact Angle Measurement

The equilibrium of a liquid drop placed on a solid surface (Fig. 2) is described by Young equation (Eq. 1) (3). The general form of this equation for the solid-liquid-air system is as follows:

$$\gamma_{LV} \mathrm{Cos}\,\theta = \gamma_{SV} - \gamma_{SL} - \pi_e \qquad (1)$$

where γ_{LV} is the liquid-vapor surface tension, γ_{SV} is the solid-vapor energy, and γ_{SL} the solid-liquid energy; π_e (external pressure) = 0 for low-energy solids (4).

For the visualization and the measurement of the contact angle, we developed a tool especially designed for the wettability in vivo (Fig. 3). This tool is based on the use of a mirror directed at a 45° angle to the skin (profile drop method).

The advancing contact angle of test liquids, i.e., the maximum value of the contact angle when the drop is inflated without the line of contact moving, is measured on the skin surface. A drop of test liquid is deposited on the skin surface using a microsyringe and inflated up to a final drop volume of 5 μL.

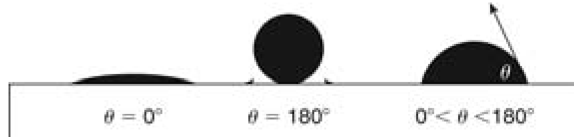


Figure 1 Solid wettability: $\theta = 0°$: total wetting, $\theta = 180°$: non wetting = dewetting, $0 < \theta < 180$: partial wetting.

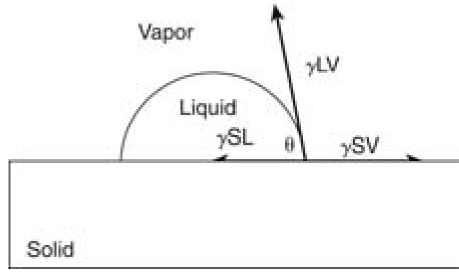


Figure 2 Equilibrium of a liquid drop on a solid surface: contact angle and surface energy.

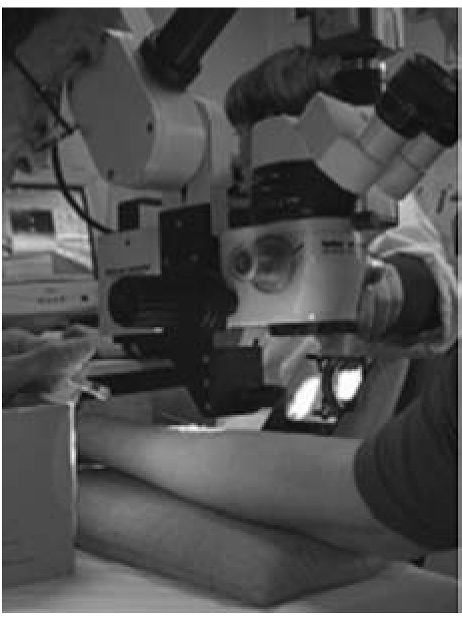


Figure 3 Contact angle visualization and measurement: tool rests on the use of a mirror directed 45° to the skin "profile drop method."

The drop's image is recorded using a video camera (CDD-Iris, Sony, France) connected to a computer and mounted on a microscope (Wild Heerbrugg M650, Switzerland), with a magnification of 16×, fitted with a slanted mirror. After visualization and storage of the drop profile, the contact angle is measured using a program that can determine θ from the tangents of both sides of the drop.

The influence of the roughness and skin temperature on the contact angle is treated in the literature (5–7). The temperature effect in the liquid in contact with the skin is minimized with the nature of the deposit (advancing contact angle) and with the very short time of deposit (15–20 seconds).

Critical Surface Tension (γ_c) and Hydrophobic/Hydrophilic Balance

Critical Surface Tension

This is the parameter that defines the capacity of a surface to be wet completely or not by a liquid. Figure 4 shows, according to Zisman's principle (8), the condition so that a liquid is spread out completely over a surface ($\gamma_{\text{liquid}} \leq \gamma_c$). γ_c is expressed according to Eq. 2.

$$\text{Cos}\theta = 1 - b(\gamma_{\text{liquid}} - \gamma_c) \quad (2)$$

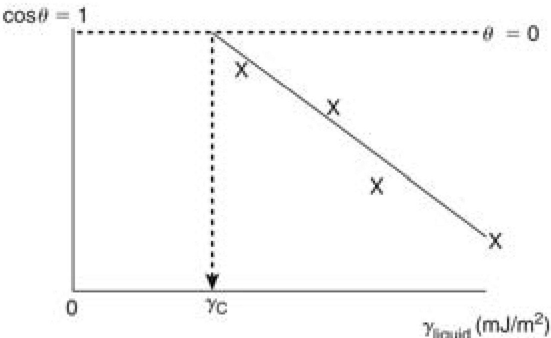


Figure 4 Critical surface tension, γ_c: total wetting condition ($\gamma_{\text{liquid}} \leq \gamma_c$).

γ_{liquid}: liquid surface tension (mJ/m^2). Note that the reduction of γ_c means the increase in the surface hydrophobia.

Hydrophobic/Hydrophilic Balance

For decades, the surface hydrophobicity has been reported to play an important role in many biological processes, such as cellular adhesion, contact inhibition, elasticity, functionality of tissue membranes, functioning of intracellular structures, and adhesion of infectious microorganisms (9).

To quantify the skin hydrophobia balance (Ho/Hi), we proposed (10) the use of the ratio γ_c/γ_{H_2O} (Eq. 3) to determine a parameter called hydrophilia (Hi). Its counterpart, hydrophobia Ho (Ho=1–Hi), characterizes the ability of the skin surface to resist wetting by water.

$$\text{Hi} = \frac{\gamma_c}{\gamma_{H_2O}} \tag{3}$$

This parameter is expressed by the ratio of its critical surface tension γ_c to the water surface tension normalized by the latter.

Surface Free Energy (SFE) (γ_s)

The γ_s of human skin is a characteristic parameter that determines most of the surface properties such as adsorption, wetting, adhesion, etc.

The γ_s of the solids cannot be directly measured because of the very weak mobility of the molecular atoms. It is necessary to resort to indirect methods such as the study of the interactions between a solid and a liquid. The γ_s is derived from measuring the contact angle of pure liquids with known surface tension parameters.

Several approaches are mentioned in the literature. The two most commonly used approaches for the skin are described below:

Geometric Mean Approach (11). The γ_s proportional to the intermolecular energy is the sum of the dispersion component γ_s^d and the polar component γ_s^p.

Acid-Base Approach (12,13). The γ_s can be expressed as the sum of Lifshitz-van der Waals γ_s^{LW} and acid-base γ_s^{AB} components: $\gamma_s = \gamma_s^{\text{LW}} + \gamma_s^{\text{AB}}$. The acid-base components can be expressed as: $\gamma_s^{\text{AB}} = 2(\gamma_s^+ \gamma_s^-)^{1/2}$, the γ_s^+ and γ_s^- components indicate, respectively, the electron-acceptor and the electron-donor components.

Data Analyses

Water Contact Angle (θ_w)

Water is an important factor for normal skin function. When the water content decreases, the skin becomes dry and itchy, and it feels uncomfortable. The degree of spreading of a water drop on the skin surface is an indication of its hydrophobic (Ho) or hydrophilic (Hi) tendency.

Water forms with the skin a semi-hydrophobic contact angle θ_w (80–91°) on the volar forearm (poor in sebum) (7,14–16). On the forehead (rich in sebum), the skin becomes less hydrophobic with $\theta_w = 57° - 73°$ (17–19).

Figure 5 Human skin wettability. Effect of sebum on hydrophobic/hydrophilic balance.

Washing (soap and water) increases significantly the hydrophobicity of the skin on the forehead (+30°) (19), and degreasing (organic solvent) increases θ_w on the forearm by +10° (7,15). It is also noted that atopic skin becomes more hydrophobic with θ_w (forearm) = 102° and that θ_w (forehead) = 73° increases in dry skin (19). In contrast, the skin (volar forearm) shows a greater affinity with thermal water ($\theta_w = 70°$) (14).

Figure 5 (17) (cartography) shows that the skin is a hydrophilic surface ($\theta_w = 60–85°$) in the presence of sebum and a hydrophobic surface in its absence ($\theta_w = 91–102°$). It was shown recently (18) that the forehead skin wettability is significantly different ($p < 0.05$) between black people (Africans or Caribbeans, $\theta_w = 71°$) and mixed races (African or Caribbean, $\theta_w = 67°$) and Caucasians ($\theta_w = 67°$).

Critical Surface Tension and Hydrophobic/Hydrophilic Balance

For a liquid to be completely spread out over the skin, it is necessary that its surface tension (γ_{liquid}) is lower or equal to the skin critical surface tension, γ_c. The absence of sebum on the volar forearm leads to $\gamma_c = 26 - 30.6$ mJ/m^2 (15) (20–24), and its presence on the forehead leads to a more wettable surface, $\gamma_c = 33.2$ mJ/m^2.

The utilization of Eq. 3 shows that the skin on the volar forearm is a more hydrophobic surface with an Ho = 62% (10), and on the forehead it is a less hydrophobic surface, Ho = 54%.

Washing and degreasing significantly increase the skin hydrophobia, γ_c varies from 27.5 to 23.7 and 21.6 mJ/m^2, respectively, and therefore Ho varies from 62% to 67% and 70%. We showed that the application of cream (w/o) decreases the skin hydrophobia; the value of γ_c is 35.5 mJ/m^2 (Hi $\approx$ Ho $\approx$ 50%) (24).

Surface Free Energy of the Skin

On the volar forearm, γ_s calculated according to both approaches is approximately 38.5 mJ/m^2 (7,10), and on the forehead, γ_s ranges between 42 and 46 mJ/m^2 according to the skin type

Table 1 Human Skin Wettability

Parameters	Volar forearm		Forehead	
	No treated	Degreasing "ether"	No treated	Degreasing "ether"
θ_w	80° (14); 84° (15) 88° (7); 91° (16)	92° (15); 101° (7)	57–73° (19); 60° (17) 67–71° (18)	84° (7)
γ_c	26 (20); 26.8 (21) 27 (22); 27.5 (23,24) 30.6 (15)	21.6 (24)	33.2[a]	22.4[a]
Ho	62% (10)	70% (10)	54%	69%
γ_s	38.5 (7,10)	32.4 (7)	42–46 (19)	34.5 (7)

[a]Data unpublished.
Abbreviations: θ_w, water contact angle; Ho, surface hydrophobicity; γ_c, critical surface tension (in mJ/m^2); γ_s, surface free energy (in mJ/m^2).

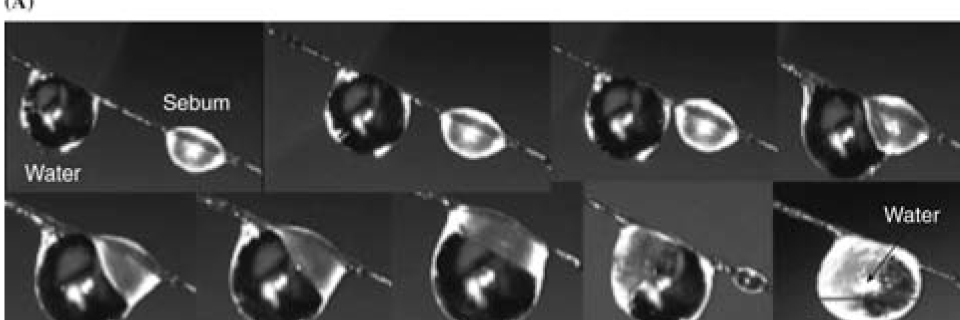
(oily, normal, dry) (19). The use of the acid-bases approach shows that the forehead (sebum-rich area) has strongly monopolar basic surfaces ($\gamma_s^- = 26$ mJ/m^2), and the forearm (sebum-poor area) has weakly basic surfaces ($\gamma_s^- = 4$ mJ/m^2) (19). Washing with soap and water affects γ_s; it decreases by –10 m/Jm^2 on the volar forearm (10) and by -8 mJ/m^2 on the forehead (19). Degreasing also decreases γ_s; it varies from 42.5 to 34.5 mJ/m^2 on the forehead and from 38.5 to 32.4 mJ/m^2 on the volar forearm (7). Table 1 summarizes the previous data.

Discussion

The capacity of the skin surface lipids to increase skin wettability was ascribed to the free fatty acids and especially to those of sebum. The skin wetting by lipids was found increased with the amount of squalene and paraffin in sebum (25).

For example, if one lets a water drop (suspended on a hair: Fig. 6A) slide toward a series of droplets of natural sebum, one notes that these droplets of sebum, by difference in surface tension ($\gamma_{sebum} < \gamma_{H_2O}$), are rolled from there on water to form a kind of sebum/water emulsion. This form of emulsion (under certain temperature conditions) leads to the protection of the aqueous phase against evaporation and transepidermal water loss (TEWL). Consequently, the water in this layer is protected against the evaporation, and therefore the skin is protected from dehydration. We consider that this layer can play the role of "G*arde d'eau*" (water storage). Figure 6B shows the same experience (filmed from another angle): drop of water placed on a glass slide + drop of natural sebum.

(A)



(B)

Figure 6 Spreading of sebum on water: hydrolipidic emulsion "Garde d'eau" formation. (**A**) Describes the meeting between a drop of water and a drop of sebum deposited on a hair, (**B**) described the same phenomenon, but the hair and the drops are placed on a glass side.

This emulsion form suggests that the quality of the skin hydration depends on the quality of its hydrolipidic layer/emulsion and consequently depends on a certain equilibrium between the two phases (aqueous and oily). This behaviour of sebum to wrap around the water is very important to understand the role of the hydrolipidic layer/emulsion in dry and atopic skin.

HUMAN SKIN FRICTION COEFFICIENT

The skin has three main mechanical roles: (*i*) to instantly or permanently match the changes in shape and volume of the underlying organs and the adipose tissue, (*ii*) to protect them against mechanical external aggressions, and (*iii*) to facilitate the adherence of palms and soles to the objects and ground (26).

The frictional behavior of the skin with different materials plays a critical role in the sensory perception of things that we come into contact with. The friction is extremely important in our perception of cosmetic application such as antiaging cream and moisturizers (27).

When touching an object, a contact is formed between our skin and the object. The tribological properties of such a contact influence how an object is perceived. Sensory perception is an important factor in the decision-making process of consumers (28). Even with closed eyes one can distinguish between a silk and a cotton fabric. A simple friction of the fingers brings considerable information on the rubbed skin: roughness, softness, dry/hydrated/fatty (oily) skin, hairs, etc. The friction is also very important for the clinician.

The friction coefficient is the measurement of the level of sliding between two surfaces. The initial force to start the slide is called "the dynamic friction coefficient," while the force necessary to continue this same slide is called "the kinetic friction coefficient." A high friction coefficient represents a weak slide, while a low friction coefficient indicates a large slide.

The review of the published literature on skin friction shows a wide range of measured values of μ (Table 2).

These differences indicate that the assessment of the friction coefficient of the skin is a highly complex problem. It involves skin elasticity, skin anisotropy, microtopography, anisotropy of the skin relief, nature of material employed, skin physicochemical nature, variation in testing conditions, and individual differences in measuring techniques. This last point can be divided into two types of designs for the test apparatus. One design is incorporated linear motion, wherein a probe is pressed onto the surface and dragged across the skin in a straight line. The other design is rotational and consists of a probe pressed onto and rotated against the skin surface. The friction coefficient does not vary significantly with gender but varies considerably among the anatomical regions of the body (33,35,37); the age effect was also measured (34,35,37). The friction coefficient is influenced by load (34,36,41,42); however, it is increased because of water application (36,39,43). On the other hand, the application of petrolatum and glycerine decreases the friction coefficient immediately on the forearm and on the hand and this effect lasts for at least one hour after application (42). The application of isopropyl alcohol (36) and washing with soap (44) dry the skin and decrease its friction coefficient. The finger has a friction coefficient μ ranged from 0.27 to 0.70 and varying among individuals because of different states of skin hydration (38). Recently, our group (18) showed a significant difference ($p < 0.05$) of μ measured on the forehead depending on the ethnic affiliation.

In 2004 (16), we showed the influence of the hydrophobic and hydrophilic characteristics of sliding and slider surfaces on μ. In this study, the wettability parameters for six surfaces [volar forearm, Teflon®, silicone impression material (Silflo®), vinyl polysiloxane impression material resin, steel, and glass] were measured and their influences were compared to the friction coefficient μ.

The tribometer used (Fig. 7) was developed and validated (16,34,45) to characterize the friction properties between skin in vivo and different sliding surfaces. A sliding ball of 10-mm diameter was pressed on the ventral forearm with a constant normal load (F_N) of 0.1 N and then moved at a constant velocity of 0.5 mm/sec. In order to maintain surfaces as flat as possible, a short sliding distance of 10 to 15 mm was selected.

In this study, we showed that when the skin is rubbed against a hydrophobic surface such as Teflon, the friction coefficient μ is lower than when rubbed against a hydrophilic surface such as glass or steel: hydrophobic surfaces = lowest friction coefficient.

Table 2 Human Skin Friction Coefficient (μ)

Author	Sliding material	Motion of test	μ
Comaish et al. (29)	Teflon$^{(1)}$ Nylon$^{(2)}$ Polyethylene$^{(3)}$ Wood$^{(4)}$	Linear	0.2$^{(1)}$–0.45$^{(2)}$–0.3$^{(3)}$–0.4$^{(4)}$ forearm
Kenins (30)	Different wool fabrics	Linear	0.32–0.48: dry skin 0.48–1.23: wet skin (forearm, finger)
El-Shimi (31)	Steel (rough^{a}, smooth^{b})	Rotational	0.2–0.4^{a} 0.3–0.6^{b} (volar forearm)
Highley et al. (32)	Nylon	Rotational	0.19–0.28 (volar forearm)
Cua et al. (33)	Teflon	Rotational	0.34 (forehead) 0.26 (volar forearm) 0.21 (palm), 0.12 (abdomen) 0.25 (upper back)
Asserin et al. (34)	Ruby	Linear	0.7 (volar forearm)
Elkhyat et al. (16)	Teflon$^{(1)}$ Steel$^{(2)}$ Glass$^{(3)}$	Linear	0.18$^{(1)}$–0.42$^{(2)}$–0.74$^{(3)}$ (volar forearm)
Elsner et al. (35)	Teflon	Rotational	0.48 (volar forearm) 0.66 (vulva)
Sivamani et al. (36)	Steel	Linear	0.56 (normal skin: dorsal finger) 0.50 (isopropyl alcohol exposure: dorsal finger) 0.2 (normal skin in vitro) 0.3 (water exposed skin in vitro)
Sivamani et al. (37)	Steel	Linear	0.4–0.6 (volar forearm)
Derler et al. (38)	Textile sample	Linear	0.27–0.7 (finger)
Egawa et al. (39)	Finger print		0.4 (volar forearm)
Lodén et al. (40)	Steel	Rotational	0.55 (dorsum of the hand) 1.1 (lower back) 0.65 (volar forearm) respectively in atopic skin 0.4–0.65–0.55
Fotoh et al. (18)	Steel	Linear	0.7–0.9 forehead

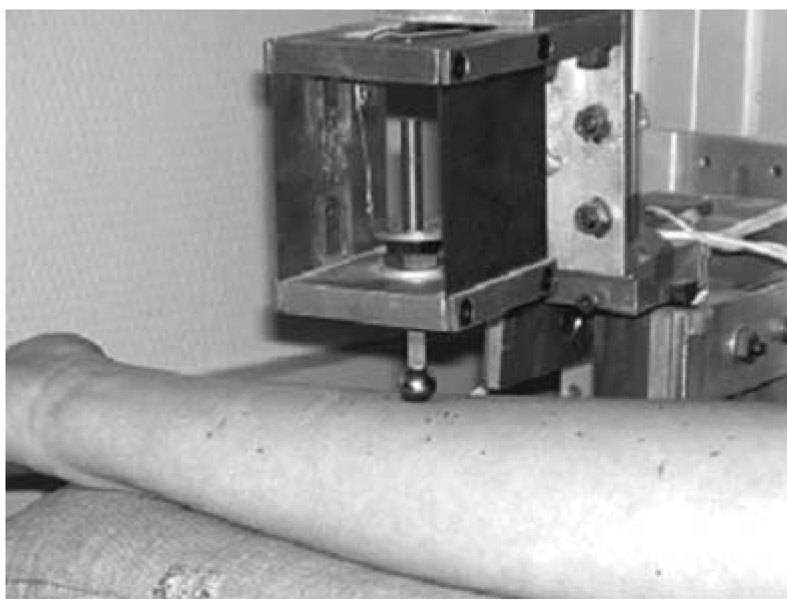


Figure 7 Linear tribometer for measuring in vivo friction coefficient.

GENERAL DISCUSSION

In this chapter, we showed that the sebum decreases the skin hydrophobia, and the wetting parameters of the surfaces in contact can play an important role in the friction phenomenon.

The largest hydrophobicity of the abdomen (Fig. 5) explains its lowest friction coefficient compared to the forehead measured by Cua et al. (33). Water application decreases the skin hydrophobia and consequently increases its friction coefficient measured by Egawa (39), Sivamani (36), and Lewis (43).

The decrease in μ after degreasing (isopropyl alcohol) (36) or after washing with soap and water (44) is quite normal; indeed these treatments increase the skin hydrophobia (Table 1), and the increased skin hydrophobia with age or in atopic skin leads to low μ reported in the literature (34,40).

The role of the surface lipids was speculated as one possible factor contributing to the frictional properties of the skin, and the correlation between μ and the skin lipid content was evaluated: Cua et al. (46) showed that the skin lipid content plays a role in the frictional properties of the skin. Moreover, in the skin, the friction resistance depends on hydrophilic and lipophilic elements present on the cutaneous surface. Fotoh et al. (18) assumed that the Ho/Hi of the cutaneous hydrolipidic film is different between the different ethnic groups studied. Black women could have a decreased skin friction coefficient as well as an increased cutaneous hydrophobicity compared with mixed race and Caucasian women.

CONCLUSION

The capacity of the skin to be wetted by water is a major parameter of the cutaneous surface ecosystem. This especially influences the equilibrium of the resident bacterial flora (either lipophilic or hydrophilic) and the possible surface colonization by pathogenic bacteria (usually hydrophilic). It also controls some functional features of the skin surface, such as triglyceride hydrolysis, skin pH, and the penetration of water into the stratum corneum, with, as major consequences, the modification of the skin mechanical behavior, permeability, and desquamation process.

We have seen that the sebum can surround water in the form of micro-emulsion to form a sort of Garde d'eau (water storage), and from this observation, we can suppose that the quality of the skin depends also on the hydrolipidic emulsion quality. This form of the hydrolipidic layer/emulsion allows the skin hydration and minimizes its TEWL.

Elias (47) noted that a significant part of the skin barrier function is provided by the stratum corneum lipids, which are suspected to play a critical role in the water-retaining properties of the stratum corneum. Grubauer (48) suggested that the TEWL serves as a signal for an increased lipid synthesis after barrier damage. Atopic skin shows an increased TEWL and indicates a defective barrier function (49,50).

The wettability and the friction coefficient of the skin provide considerable information: They can assist in the assessment of the skin nature (normal/dry/greasy skin) and its hydrolipidic layer/emulsion. They can also help to evaluate the effects of cosmetics and pharmaceuticals application in the Ho/Hi of the cutaneous surface.

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41 Classification of Irritant Contact Dermatitis

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INTRODUCTION

Contact dermatitis is defined as inflammation of the skin invoked as a result of exposure to an exogenous agent and constitutes a key portion of occupational disorders in industrialized societies.

In 1898, contact dermatitis was first appreciated to have more than one mechanism and is now generally divided into *irritant contact dermatitis* (ICD) and *allergic contact dermatitis* (ACD) on the basis of these mechanistic differences. ACD is a delayed (type IV) hypersensitivity reaction, mediated by T cells and requiring prior sensitization, while ICD has a non-immunological mechanism, thus not requiring sensitization. Clinical distinction of the two processes is often challenging, as morphology and histopathology of irritant and allergic dermatitis reactions can be virtually indistinguishable. The two processes may, and often do, coexist, thereby further complicating matters.

The morphological spectrum of ICD is broad and frequently impossible to distinguish from ACD and even endogenous (atopic) dermatitis. Chronological descriptions of these processes are often clinically used. "Acute, subacute, and chronic dermatitides" are terms applicable to ACD and ICD as well as atopic dermatitis. The erythema, edema, and vesiculation seen in acute dermatitis or the hyperkeratosis, lichenification, and fissuring seen in the chronic phase are largely nonspecific signs. Although chronological classification has its uses, the main classification of irritation is now based on both morphology and clinical course of the dermatitis.

CLINICAL CLASSIFICATION OF ICD

ICD (*synonyms: cutaneous irritation, irritant dermatitis*) is the biological response of the skin to a variety of external stimuli that induce skin inflammation without the production of specific antibodies. Formerly considered a monomorphous process, it is now understood to be a complex biological syndrome, with a diverse clinical appearance, pathophysiology, and natural history. The clinical appearance and course of ICD vary depending on multiple external and internal factors. This diversity in clinical presentation has generated a classification scheme, on the basis of both morphology and mode of onset. The various subtypes of ICD and their respective prognoses are tabulated in Table 1.

Acute ICD

When exposure is sufficient and the offending agent is potent, classic signs of acute skin irritation are seen. Erythema, edema, inflammation, and vesiculation are typical features, although acute irritation may range from mild erythema through exudative cutaneous inflammation to ulcerative lesions and frank epidermal necrosis, depending on factors such as the chemical and exposure time (1). At the extreme end of this spectrum is the "chemical burn"—this entity is recognized by severe tissue damage as a result of exposure to highly alkaline or acidic compounds—most often as a result of an industrial accident. Symptoms of acute ICD are pruritus, burning, stinging, and pain.

In keeping with an exogenous dermatosis, acute ICD usually exhibits an asymmetrical distribution and sharply demarcated borders. These borders delineate the area of exposure to the

Table 1 Ten Subtypes of ICD

	Irritation	Onset	Prognosis
1.	Acute ICD	Acute—often single exposure	Good
2.	Delayed acute ICD	Delayed—12–24 hr or longer	Good
3.	Irritant reaction	Acute—often multiple exposures	Good
4.	Chronic ICD	Slowly developing (wk–yr)	Variable
5.	Traumatic ICD	Slowly developing after preceding trauma	Variable
6.	Acneiform ICD	Moderately slowly developing (wk–mo)	Variable
7.	Nonerythematous (suberythematous) irritation	Slowly developing	Variable
8.	Subjective (sensory) irritation	Acute	Excellent
9.	Friction dermatitis	Slowly developing	Variable
10.	Asteatotic irritant eczema	Slowly developing	Variable

offending chemical. Contact with a potent irritant is often accidental, and an acute ICD is elicited in almost anyone, independent of constitutional susceptibility—in contrast to chronic ICD.

This classic, acutely developing dermatitis usually heals soon after exposure, assuming there is no reexposure—this is known as the "decrescendo phenomenon." In contrast, ACD usually exhibits a "crescendo phenomenon," i.e., transient worsening of symptoms and signs, despite removal of the allergen. In unusual cases, ICD may persist for months after exposure, followed by complete resolution.

The availability of the material safety data sheet and data from the single application Draize rabbit test combined with activities of industrial hygienists and other informed personnel have greatly decreased the frequency of such dermatitis in industry.

Delayed Acute ICD

Some chemicals produce acute irritation in a delayed manner so that inflammation is retarded until 8 to 24 hours or more after exposure (2). Except for the delayed onset, the clinical appearance and course resemble those of acute ICD. The delayed acute irritant dermatitis, because of its delayed onset and atypical crescendo periodicity, is often confused with ACD; appropriately performed diagnostic patch tests easily separate the two, i.e., the substances implicated in delayed, acute ICD would result in negative patch test results. In delayed acute ICD, a burning sensation predominates, rather than pruritus. Examples of substances causing delayed irritation are hexanediol and butanediol diacrylates (2), dithranol (anthralin), calcipotriol, and benzalkonium chloride.

Irritant Reaction

Individuals extensively exposed to irritants often develop erythematous, chapped skin in the first months of exposure. This irritant reaction may be considered a pre-eczematous expression of acute skin irritation. The term "irritant reaction" is now increasingly used if the clinical picture is monomorphic, rather than the usual polymorphic appearance of ICD, i.e., only one of the parameters usually seen in ICD are present, e.g., scaling, erythema, vesiculation, pustules, or erosions. This pattern is frequently seen in hairdressers and other wet workers. Frequently, this condition heals spontaneously, with hardening of the skin. However, repeated irritant reactions can sometimes lead to contact dermatitis, usually with good prognosis. Compounds that cause irritant reactions are typically mild irritants, such as detergents, soaps, and water.

Chronic ICD

When exposure inducing an acute irritant dermatitis is repeated, the dermatitis tends to persist and becomes chronic (more than 6 weeks has been suggested as an arbitrary threshold period). In chronic ICD (*synonyms: cumulative ICD, traumiterative dermatitis, wear and tear dermatitis*), the frequency of exposure is too high in relation to the skin recovery time.

Multiple subthreshold skin insults lead to a manifest dermatitis when the irritant load exceeds the individual's elicitation threshold for visible effects. Chronic ICD was called "traumiterative dermatitis" in the older German literature ("traumiterative" = traumas repeating) (3,4). Classic signs are erythema and increasing xerosis (dryness), followed by hyperkeratosis with frequent fissuring and occasional erythema. The lesions are usually localized but ill

defined. Pruritus and pain due to fissures are symptoms of chronic ICD. Chronic ICD often presents as hand eczema ("housewives" eczema').

Chronic ICD is the most common type of ICD. This clinical picture may develop after days, weeks, or years of subtle exposure to chemical substances. Variation in individual susceptibility and the physical properties of the irritating substance increase the multiplicity of clinical findings. Delayed onset and variable attack lead to confusion with ACD. To rule out an allergic etiology, appropriate diagnostic patch testing is indicated. Models of chronic ICD have been developed, contributing to product evaluation and mechanistic insights (5,6).

Traumatic ICD

Traumatic ICD develops after acute skin trauma, such as burns, lacerations, or acute ICD. The skin does not completely heal, but erythema, vesicles, papules, and scaling appear at the site of injury. The clinical course later resembles discoid (nummular) dermatitis. It may be compounded by a concurrent allergen exposure. The healing period is generally prolonged.

Often these patients are considered to have factitial dermatitis because of a healing phase followed by exacerbation. Although factitial aspects may occur in some patients, this peculiar form of irritation appears to be a disease sui generis. Its chronicity and recalcitrance to therapy provides a challenge to both patient and physician.

Acneiform ICD

Certain exogenous substances have the capacity to elicit an acneiform eruption (7,8), and even allergic reactions may sometimes be pustular or follicular (9). Acneiform ICD (*synonyms: pustular ICD, follicular ICD*) should always be considered in the differential diagnosis of an adult with acneiform lesions. The pustules are usually sterile and transient.

In occupational exposure, only a minority of subjects develop pustular or acneiform dermatitis. Thus, the development of this type of ICD appears to be dependent on both constitutional and chemical factors. *Chloracne* is an industrial disease caused by exposure to chlorinated aromatic hydrocarbons, in particular chlorinated dioxins, which are the most potent acnegenic agents. Many of the chloracnegens are also hepatotoxic; therefore, this is a disease of medical importance. Acneiform ICD may also develop from exposure to metals, mineral oils, greases, tar, asphalt, cutting oils, and metalworking fluids.

Acne cosmetica represents acneiform ICD caused by cosmetics. *Pomade acne* is a well-known form of acne cosmetica, seen in Afro-Caribbean women who apply vegetable oils to their skin (10). A similar problem has been reported with applications of white petrolatum (11). Nowadays, most cosmetics available in Western countries are noncomedogenic and nonacnegenic.

Non-erythematous or Suberythematous Irritation

In the early stages of skin irritation, subtle skin damage may occur without visible inflammation. As a correlate of non-visible irritation, objectively registered alterations in the damaged epidermis have been reported via cutaneous bioengineering techniques (12–14). It is customary in Japan to screen new chemicals, cosmetics, and textiles for subtle signs of stratum corneum (SC) damage, employing replicas of SC (the Kawai method; Kawai 1971). A similar technique, squamometry or corneosurfametry, has now been refined to detect subtle subclinical alterations in the SC caused by application of mild irritants (15).

Subjective or Sensory Irritation

Some individuals ("stingers") experience itching, stinging, burning, or tingling sensations on contact with certain chemicals (14,16), despite a distinct lack of objective signs on clinical examination. Despite the lack of clinical manifestations, the subjective sensations are reproducible, typically occurring within seconds to minutes following exposure; this type of irritation is known as subjective or sensory irritation. Lactic acid is a model for this non-visible cutaneous irritation. The threshold for this reaction varies between subjects, independent of susceptibility to other irritation types. The quality as well as the concentration of the exposing agent is also important, and neural pathways may be contributory, but the pathomechanism is unknown. Some sensory irritation may be subclinical contact urticaria. Screening raw ingredients and final formulations in the guinea pig ear swelling test (17) or the human forehead assay allows us to minimize the amount of subclinical contact urticaria.

Although subjective irritation may have a neural component, recent studies suggest that cutaneous vasculature may be more responsive in stingers than non-stingers (14,18). At least 10% of women complain of stinging with certain facial products; thus, further work is needed to develop a strategy to overcome this type of discomfort.

Friction Dermatitis

Repeated friction of low intensity is known to induce callus formation (hyperkeratosis and acanthosis), hardening of the skin, hyperpigmentation, and friction blisters in normal skin. In atopic people, lichenification and lichen simplex chronicus may ensue a result of friction. All of the above may be considered as adaptive phenomena to friction and should not be confused with friction dermatitis.

True friction dermatitis is the development of ICD in response to low-grade friction—this is seen clinically as erythema, scaling, fissuring, and itching surrounding the area of frictional contact. The syndrome has been characterized by Susten (19). Cases of occupational friction dermatitis in the literature are seldom documented, but most often reported in association with paper work (20). More recently, a short collection of further cases of friction dermatitis has been published (21).

Asteatotic Irritant Eczema

Asteatotic eczema (*synonyms: asteatotic dermatitis, exsiccation eczematid, eczema cracquele*) is a variant of ICD seen in elderly individuals, as a result of worsening xerosis, particularly during dry winter months. Clinically, the skin is dry (xerosis), with loss of smoothness, ichthyosiform scale, and cracking of the superficial epidermal layers, often associated with eczematous changes. The term "eczema cracquele" refers to the cracked, patchy eczematous appearance (likened to cracked porcelain, or "crazy paving") usually seen on the lower legs of these individuals. An uncomfortable sensation of "tightness" and pruritus is often felt.

Xerosis is a result of low water content in the SC, causing the SC to lose its suppleness and the corneocytes to be shed in large polygonal scales. Xerosis is usually more pronounced in the elderly and in atopic individuals. Environmental insults, such as low humidity, low temperatures, and very high doses of ultraviolet radiation (UVR) [>3 or 4 minimal erythema doses (MEDs)] can help accelerate this process. In an occupational setting, this is sometimes combined with repeated exposure to wet work, chemical insults, and friction, cumulating in perturbation of the skin barrier. Skin barrier dysfunction then leaves the skin even more vulnerable to exogenous insults and asteatotic irritant eczema ensues.

Miscellaneous

Airborne ICD is not included as one of the 10 genotypes as the mechanisms are similar to acute or chronic ICD; the only difference is that the irritant substance is dispersed and transported in the air before contact with skin. This causes dermatitis on exposed areas of skin, most commonly on the face, and may mimic photoallergic reactions.

Phototoxicity or photoirritation is another form of skin irritation following cutaneous or systemic exposure to a phototoxic agent in combination with appropriate radiation (most often in the UVA spectrum). *Phytophotodermatitis* specifically represents phototoxic dermatitis in response to plants or plant derivatives, such as species in the *Umbelliferae* (e.g., celery, carrot) and Rutaceae (e.g., lime, lemon, bergamot) families. *Berloque dermatitis* refers to fragrance dermatitis because of bergapten, the photoactive compound found in oil of bergamot, an ingredient found in fragrances; this compound has now been removed from most perfumes and substituted with artificial or highly refined bergamot oil.

Other reactions, which can be caused by contact with irritant substances, but do not fall within the scope of this chapter, include pigmentary alterations, nonimmunological contact urticaria, granulomatous reactions, and alopecia.

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42 Principles and Mechanisms of Skin Irritation

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INTRODUCTION

In contrast to allergic contact dermatitis (ACD), irritant contact dermatitis (ICD) is the result of unspecified damage attributable to contact with chemical substances that cause an inflammatory reaction of the skin (1) and individual susceptibility (2). The clinical appearance of ICD is extremely variable. It is determined by the type of irritant and a dose-effect relationship (3). The clinical morphology of acute ICD as one side of the spectrum is characterized by erythema, edema, vesicles that may coalesce, bullae, and oozing. Necrosis and ulceration can be seen with corrosive materials. Clinical appearance of chronic ICD is dominated by redness, lichenification, excoriations, scaling, and hyperkeratosis.

Any site of skin may be affected. Most frequently the hands as human "tools" come into extensive contact with irritants, whereas most adverse reactions to cosmetics occur in the face because of the particular sensitivity of this skin region. Airborne ICD develops in uncovered skin areas, mostly in the face and especially the periorbital region after exposure to volatile irritants or vapor (4–6).

Despite their different pathogenesis, ACD and ICD, particularly chronic conditions, show a remarkable similarity with respect to clinical appearance, histopathology (7,8), and immunohistology (9,10). Therefore, ICD can be regarded as an exclusion diagnosis after negative patch testing. The histological pattern of chronic ICD is characterized by hyper- and parakeratosis, spongiosis, exocytosis, moderate to marked acanthosis, and mononuclear perivascular infiltrates with increased mitotic activity (11,12).

MOLECULAR MECHANISMS OF SKIN IRRITANCY

As mentioned, striking clinical similarities exist between ICD and ACD, and even extensive immunostaining of biopsies does not allow discrimination between the two types of dermatitis (10). Nevertheless, the underlying pathophysiological mechanisms are thought to be substantially different (13). Attempts to differentiate the types of contact dermatitis with new methods are constantly under way. Recently, in vivo reflectance confocal microscopy has been suggested as an adjunctive tool in contact dermatitis diagnosis (14). With this technique, features of ACD and ICD that include spongiosis, exocytosis, vesicle formation, and blood vessel dilatation can be visualized. Hallmarks of ICD that are stratum corneum disruption, epidermal necrosis, and hyperproliferation, whereas ACD is supposed to present more typically with vesicle formation (14). However, these findings probably relate to acute dermatitis, whereas chronic allergic and irritant dermatitis can be expected to be undistinguishable by in vivo reflectance confocal microscopy just as they are by light microscopy.

In contrast to ACD, ICD lacks hapten-specific T-lymphocytes. The pathogenic pathway in the acute phases of ICD starts with the penetration of the irritant into the barrier, either activation or mild damage of keratinocytes, and release of mediators of inflammation with unspecific T-cell activation (15). In a recently published study of Meller et al., it was demonstrated that chemokine responses are helpful characteristics to distinguish the chemical-induced allergic from the irritant skin inflammation. They found that allergic and irritant skin responses have distinct molecular expression profiles. Chemokine genes predominantly regulated by T-cell effector cytokines demonstrated differential upregulation in hapten-specific skin inflammation. CXCR3 ligands, such as CXCL9 and CXCL10, were selectively induced during hapten-specific, but not irritant-induced skin inflammation. It was demonstrated that effector cytokines released by a small number of activated hapten-specific memory T cells

stimulate gene expression of a large number of surrounding resident cells, leading to the production of a discriminative chemokine signature. In contrast, the absence of antigen-specific T-cell activation in irritant skin responses results in only negligible amounts of T-cell-derived effector cytokines (16).

Epidermal keratinocytes play a crucial role in the inflammation of ICD; they can be induced to produce several cytokines and provoke a dose-dependent leukocyte attraction (17). In response to the impairment of stratum corneum barrier with direct toxic effect on keratinocytes (14,18,19), preformed IL-1α is released. It stimulates other keratinocytes and fibroblasts to produce more Il-1α, IL-1β, IL-6 IL-8, and TNF-α (13,20–23). The cytokine-induced cascade leads to vasodilatation in the dermis and cellular infiltrate in the epidermis (13,24,25). But keratinocytes also produce anti-inflammatory cytokines to counteract these inflammatory processes. The IL-1 receptor antagonist (IL-1RA) blocks IL-1 activity by competitive binding to the IL-1 receptor without triggering a signal cascade. IL-10 is another anti-inflammatory cytokine (20,23).

The upregulation of certain adhesion molecules like α6 integrin or contact dermatitis 36 is independent from the stimulus and not cytokine induced (18,26). A number of agents and cytokines themselves are capable of mediating cytokine production in keratinocytes. IL-1 and TNF- α play a role as inflammatory cytokines, IL-8 and IP-10 are known to act as chemotaxins, and IL-6, IL-7, IL-15, granulocyte-macrophage colony-stimulating factor (GM-CSF), and TGF-α can promote growth. Other cytokines, such as IL-10, IL-12, and IL-18, are known to regulate humoral versus cellular immunity (27). It is still controversial whether the cytokine profile induced by irritants differs from that induced by allergens (28–31). In irritant reactions, TNF-α, IL-6, IL-lβ, and IL-2 have been reported to be increased (32,33). De Jongh et al. recently investigated stratum corneum cytokines and skin irritation responses after single and repetitive exposures to sodium lauryl sulfate (SLS) (34). They found an IL-1α decrease of 30% after repeated exposure, while IL-1RA increased 10-fold and IL-8 increased 4-fold. Baseline IL-1RA and IL-8 values after single exposure were predictors of transepidermal water loss (TEWL) and erythema. Their results suggest that subjects with higher baseline stratum corneum levels of IL-1RA and IL-8 have a stronger response to skin irritation and that baseline levels of these cytokines can serve as indicators of skin irritability (34).

In subliminal contact to irritants, barrier function of the stratum corneum and not the keratinocyte is the main target of the insulting stimulus. Damage of the lipid barrier of the stratum corneum is associated with loss of cohesion of corneocytes and desquamation with increase of TEWL. This is one triggering stimulus for lipid synthesis, and it promotes barrier restoration (35). Nevertheless, recent studies show that the concept of TEWL increase after SLS being directly related to a delipidizing effect of surfactants on the stratum corneum cannot be kept up without limitation. Moreover, SLS exposure for 24 hours causes damage in the deeper nucleated cells of the epidermis, leaving the lamellar arrangements of lipids intact (36).

However, lipids of the stratum corneum play an important role for barrier function. Proksch et al. observed an increase in skin lipid synthesis after acute irritation with acetone treatment (37,38). Heinemann et al. observed an upregulation of the production of ceramide 1 in response to repeated irritation with SLS, thus suggesting that this upregulation might play a major role in the development of a hardening phenomenon (39). The hardening effect is understood as the adaptation of skin to repeated exogenous irritative noxes clinically resulting in stabilized skin state in spite of ongoing irritant exposure.

The stratum corneum influences epidermal proliferation after contact to irritants by increasing the mitotic activity of basal keratinocytes and in this way enhancing the epidermal turnover (40,41). Disruption of the stratum corneum can stimulate cytokine production itself, and in this way promote the inflammatory skin reaction, as shown by Wood et al. (42). They found an increase of TNF-α, various interleukins, and GM-CSF.

It has been shown that chemically different irritants induce differences in the response in the epidermis during the first 24 hours with respect to cytokine expression, indicating different "starting points" for the inflammatory response that results in the same irritant response clinically after 48 hours. Nonanionic acid, but not SLS, induced an increase in mRNA expression for IL-6, whereas mRNA expression for GM-CSF was increased after SLS (43). Forsey et al. saw a proliferation of keratinocytes after 48 hours of exposure, and apoptosis of keratinocytes after 24 and 48 hours of exposure to SLS. In contrast, nonanionic acid decreased keratinocyte proliferation after 24 hours of exposure and epidermal cell apoptosis after only 6 hours of exposure (44).

Other interesting details for understanding the molecular mechanisms of skin irritation have been contributed by Ma et al. (45). They investigated the role of metallothioneins (MTs) in SLS-induced skin irritation in MT genes I and II knockout mice [MT(–/–)] and demonstrated that MT (–/–) mice showed a much higher degree of skin inflammation than MT (+/+) mice did. With this result, they suggested that MT I and II genes presumably play an important role in skin irritation.

FACTORS PREDISPOSING TO CUTANEOUS IRRITATION

The skin of different individuals differs in susceptibility to irritation in a remarkable manner, and a number of individual factors influencing development of irritant dermatitis that have been identified include age, genetic background, anatomical region exposed, and preexisting skin disease.

Although experimental studies did not support sex differences of irritant reactivity (46,47), females turned out to be at risk in some epidemiological studies (48,49). Presumably increased exposure to irritants at home, caring for children younger than four years, lack of dishwashing machine (50), and preference for high-risk occupations contribute to the higher incidence of ICD in females (47).

The most established individual risk factor of several epidemiological studies concerning irritant hand dermatitis, is atopic skin diathesis (48,51–54). On the other hand, experimental studies concerning the reactivity of atopics and nonatopics to standard irritants have given contradictory results (55,56) and, as shown in a Swedish study, about 25% of the atopics in extreme-risk occupations, such as hairdressers and nursing assistants, did not develop hand eczema (57). Age is as well related to irritant susceptibility insofar as irritant reactivity declines with increasing age. This is true not only for acute but also for cumulative irritant dermatitis (58,59). Fair skin, especially skin type I, is supposed to be the most reactive to all types of irritants, and black skin is the most resistant (60–63).

Clinical manifestation of ICD is influenced by type and concentration of irritant, solubility, vehicle, and length of exposure (64) as well as temperature and mechanical stress. Pathogenesis of ICD is complex and may be related to a combination of different types of irritants as well as to different types of irritation. Sequential ("tandem") exposure to different irritants often occurs in the workplace and modifies the cutaneous response, in contrast to repeated exposure to each irritant alone, indicating a potential aggravating effect of the combination of chemically different irritants (65,66). In several studies, the synergistic or additive effect on skin response of irritants in combination was investigated (67). It has been demonstrated that the repeated sequential application of occlusion (with gloves, water, or SLS) and mechanical irritation enhances the effect on barrier disruption caused by single application (68). It was also described that concurrent application of an anionic detergent and a mild acidic irritant can lead to disruption of the barrier function, which, although not additive, is still considerable. The combined application of SLS and mild acids (ascorbic and acidic acid) did not prevent SLS-induced irritation. NaOH in low concentrations may also act as a potent irritant, but its effect is not enhanced by SLS (69). The contact with substances that are potentially barrier disruptive, especially in combination with other irritants, boosts the susceptibility for ICD. In contrast, exposure to low concentrations of organic fruit acids either alone or in combination with SLS did not significantly contribute to the development of ICD or increase susceptibility to SLS-induced irritation (70).

Changes in climatic conditions are known to influence barrier function and to induce ICD (71–76) or to aggravate preexisting skin irritation. Sequential treatment with airflow and SLS led to an impairment of barrier function and irritation stronger than caused by SLS alone (76). Similar effects might occur under low humidity conditions, which are known to desiccate skin, such as during the winter months (73,74,77,78).

EPIDEMIOLOGY

Population-based data on the incidence and prevalence of ICD are rare. The figures on the incidence of ICD vary considerably, depending on the study population. Most data stem from studies about occupational hand dermatoses.

Coenraads and Smit reviewed international prevalence studies for eczema attributable to all causes conducted with general populations in different countries (England, the Netherlands, Norway, Sweden, the United States) and found point prevalence rates of 1.7% to 6.3%, and one- to three-year period prevalence rates of 6.2% to 10.6% (79).

An extensive study of Meding on hand eczema in Gothenburg, Sweden, included 20,000 individuals randomly selected from the population register (48). She estimated a one-year period prevalence of hand eczema of 11% attributable to all causes, and a point prevalence of 5.4%. ICD contributed to 35% of the cases, whereas 22% were diagnosed as atopic hand dermatitis and 19% as ACD. In a multicenter epidemiological study on contact dermatitis in Italy by GIRDCA (Gruppo Italiano Ricerca Dermatiti da Contatto e Ambientali), 42,839 patients with contact dermatitis underwent patch testing. In accordance with the findings of Meding, nonoccupational as well as occupational ICD affected women in a higher percentage compared with males (48,49). In Heidelberg, Germany, a retrospective study of 190 cases of hand dermatitis revealed 27% as ICD, 15.8% as ACD, and the majority (40%) as being of atopic origin, and 10% micellaneous diagnoses (80). Even still higher rates of ICD were found by Soder et al. in cleaning and kitchen employees. One hundred and sixty-eight (79.2%) of 212 participants suffered from hand dermatitis, and ICD with 46.2% ($n = 98$) was the predominant diagnosis (81). A Danish study on occupational hand eczema revealed rates of 61.9% for ICD and 21.2% for ACD (82). The proportion of occupational ICD was similar for males and females (59.7% and 63.1%, respectively), even though females were overrepresented in wet occupations (83). In accordance with these findings, a retrospective epidemiological study of occupational skin disease in Singapore over a two-year period also demonstrated that ICD is more common than ACD: ICD made up for 62.4% of all cases of occupational contact dermatitis, ACD constituted 37.6% (84).

Interesting findings result from investigations of the severity of irritant hand dermatitis five years after initial diagnosis (85). Fifty percent of 124 ICD cases had still medium and 32% severe hand dermatitis demonstrating that irritant hand dermatitis is chronic in duration. Skoet et al. found a mean disease duration of 4.4 years for males and 4.9 years for females (83).

Reports on adverse reactions to cosmetics, including those with only subjective perceptions without morphological signs, are more frequent than assumed. In a questionnaire carried out in Thuringia, eastern Germany, even 36% of 208 persons reported adverse cutaneous reactions against cosmetics, 75% of them being female (86). Adverse reactions to cosmetics and hygiene products occur predominantly in females (87). Clinical examinations have revealed that the majority of self-reported reactions are of irritant type (88,89). Most untoward reactions caused by cosmetics occur on the face, including the periorbital area (90).

In a study by Broeckx et al., 5.9% of a test population of 5202 patients with possible contact dermatitis had adverse reactions to cosmetics. Patch testing classified only 1.46% as irritant reactions, whereas 3.0% could be classified as ACD. More than 50% of the cases of irritation were attributable to soaps and shampoos (91). In Sweden, the top-ranking products causing adverse effects, as reported by the Swedish Medical Products Agency, were moisturizers, hair care products, and nail products (87). In a Danish population survey with persons aged 19 to 80 years asked for self-diagnosed dermatitis, the reported one-year prevalence of skin symptoms on the face (acne excluded) was 14%. Of those who reported skin symptoms on the face, 33% also reported hypersensitivity to cosmetics (92).

In other studies, the incidence of cosmetic intolerance varied between 2.0% and 8.3%, depending on the test population (90,93,94). In a large multicenter prospective study on reactions caused by cosmetics, Eiermann et al. found irritancy to account for only 16% of 487 cases of contact dermatitis caused by cosmetics. Of 8093 patients tested for contact dermatitis, 487 cases (6%) were diagnosed as contact dermatitis caused by cosmetics (95). Since most consumers just stop using cosmetics and hygiene products when experiencing mild irritant or adverse reactions and seldom consult a physician, it can be assumed that mild irritant reactions to cosmetic products are still underestimated (92,96).

The symptoms of discomfort such as stinging, burning, itching noticed by many persons following product applications are summarized in the term “sensitive skin.” Only little epidemiological evidence exists with respect to its prevalence. In 2001, Willis et al. (97) published an epidemiological study in the United Kingdom to assess the prevalence of sensitive skin in the population and to examine possible factors that may be associated with sensitive skin. They found that sensitive skin is a common phenomenon with about 50% of women and 40% of men regarding themselves as having a sensitive skin. 10% of women and

5.8% of men described themselves as having very sensitive skin. Jourdain et al. (98) reported that 52% of women aged between 18 and 45 years agreed with the statement: "I have a sensitive facial skin." Approximately 30% of the total population strongly agreed with this statement.

CLINICAL TYPES OF ICD

According to the highly variable clinical picture, several different forms of ICD have been defined. The following types of irritation have been described (15,99):

- Acute ICD
- Delayed acute ICD
- Irritant reaction
- Cumulative ICD
- Traumiterative ICD
- Exsiccation eczematid
- Traumatic ICD
- Pustular and acneiform ICD
- Nonerythematous
- Sensory irritation

Acute ICD

Acute ICD is caused by contact to a potent irritant. Substances that cause necrosis are called corrosive and include acids and alkaline solutions. Contact is often accidental at the workplace. Cosmetics are unlikely to cause this type of ICD because they do not contain primary irritants in sufficient concentrations.

Symptoms and clinical signs of acute ICD develop with a short delay of minutes to hours after exposure, depending on the type of irritant, concentration, and intensity of contact. Characteristically, the reaction quickly reaches its peak and then starts to heal; this is called "decrescendo phenomenon." Symptoms include burning rather than itching, stinging, and soreness of the skin and are accompanied by clinical signs such as erythema, edema, bullae, and even necrosis. Lesions are usually restricted to the area that came into contact, and sharply demarcated borders are an important sign of acute ICD. Nevertheless, clinical appearance of acute ICD can be highly variable and sometimes may even be indistinguishable from the allergic type. In particular, combination of ICD and ACD can be troublesome. Prognosis of acute ICD is good if irritant contact is avoided.

Delayed Acute ICD

For some chemicals, such as anthralin, it is typical to produce a delayed acute ICD. Visible inflammation is not seen until 8 to 24 hours or more after exposure (100). Clinical picture and symptoms are similar to acute ICD. Other substances that cause delayed acute ICD include dithranol, tretinoin, and benzalkonium chloride. Irritation to tretinoin can develop after a few days and result in a mild to fiery redness followed by desquamation, or large flakes of stratum corneum accompanied by burning rather than itching. Irritant patch-test reactions to benzalkonium chloride may be papular and increase with time, thus resembling allergic patch-test reactions (101). Tetraethylene glycol diacrylate caused delayed skin irritation after 12 to 36 hours in several workers in a plant manufacturing acrylated chemicals (102).

Irritant Reaction

Irritants may produce cutaneous reactions that do not meet the clinical definition of "dermatitis." An irritant reaction is therefore a subclinical form of irritant dermatitis and is characterized by a monomorphic rather than polymorphic picture. This may include one or more of the following clinical signs: dryness, scaling, redness, vesicles, pustules, and erosions (103). Irritant reactions often occur after intense water contact and in individuals exposed to wet work, such as hairdressers or metal workers, particularly during their first months of training. It often starts under rings worn on the finger or in the interdigital area, and may

spread over the dorsum of the fingers and to the hands and forearms. Frequently, the condition heals spontaneously, resulting in hardening of the skin, but it can progress to cumulative ICD in some cases.

Cumulative ICD

Cumulative ICD is the most common type of ICD (99). In contrast to acute ICD that can be caused by single contact to a potent irritant, cumulative ICD is the result of multiple subthreshold damage to the skin when time is too short for restoration of skin-barrier function (104). Clinical symptoms develop after the damage has exceeded a certain manifestation threshold, which is individually determined and can vary within one individual at different times. Typically, cumulative ICD is linked to exposure of several weak irritants and water contact rather than to repeated exposure to a single potent irritant. Because the link between exposure and disease is often not obvious to the patient, diagnosis may be considerably delayed, and it is important to rule out an allergic cause. Symptoms include itching and pain caused by cracking of the hyperkeratotic skin. The clinical picture is dominated by dryness, erythema, lichenification, hyperkeratosis, and chapping. Xerotic dermatitis is the most frequent type of cumulative toxic dermatitis (105). Vesicles are less frequent in comparison to allergic and atopic types (48); however, diagnosis is often complicated by the combination of irritation and atopy, irritation and allergy, or even all three. Lesions are less sharply demarcated in contrast to acute ICD.

Prognosis of chronic cumulative ICD is rather doubtful (47,83,85,106–108). Some investigators suggest that the repair capacity of the skin may enter a self-perpetuating cycle (104).

Traumiterative ICD

The term “traumiterative ICD” has often been used similarly to cumulative ICD in the past (99,103). Clinically, the two types are very similar as well. According to Malten and den Arend, traumiterative ICD is a result of too-early repetition of just one type of load, whereas cumulative ICD results from too-early repetition of different types of exposures (3).

Exsiccation Eczematid

Exsiccation eczematid is a subtype of ICD that mainly develops on the extremities. It is often attributable to frequent bathing and showering as well as extensive use of soaps and cleansing products. It often affects elderly people with low sebum levels of the stratum corneum. Low humidity during the winter months and failure to remoisturize the skin contribute to the condition. The clinical picture is typical, with dryness, ichthyosiform scaling, and fissuring. Patients often suffer from intense itching.

Traumatic ICD

Traumatic ICD may develop after acute skin traumas such as bums, lacerations, and acute ICD. The skin does not heal as expected, but ICD with erythema, vesicles and/or papulovesicles, and scaling appears. The clinical course of this rare type of ICD resembles that of nummular dermatitis (99).

Pustular and Acneiform ICD

Pustular and acneiform ICD may result from contact to irritants such as mineral oils, tars, greases, some metals, croton oil, and naphthalenes. Pustules are sterile and transient. The syndrome must be considered in conditions in which acneiform lesions develop outside typical acne age and locations. Patients with seborrhoea, macroporous skin, and prior acne vulgaris are predisposed along with atopics.

Nonerythematous ICD

Nonerythematous ICD is an early subclinical stage of skin irritation that lacks visible inflammation but is characterized by changes in the function of the stratum corneum that can be measured by noninvasive bioengineering techniques (99,109).

Sensory Irritation

Sensory irritation is characterized by subjective symptoms without morphological changes. Predisposed individuals complain of stinging, burning, tightness, itching, or even painful sensations that occur immediately or minutes/hours after contact. Those individuals with

hyperreactive skin often report adverse reactions to cosmetic products, with most reactions occurring on the face. Fisher defined the term "status cosmeticus," which describes a condition in patients who try a lot of cosmetics and complain of being unable to tolerate any of them (110,111). Lactic acid serves as a model irritant for diagnosis of so-called stingers when it is applied in a 5% aqueous solution on the nasolabial fold after induction of sweating in a sauna (111). Other chemicals that cause immediate-type stinging after seconds or minutes include chloroform and methanol (1:1) and 95% ethanol. A number of substances that have been systematically studied by Frosch and Kligman may also cause delayed-type stinging (111,112). Several investigators tried to determine parameters that characterize those individuals with "sensitive skin,"s a term that still lacks a unique definition (113,114). It could be shown that individuals who were identified as having sensitive skin by their own assessment have altered baseline biophysical parameters, showing decreased capacitance values, increased TEWL, and higher pH values accompanied by lower sebum levels (114–116). Possible explanations for hyperirritability (other than diminished barrier function) that have been discussed are heightened neurosensory input attributable to altered nerve endings, more neurotransmitter release, unique central information processing or slower neurotransmitter removal, enhanced immune responsiveness, and increased sweat glands (113,117,118). It is not clear whether having sensitive skin is an acquired or inherited condition; most probably it can be both. As in other forms of ICD, seasonal variability in stinging with a tendency to more intense responses during winter has been observed (119). Detailed recommendations for formulation of skin care products for sensitive skin have been given by Draelos (113). Recent reviews on experimental studies on the nature of sensitive skin and on host factors were published by Kligman et al. (120) and Farage et al. (116) in 2006.

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43 Mechanism of Skin Irritation by Surfactants and Anti-Irritants[a] for Surfactant-Based Products

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INTRODUCTION

Each day our skin is in contact with a multitude of aggressions that we need to minimize. This can be done by decreasing the intrinsic irritation potential of the insult, by placing an additional barrier between the irritant and our skin, or by changing our behavior. Chemical irritants are usually the best-known irritants that inflame our skin, but physical, biological, and environmental factors are also important causes of irritation (Table 1)

In some cases, several irritant categories may act simultaneously on the skin to potentiate their effect. For instance, scrubbing products involve a mechanical stress of the skin by rubbing the skin with solid particles and a chemical stress by the surfactants used to formulate the vehicle. With so many types of potential irritants, it is obvious that skin irritation can be induced through different pathways.

SURFACTANTS

Surfactants: A Good Model to Investigate Skin Irritation

Surfactants are frequently used as a model to investigate skin irritation and the effect of anti-irritants for three main reasons.

Surfactants are a Major Cause of Skin Irritation

As a result of their detergent and foaming properties, surfactants find broad use in many domestic products that contact the skin (Table 2). Furthermore, many subjects take several showers/baths a day for cleansing as well as for relaxation and pleasure.

It is Quite Easy to Obtain Very Well-Standardized Surfactants to Work With

In the scientific literature, sodium lauryl sulfate (SLS) is regularly used as the "gold" standard to induce skin irritation (18) for several reasons:

- SLS is classified as a skin irritant, Xi-R38 (19).
- SLS can be obtained in a very pure form, which allows different laboratories to work on the same material.
- SLS can be easily formulated in various vehicles.
- Allergic reactions to SLS are not common, although a few cases have been reported (20).
- The level of induced irritation can be controlled by adjusting the concentration (21,22).
- Any skin damage is rapidly reversible.

Unlike Other Irritants, Surfactants May Induce Irritation Through Several Pathways

Because of their structure and physicochemical properties, surfactants interact with various targets of the skin: constitutive and functional proteins, intercellular or cell membrane lipids, and living cells.

[a]The term 'anti-irritant' is used to express a reduction of the irritation potential; it does not mean a total suppression of skin irritation.

Table 1 Examples of Potential Skin Irritants

Chemicals
Surfactants (1), solvents (2,3), acids and alkalis (3), dessicants (4), concentrated salt solutions (5), alcohol (6), oils (7), water in wet work conditions (8)
Environmental conditions
Extreme weather conditions [very warm, very cold, dry atmosphere (9), UV radiations or pollution (10)]
Physicals
Abrasives (11), occlusion (12) needles (13), burns (14), rubbing (15)
Biological factors
Some enzymes or combination of enzymes (16), some plants (17)

Table 2 Surfactant-Containing Products

Cosmetics and toiletries
Body-cleansing liquids (shower gels, facial cleansers, liquid soaps, foam baths)
Body-cleansing solids (soap bars, syndet bars, combars)
Shampoos
Shaving products
Toothpastes
Deodorants
Household products
All purpose cleaners
Windows cleaners
Hand dishwashing liquids
Automatic dishwashing products
Fabric detergents
Fabric softeners

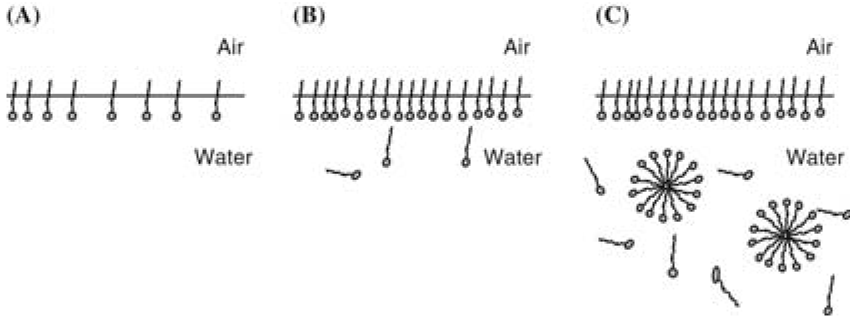

Figure 1 Surfactant behavior in solution. (**A**) Surfactant molecules in aqueous solution concentrate at the air–water interface with the hydrophobic part oriented toward the air side. (**B**) When the concentration of surfactant increases, the interface becomes saturated with surfactant molecules that penetrate into the solution. (**C**) To minimize their interaction with water, the hydrophobic parts of the surfactants interact together and form micelles in solution. These latter are unstable structures that form and disaggregate to establish a balance between monomers and micelles in the solution.

Surfactant Behavior in Solution: Their Physicochemical Properties

Surfactants are amphiphilic molecules, meaning that they contain two opposing parts: hydrophilic (water-loving) and hydrophobic (water-hating). When dissolved in water, the former is readily hydrated while the latter avoids water. As a surfactant is added to water, it concentrates as independent molecules (called monomers) at the air/water interface with the hydrophobic part trying to avoid the water environment. At a certain concentration, called the "critical micellar concentration" (CMC), the surfactant can no longer concentrate at the surface and goes into the bulk of the solution. In order to avoid contact with water, the hydrophobic part of the surfactant molecules tends to aggregate together into larger particles called "micelles" (23) (Fig. 1). However, the hydrophilic part of the surfactant either by repulsive forces between similar charges (for anionic or cationic surfactants) or by trying to interact better with water (all surfactant types) tends to work to disaggregate the micelles. On the basis of those attractive and repulsive forces, micelles are dynamic structures that continuously form and disrupt to define an overall relative proportion

of monomers and of micelles in the bulk. As a consequence, any system that is able to stabilize the micelles or facilitate the incorporation of free monomers into the micelles will reduce the relative proportion of monomers in the solution (24).

MECHANISM OF INTERACTION BETWEEN SURFACTANTS AND THE SKIN

When surfactants come into contact with the skin, they can interact with it in different ways (25):

- By binding to the surface proteins of the skin
- By denaturing skin surface proteins
- By solubilizing or disorganizing the intercellular lipids of the skin
- By penetrating through the epidermal lipid barrier
- By interacting with living cells

All these interactions may lead to irritation. Whatever the mechanism of interaction between the surfactant and the skin, the free monomeric form will be the key driver to initiate irritation as illustrated hereafter.

Interactions with Skin Proteins

Binding of surfactant to isolated stratum corneum (SC), the most external layer of the skin, saturates at or near the CMC (26), which is consistent with the fact that only monomers of surfactants can adsorb to the proteins of the skin (27). After binding to the proteins, surfactants cause the proteins to denature, leading to a swelling of the SC (28). Rhein et al. (29) investigated the swelling of isolated SC when exposed to various single surfactant solutions and showed that the swelling was concentration and time dependent up to the CMC before leveling off. The authors interpreted their results as support for a single interaction between the surfactant monomer and skin proteins.

Denaturation of functional proteins and especially enzymes have multiple consequences such as impaired desquamation process, maturation of lipids and proteins in the epidermis, defense system against free radicals, and enhancement of oxidative stress (30,31).

However, even if the interaction of surfactants with skin surface proteins is related to the CMC of the surfactants or surfactant mixtures, above the CMC there is no more a direct relationship. For surfactants mixtures, it is proposed that above the CMC the affinity of individual surfactants for skin surface proteins also plays a critical role on skin irritation (32).

Interaction with the Intercellular Skin Lipids

The protective lipidic barrier of the skin is composed of highly organized lipid layers located between the cells of the SC. In order to disorganize these lipids and alter the skin barrier function, surfactants have to integrate into the lipidic layers that are mostly hydrophobic. Because of their small size, monomers of surfactants can easily reach the intercellular lipids and disturb the skin barrier function, making such an effect depending on the relative proportion of monomers in solution. However, it has been recently shown (33) that micelles formed from sodium dodecyl sulfate (same as SLS) have a hydrodynamic radius size that is compatible with partial penetration into the SC, and should be capable of interacting with the intercellular lipids. This would partly explain why increasing the concentration of single SLS surfactant solutions above the CMC leads to increased irritation. For other surfactant types, micelles have to release their monomers to interact with the lipidic barrier. The dose-related level of irritation caused by such surfactants above their CMC (34) should thus be related to another mechanism.

Interaction with Living Cells

Once the lipidic barrier has been disrupted or weakened, monomers of surfactants can reach the living part of the epidermis and interact with the keratinocytes and Langerhans cells, leading to the following:

- A lysis of the cells in the case of severe irritants and the release of chemical mediators into the intercellular space

- An alteration of the cellular membrane and passive diffusion of chemical mediators from the cytoplasm into the intercellular space
- A stimulation of the cells with subsequent active release of chemical mediators into the intercellular space or synthesis of new mediators

Whatever the pathway, these mediators will initiate a multitude of reactions at the site of irritation such as a stimulation of cell proliferation, a stimulation of neighboring cells to produce additional mediators, a vasodilatation of blood capillaries in the dermo-epidermal papillae, and an attraction of blood cells. Many different chemical mediators will also be upregulated at the site of irritation such as interleukin (IL)-1 α and β, IL-2, -6, -8, and -10, granulocyte macrophage colony-stimulating factors (GM-CSF), tumor necrosis factor α (TNF-α), interferon-γ, and others. This cascade of chemical messengers responsible for the inflammatory reactions is detailed elsewhere in this book (chap. 42).

Interaction with Neuroreceptors

In sensitive persons, initial contact with some surfactants result in sensory irritation characterized by stinging, itching, or a burning sensation. Such an early signal of irritation was exploited a long time ago with the development of the so-called lactic acid stinging test (35) to detect subjects with an "upper level of skin sensitivity" in the face.

This type of sensory irritation occurs when thin, unmyelinated, chemically sensitive type-C nociceptors are activated and transmit a depolarizing signal via the dorsal root ganglia in the spinal cord to the brain where the sensation is appreciated (36). These receptors are extensively distributed through the dermis and the epidermis allowing excitation, even by faint stimuli. For more intense irritants, a retro-signal is transmitted from the dorsal root ganglia to the inflammation site and contributes to the erythematous reaction.

ANTI-IRRITANTS FOR SURFACTANT-BASED PRODUCTS

Fortunately, nowadays many systems have been developed to minimize the risks of intolerance to cosmetics or surfactant-based products. This is extremely important because of the increased use of toiletry products. They must be as mild as possible to the skin. Not only the mildest ingredients are used, but also finished hygiene products often contain one or more anti-irritant systems.

Anti-Irritation by Using Only Mild Surfactants

The first approach to develop a mild, surfactant-based product is to carefully select the mildest surfactants. Nonionic surfactants are generally considered as the mildest and are typical ingredients in body-cleansing products for babies, for sensitive skin subjects, and for face-cleansing products. However, several anionic surfactants are also extremely respectful of the skin condition and are often introduced in the same categories of products. These are, for instance, highly ethoxylated (at least 5-EO) alkyl sulfates, sulfosuccinate esters, sarcosinates, fatty acid-protein condensate, alkyl phosphate ester, alkyl glutamate, taurates, and others. Amphoteric surfactants are rarely used alone, but rather as secondary surfactant; thus, their intrinsic irritation potential has no real meaning. Cationic surfactants are essentially used for their antibacterial properties rather than their detergent properties and are often described in the literature as the most irritating surfactants. However, like anionic surfactants, it is also possible to find very mild cationic surfactants (e.g., salts of alkyl amine, quaternized alkyl polyglycosides). Because of their low usage, the cationic surfactants will not be discussed in this chapter.

Anti-Irritation by an Appropriate Combination of Surfactants

The best counterirritants for surfactants are other surfactants. Several authors have clearly demonstrated such a positive interaction between various surfactants in vitro (29,37) and in vivo (32,38,39), with diluted (29,37,38) or with highly concentrated solutions (32,39). Amphoteric surfactants are well known to decrease the irritation potential of anionic surfactants (40), but nonionic surfactants can display the same effect when used at a sufficiently high concentration. More surprisingly certain anionic surfactants can reduce the irritation potential of another anionic surfactant, instead of cumulating their effects (39).

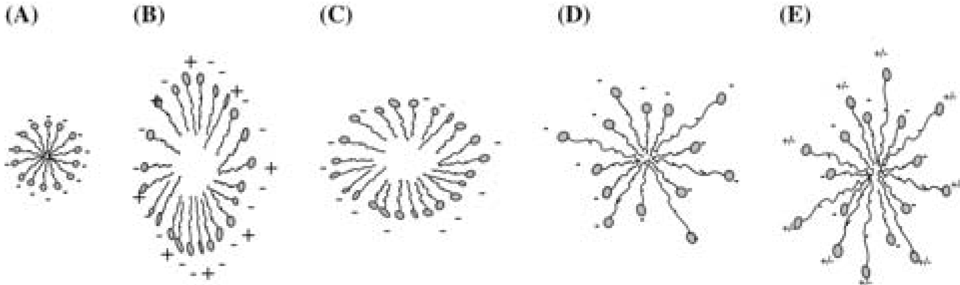

Figure 2 Mixed micelles of surfactants in solution. In aqueous solution, the hydrophobic tail of surfactant monomers form self-assembled aggregates within the core of the micelle, while the hydrophilic head interacts more with the water molecules. This structural arrangement is more energetically favorable because it reduces the unfavorable hydrocarbon/water contact energy. (**A**) When micelles are formed of only one single type of surfactants (e.g., anionic surfactants), electrostatic repulsion forces tend to disrupt the micelles that are not stable. (**B**) Adding cationic surfactants into the micelles increases the size of the micelles, modifies their form, and stabilizes the micelle by introducing attractive charges between the positive and negative polar head groups. (**C**) When adding nonionic surfactants to solution A, uncharged surfactant heads incorporate into the micelles, increasing the size of the micelles as well as the distance between the anionic polar heads. As a result of this increased distance, the repulsive forces between the monomers are reduced, which leads to a stabilization and change of micelle form. (**D**) When a second type of anionic surfactant is incorporated to solution A, the hydrophobic tail should be different from the primary surfactant tail. Consequently, the distance between the anionic surfactant heads is greater, repulsive forces are lower, and the micelles become more stable, are larger and of a different form. (**E**) When amphoteric surfactants are added to the solution of anionic surfactants, their behavior depends on the electronic charge of the surfactant [positive at a pH below the lowest pKa of the surfactant (case B), negative at a pH higher than the highest pKa of the surfactant (case D), and zwitterionic at a pH between the lowest and highest pKa of the surfactant (case E)].

How Can Secondary Surfactants Reduce the Irritation Potential of Primary Surfactants: The Principle of Surfactant Antagonism

Skin is a complex organ with different potential targets for surfactants. Several mechanisms may, thus, occur to explain the reduced irritation observed by mixed surfactant systems as compared with single surfactant solutions.

Overall mechanism for all targets. On the basis of the fact that mainly monomers irritate the skin and that there is an equilibrium in solution between micelles and monomers, any factor able to stabilize the micelles, and hence decrease the relative proportion of monomers, plays a major role in reducing irritation (24). This is the case for secondary surfactants added to the system as explained in Figure 2, but also for other kinds of macromolecules such as proteins or other polymers (41).

As shown in Figure 2, any kind of secondary surfactant is able to stabilize the micelles and reduce the relative amount of irritant monomers in the solution.

Furthermore, in the case of mixed micelles of types B, C, or E (Fig. 2), the overall electrical charge density at the surface of the micelles is lowered. This effect allows the micelles to be less repulsive to surfactant monomers in the surrounding bulk, thus allowing the monomers to more easily incorporate into an ever-expanding micelle. This mechanism explains the reduction in the amount of free monomers in the solution.

Additional mechanism for interactions with surface proteins. The affinity of the monomers for proteins may also displace the monomer–micelle equilibrium. This affinity depends on the intrinsic properties of the surfactant (e.g., ionic charge, tertiary structure, hydrophobic domains, carbon chain length, level of ethoxylation) on the mobility of the monomer in the solution (which is related to the size of the monomer), and on the availability of the protein's binding sites. This latter parameter is mainly significant in the case of concentrated solutions of surfactant mixtures, because their monomers compete for binding sites on the surface proteins as well as with interactions within the micelle. The amount of available monomers that will bind to the protein will thus be decreased, and the irritation potential of the mixture lowered. Such a decrease in the binding of the anionic surfactants to the skin surface has been

demonstrated by attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) in the presence of a secondary surfactant of any type (42).

Similarly, proteins or polymers added to a surfactant solution may also compete for the same binding sites as the surfactant monomers at the surface of the skin and are often counterirritants for the surfactants (42).

Additional mechanism for interactions with intercellular skin lipids. Micelles formed from a single surfactant type are smaller than micelles formed from several surfactant types. While the former category may have a size allowing them to partially penetrate through the SC [e.g., the case for SLS, (33)], the latter examples should be sterically hindered from penetrating into the skin and interacting with the intercellular lipids (43). This effect results in a partial protection of the skin barrier function when adding a secondary surfactant to the primary one.

Scientific Cases of Reduced Irritation in Surfactant Mixtures

Many peer-reviewed scientific publications have reported that mixtures of surfactants are less irritating than expected by the sum of the irritation potential of each species taken separately. Several review papers by Goldemberg (44,45), Effendy and Maibach (46), and Paye (47–49) have illustrated examples of antagonisms between surfactants. The following section focuses on giving additional concrete examples grouped by the type of interaction between the skin and the surfactant investigated by the author.

Interaction of surfactant with proteins in vitro or in vivo

- Ohbu et al. (50) evaluated the protein denaturation properties of surfactants using circular dichroism and demonstrated that the sodium dodecyl sulfate–induced denaturation of bovine serum albumin (BSA) was counteracted by dodecyltrimethylammonium chloride or by *N*, *N′*-dimethyldodecylaminoxide.
- Dominguez et al. (40), using human callus as a skin model, demonstrated a considerable inhibition of adsorption of SLS on the callus when alkyl amido betaine (AAB) was present in the same solution. They deduced from their data that the two individual surfactants were more irritating than any of the combinations tested. They explained their data by a stabilization of the micelles of mixed surfactants and hence a reduction of bioavailable monomers.
- Miyazawa et al. (51) showed in vitro that mixed surfactants reduced protein denaturation compared with single surfactant solutions. Again this was explained on the basis of the reduced level of free surfactant monomers in the mixed surfactant solution as compared with single surfactant solutions.
- Blake-Haskins et al. (52) showed, using an in vitro protein denaturation assay (collagen swelling), that the addition of an amphoteric surfactant to an anionic surfactant reduced the denaturation potential of the anionic surfactant.
- Paye and Jacobs (42), using attenuated total reflection–Fourier transform infrared (ATR-FTIR) spectroscopy, demonstrated, by a study on human volunteers, that the binding of anionic surfactants, SLS, and linear alkyl benzene sulfonate (LAS), to skin surface proteins was significantly reduced when amphoteric or nonionic surfactants were added in the solution. This study illustrated the competition between the two types of monomers for the binding sites on skin surface proteins.
- Tadenuma et al. (53) showed that when alcohol ethoxylate (AE) was added to SLS, the BSA was less denatured. The higher the concentration of AE for a fixed concentration of SLS, the greater the inhibitory effect of AE on SLS-induced protein denaturation. By measuring the adsorption isotherms of SLS onto agarose-immobilized BSA in the presence and absence of AE, the authors correlated reduced protein denaturation by AE with a dramatic reduction of binding of SLS to BSA because of the adsorption of AE onto the protein.
- Paye et al. (54), using commercial surfactants (as provided by manufacturers) and in the exact proportions as in standard commercialized laundry detergents, demonstrated that the protein denaturation potential (using the in vitro zein test) of the mixtures of surfactants was in all cases lower than expected by the cumulative protein denaturation effect measured for the surfactants separately.

Interaction of surfactant with lipids or membranes

- Charaf and Hart (55) investigated in vitro the interaction of surfactants with membranes and demonstrated that the addition of lauryl ether sulfosuccinate to a given concentration of SLS decreased the aggressiveness of the latter surfactant for the membrane.
- Garcia et al. (24) demonstrated that mixtures of surfactants diffused less through a membrane than the same surfactants tested separately. This observation was interpreted by the fact that micelles were too big to penetrate through the membrane and that the relative proportion of monomers was lowered in the mixed solutions.
- Kawasaki et al. (56), using the electron paramagnetic resonance (EPR) technique, demonstrated an increased fluidity of the SC intercellular lipid structure after application of a solution of SLS. This increased disordering was most likely because of an intercalation of SLS monomers into the intercellular lipids organization. The addition of sodium lauryl glutamate (SLG), another anionic surfactant, to SLS inhibited the fluidization of the intercellular lipids caused by SLS alone.
- Moore et al. (43), using dynamic light scattering measurements, determined the size of the hydrodynamic radius for SLS micelles that was compatible with partial penetration inside the SC, while the size of the mixed micelles from SLS and dodecyl hexa(ethylene oxide) (C12E6) was higher and hindered the penetration of the mixed micelles inside the SC. They confirmed their hypothesis by measuring the hydrodynamic radius of surfactants evaluated in skin penetration studies and showed that the addition of C12E6 to the SLS solutions was found to decrease the amount of SLS penetrating into the epidermis. They attributed this decreased penetration to two causes: a decrease in the concentration of SLS monomers because of a stabilization of the micelles and a decreased penetration of the mixed micelles because of an increase in their steric size.

Interaction of surfactants with living cells in vitro

- Earl et al. (57) showed in a three-dimensional cell culture model of human skin that equal mixtures of SLS and *N*,*N*-dimethyl-*N*-dodecylaminobetaine have reduced cytotoxicity potential compared with their single applications at the same concentrations. Their observation correlated well with the results of a four-hour human patch study in which the same single surfactants were tested versus the surfactant combinations at high concentrations.
- Benassi et al. (58), using cell culture models, demonstrated that the cytotoxic effect of SLS was reduced when it associated with different tensides such as cocamidopropyl betaine, polysorbate-20, and polysorbate-80. They compared their results to previous data showing that the barrier damage caused by SLS in vivo was lower when SLS was used in combination with other tensides because they were able to reduce the CMC of SLS.

Interaction of diluted surfactant solutions with skin in vivo

- Rhein et al. (59) used in vivo skin irritation studies (21-day cumulative irritation test) to show that the addition of (C12-C14) alkyl, 7-ethoxy sulfate (AEOS-7EO) to a constant dose of SLS resulted in a significant reduction of erythema, hence producing a milder system.
- Marti (60), using four in vitro and two in vivo models for skin and mucous membrane irritation prediction, showed that the irritation potential of sodium lauryl ether sulfate used as a primary surfactant could be significantly decreased by adding cocamidopropyl betaine, or coco amphocarboxypropylate, or protein fatty acid condensate as secondary tensides for mildness synergy in shampoo formulations.
- Zenhder et al. (61) evaluated the effect of sodium laureth carboxylate with two different levels of ethoxylation (5 and 13 ethoxylations) for their effectiveness in reducing the irritation potential of SLS in a five-day human patch test. Both carboxylates were counterirritants to SLS as shown by clinical examinations, measurements of superficial blood flow, skin barrier alterations, and skin electrical conductance.

- Lee et al. (38), in a 24-hour patch test, showed that adding SLG to a solution of SLS decreased the irritation potential of the latter.
- Teglia and Secchi (62), using a three-week arm-soaking test on human volunteers, showed that the amphoteric surfactant, cocamidopropyl betaine, had a similar anti-irritant effect versus wheat protein when added to a solution of SLS. Both the wheat protein and cocamidopropyl betaine protected the skin against alteration of the skin barrier and subsequent irritation.
- Teglia and Secchi (41) reported that using SLS, sodium laureth sulfate (SLES), and olefin sulfonate as primary surfactants, and obtaining some new formulations by mixing these primary surfactants with four different auxiliary surfactants and protein hydrolyzates, reduced the damage to the SC.
- Paye and Cartiaux (63) showed in a short-term patch test on human volunteers that alkyl betaine (amphoteric surfactant) and AE (nonionic surfactant) reduced the alteration of the SC caused by SLS or by LAS (anionic surfactants).
- McFadden et al. (64) mentioned in one of their publications that they had run an unpublished clinical study demonstrating that the direct addition of benzalkonium chloride (BC) to a solution of SLS reduced the irritant inflammatory response of the volunteers to SLS. They explained their observation by a stabilization of the mixed micelles by BC.
- Vilaplana et al. (65) emphasized the importance of the physicochemical behavior of surfactants in solution as a way of minimizing their irritant properties. In a 48-hour patch test on human volunteers, the authors showed that the addition of disodium cocoyl glutamate or of sodium PEG-4 lauramide carboxylate to a solution of SLS produced a significant reduction in the transepidermal water loss (TEWL), skin color reflectance, and laser Doppler velocimetry, even though there was a two-time increase in the total surfactant concentration.

Interaction of concentrated surfactant preparations with skin in vivo

- Dillarstone and Paye (39), using the four-hour human patch test with concentrated surfactant systems, demonstrated that the addition of 10% of the following surfactants: cocoamidopropylbetaine, ethanolamide, SLES, or AE to a solution of 20% SLS, or of LAS decreased in all cases the level of erythema induced by the anionic surfactant alone, even though the overall concentration of surfactant in the mixture was increased. Even more, a solution with 20% LAS + 10% SLES + 10% AE (total concentration of 40%) was found to be less irritating than a solution of only 20% LAS.
- Hall-Manning et al. (32), using the four-hour human patch test, investigated the interaction between highly concentrated anionic and amphoteric surfactants and showed that the irritant effect on skin of the mixtures (20% of sodium dodecyl sulfate + 20% of dimethyl dodecyl amido betaine) was significantly lower than the effect of the anionic surfactant (at 20%) tested alone. The authors pointed out the correlation between the reduced irritation and the reduced CMC for the mixture of surfactants. However, at such a high surfactant concentration, they also attributed the lowered irritation potential to a reduced affinity of the individual surfactants for the skin proteins.

Anti-Irritation by Polymers or Proteins/Peptides

The counterirritant capability of polymers or proteins on surfactants has been reported in the literature (61,66–68). The mechanism by which polymers and proteins function is similar to the one described above for surfactant mixtures. They incorporate into the micelles and thus decrease the relative amount of free monomers in solution. Their skin substantivity can also involve blocking binding sites at the surface of the skin, thus making them nonaccessible to surfactants.

Polymers or proteins differ in their ability to interact with the skin surface and to be incorporated into the micelles. The following parameters should be considered when selecting a polymer/protein:

- Better interaction with the micelles correlates with increased hydrophobicity (66).
- Better substantivity to the skin correlates with higher hydrophobicity when the polymer is quaternized or is cationic or when the net charge or the size of the polymer/protein increases (67,68).

As stated above, more hydrophobic and/or larger polymers/proteins are much more effective to depress the skin irritation potential of surfactants.

However, in the literature, the anti-irritant effect of proteins/polymers in the presence of surfactants has been demonstrated mostly in single surfactant solutions, and at a high polymer-surfactant ratio, not always compatible with other properties of the finished product. When they are formulated into finished products already optimized for skin compatibility through an appropriate combination of surfactants, most polymers or proteins do not bring any further mildness benefit to the product (unpublished data).

Anti-Irritation by Refattening Agents

One of the negative effects of surfactants on skin is the alteration of its lipid barrier. This can be easily assessed by measuring the TEWL (56,69), which increases with an impairment of the barrier. Using refattening ingredients or skin barrier–repairing ingredients in surfactant-based products can reduce the disruption of the barrier function if those ingredients are appropriately delivered to the skin surface. Such ingredients are often the basis for the barrier cream effect of creams (lotions) topically applied before or after contact with an irritant. Some of these ingredients can also be formulated into a surfactant system and act directly as anti-irritants in the mixture. The occlusive effect they bring at the surface of the skin delays the water loss and maintains the skin in a less-dehydrated state. Furthermore, they can progressively form an additional barrier protecting the skin against the surfactants in repetitive product applications conditions. Several types of refattening ingredients are available and can be formulated in surfactant systems. Among these are ethoxylated mono-, di-, and tri-glycerides, fatty alcohols and ethoxylated fatty alcohols, fatty acid esters, lanolin derivatives, or silicone derivatives. A few products containing a high percentage of oil also exist and can possibly be added to surfactant systems to serve in a barrier protection role.

Anti-Inflammatory Effect

Anti-inflammatory ingredients are not specific for surfactant-induced irritation, and most of them are used in pharmacology rather than in cosmetology. Because of the complexity of the inflammatory process, several families of anti-inflammatory ingredients have been developed such as glucocorticoids, nonsteroidal anti-inflammatory drugs (tacrolimus, cyclosporin, rapamycin, ascomycin, and leflunomide), flavonoids, essential oils, or α-bisabolol (70–72). In order to be effective, such ingredients must be delivered to the skin in a bioavailable form and in a sufficient amount. The case of essential oils, flavonoids, and α-bisabolol is discussed in more details in other sections of this handbook.

Antioxidants

In biological systems, antioxidants processes have a protective role against oxidative stress through three different mechanisms:

- By scavenging the early pro-oxidant species
- By preventing the initiation or the propagation of the free-radical reactions
- By returning oxidized groups to their reduced state

In dermatology and cosmetology applications, antioxidants belong to a relatively new field of investigation and interest. Some of the most important antioxidants with known applications are vitamin E, vitamin C, thiols, polyphenols, and flavonoids. Their mechanism of action in the antioxidant process is reviewed by Weber et al. [Chapter 28]. In surfactant-based products, antioxidants are only occasionally used to reduce the skin irritation potential of the product (73). However, several cutaneous enzymes are involved in the protection of the skin

against free radicals and reactive oxygen species (ROS). Such enzymes are partly denatured once surfactants penetrate the skin and the natural defense mechanisms of the skin may then become overwhelmed leading to an oxidative stress situation. Any supplementation of the skin with scavenging systems to, for example, combat surfactant irritation could result in a reduced irritation response.

Anti-Sensory Irritation

Although much less discussed than the clinical irritation, which is characterized by observable or functional alterations, subjective irritation also exists. It does not have great interest for the dermatologists, but for cosmetologists it can be the reason consumers like or reject their product.

Three different categories of sensory signals of irritation have been identified. Briefly, these are

- stinging, burning, and itching signals;
- dryness and tightness perception preceding clinical signs of irritation; and
- peculiar "irritated skin"–perceived signals unrelated with a true irritation process.

These types of irritant signals will require different "anti-irritant" systems.

Anti-Irritants for Stinging, Burning, and Itching Sensations

Strontium salts have been demonstrated to be effective and selective anti-irritants for chemically induced sensory irritation associated with stinging, burning, or itching manifestations (36). Strontium salts (nitrate or chloride) are claimed to be especially indicated for subjects with sensitive facial skin and prone to stinging sensations (36,74). The interest of strontium salts, as described by Hahn (36), is that they are very specific and selective inhibitors of the sensory signals of irritation, without suppressing other receptors (such as temperature, tactile, pressure, etc.).

Several controlled clinical studies (36,75) were run to show that strontium nitrate or chloride, at concentrations from 5% to 20%, effectively suppressed or reduced sensory irritation caused by chemical or biological irritants over a wide range of pHs from 0.6 to 12. In tests, the strontium salts were included in the solution with the irritant, or before or after the application of the irritant as shown in Table 3.

Although not tested in surfactant systems, strontium salts may play a similar beneficial effect on surfactant-induced sensory irritation, mainly in certain classes of sensitive skin subjects.

As described within the mechanism of sensory irritation, it has been observed in some studies that, on top of reducing the sensorial signs of irritation, strontium salts could also decrease the level of erythematous reactions generated by the irritant.

Table 3 Clinical Tests Support the Anti-Sensory Irritant Potential of Strontium Salts

Irritant	Test site	Timing of application[a]
Lactic acid, 7.5%, pH 1.9 (solution)	Face	Mixed, pre or post
Lactic acid, 15%, pH 3.0 (solution)	Face	Mixed
Glycolic acid, 70%, pH 0.6 (peeling solution)	Arm	Mixed
Capryloyl salicylic acid, 1% (exfoliant cream)	Cheek	Mixed
Ascorbic acid, 30%, pH 1.7 (solution)	Face	Mixed
Aluminum chloride, 20% (antiperspirant preparation)	Axilla	Pre
Aluminum/zirconium salt, 25% (antiperspirant solution)	Arm	Mixed
Calcium thioglycolate, pH 9–12 (depilatory lotion)	Leg	Post
Histamine (intradermal injection, 100 μg)	Forearm	Pre

[a]"Pre" means that strontium salts were applied to skin prior to the irritant, "post" means that the salts were applied after skin had been irritated by the irritant, and "mixed" means that strontium salts were included in the preparation with the irritant.
Source: From Ref. 36.

Although several hypotheses have been communicated to explain the mechanism of action of strontium salts (36,76), the mode of action still remains unclear. Below, please find some of author's thoughts on this matter:

- Because strontium salts mitigate the irritant event immediately after application, it is assumed that they act directly on the type-C nociceptor and suppress the neuronal depolarization that normally transmits the sensory signal to the brain.
- By their analogy to calcium, strontium salts could also use calcium channels to induce the release of neurotransmitters in synapsis or could antagonize the usual calcium-induced depolarization.
- It is also not impossible that strontium salts could directly influence keratinocytes or inflammatory cells and regulate the release of some cytokines.

Anti-Irritants for Dryness/Tightness Perception

Tightness and dryness perception are usually the earliest warning signs detected by highly receptive subjects using products that are not irritating with one single use but can become slightly irritating or skin drying after multiple exposures. These signs are generally followed, if the product is not discontinued, by the progressive development of clinical signs of intolerance such as scaling, flaking, or even erythema (77).

This kind of subclinical irritation is essentially observed for surfactant-based products and refattening agents, as described above, should be incorporated into the formula at a high concentration to mitigate the drying effect. Additionally, topical skin rehydrating preparations can also be effective in some cases to decrease the dryness/tightness perception.

Anti-Irritants for Negative Sensory Skin Feel

Negative subjective sensory signals that are translated as "irritated skin" by the consumers while totally independent of irritation can be addressed in two ways:

- If these signals are induced by the surfactant-based product, the surfactant system should be reformulated. Indeed, each surfactant is associated with a specific perception to the skin such as slipperiness, smoothness (perception of a mild product) or, at the extreme, roughness, and drag (perception of an irritant product). A good combination of surfactants can provide the desired skin feel and signal.
- Skin feel additives may be added to the product to deliver smoothness, silkiness, and a hydrated feel associated with a "non-irritated" skin signal. A review of the skin feel additives has been made by Zocchi in another section of this handbook (Chapter 34).

The Effect of Divalent Cations on Skin Irritation

Magnesium is not an Anti-Irritant for Surfactants (78)

Magnesium is frequently described as a depressor of skin irritation. Such a false idea is essentially arising from in vitro data based on protein denaturation tests. In those tests, the more a surfactant solution denatures a protein, the more it is predicted to be an irritant to the skin; and magnesium clearly depresses surfactant-induced protein denaturation in vitro (79). However, when well-controlled in vivo tests were performed to investigate the effect of magnesium directly on human volunteers, it was confirmed that magnesium does not decrease the skin irritation potential of surfactants or surfactant-based products (78). The in vivo studies included both acute irritation by occlusive patch tests and chronic irritation by repetitive short-term applications of the products. The study compared sodium and magnesium salts of surfactants (e.g., magnesium and SLS) in single solutions or incorporated into finished products and investigated the effect of adding magnesium sulfate to a solution of surfactant.

Some preliminary studies with calcium showed a behavior similar to magnesium (personal data) with an inhibition of protein denaturation in vitro while no reduction of irritation in vivo.

Zinc Salts May be Potent Anti-Irritants for Surfactants

Zinc is a key co-element in more than 200 enzymatic reactions that happen in the skin and is, as such, of critical importance to the skin (80). A few publications have shown the beneficial

protective effect of topically applied zinc oxide on skin irritation (81) and as a mediator of oxidative stress (82). Zinc oxide has also been incorporated into skin protective aerosol compositions to protect a baby's bottom from erythema (83), and in surfactant-based liquid products to exert a substantial anti-irritant effect on skin (84). Other zinc salts have been incorporated in leave-on products (gels, creams, lotions, or ointments) to reduce or prevent dermal or mucosal irritation (85).

More recently, Rigano L et al. have shown that zinc salts of coceth sulfate were very mild to the skin and that zinc salts of lauryl ether sulfate were milder than their sodium counterpart (86).

In view of the many situations in skin where zinc plays an essential role, the exact mechanism by which the zinc element exerts its beneficial effect on skin irritation has still, however, not been elucidated.

CONCLUSION

This chapter describes how surfactants interact with the skin and briefly reviews several systems by which it is now possible to control the skin irritation potential of surfactant-based products. This can be done through a

- modification of surfactant behavior in solution;
- modification of surfactant interaction with the skin surface;
- protection of the skin surface via ingredients (e.g., lipids, proteins, and polymers); and
- control of their subjective perception by the consumer using strontium salts or skin feel agents.

These anti-irritant systems, combined with a selection of mild surfactants allow the cosmetic formulator to design very mild hygiene products.

Other anti-irritant systems also exist for leave-on cosmetics and in pharmacology such as antioxidants and anti-inflammatory ingredients. They are still not yet commonly used in surfactant-based products but, if correctly delivered to the skin during the use of the product, they could provide a new field of research for improving the tolerance of cleansing products.

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44 In Vivo Irritation

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INTRODUCTION

Irritant Dermatitis

Skin irritation is a localized nonimmunologically mediated inflammatory process. It may manifest objectively with skin changes such as erythema, edema, and vesiculation or subjectively with the complaints of burning, stinging, or itching, with no detectable, visible, or microscopic changes. Several forms of objective irritation exist (Tabel 1). Acute irritant dermatitis may follow a single, usually accidental, exposure to a potent irritant and generally heals soon after exposure. An irritant reaction may be seen in individuals such as hairdressers and wet-work-performing employees, who are more extensively and regularly exposed to irritants. Repeated irritant reactions may develop into a contact dermatitis, which generally has a good prognosis. Other forms of irritant dermatitis include delayed acute irritant contact dermatitis, which occurs when there is a delay between exposure and inflammation, and cumulative irritant dermatitis, which is the most common form of irritant contact dermatitis. After exposure, an acute irritant dermatitis is not seen, but invisible skin changes occur, which eventually lead to an irritant dermatitis when exposure reaches a threshold point. This may follow days, weeks, or years of exposure (1). These various forms require specialized models to predict their occurrence after exposure to specific products.

Need for Models

Prevention of skin irritation is important for both the consumer who will suffer from it and for the industry, which needs a licensable and marketable product. Accurate prediction of the irritation potential of industrial, pharmaceutical, and cosmetic materials is therefore necessary for the consumer health and safety and for product development. Presently, animal models fulfill licensing criteria for regulatory bodies. In the European Union (EU), animal testing for cosmetics was to be banned in 1998; however, the deadline was extended to June 30, 2000, because scientifically validated models were not available. Until alternative models can be substituted, in vivo models provide a means by which a cosmetic can be tested on living skin, at various sites, and under conditions that should closely mimic the intended human use.

Many aspects of irritation have been described, ranging from the visible erythema and edema to molecular mediators such as interleukins and prostaglandins. Therefore, a variety of in vivo and in vitro approaches to experimental assay are possible. However, no model assays inflammation in its entirety. Each model is limited by our ability to interpret and extrapolate the features of inflammation to the desired context. Therefore, predicting human responses on the basis of data from nonhuman models requires particular care.

Various human experimental models have been proposed, providing irritant data for the relevant species. Human models allow the substance to be tested in the manner that the general public will use it, e.g., wash testing (see the following section) attempts to mimic the consumer's use of soaps and other surfactants. Also, humans are able to provide subjective data on the degree of irritation caused by the product. However, human studies are also limited by pitfalls in interpretation, and by the fear of applying new substances to human skin before their irritant potential has been evaluated.

ANIMAL MODELS

Draize Rabbit Models

The Draize model (2) and its modifications are commonly used to assay skin irritation using albino rabbits. Various governmental agencies have adopted these methods as standard test procedure. The procedure adopted in the U.S. Federal Hazardous Substance Act is described

Table 1 Classification of Irritant Dermatitis

Classification	Features	Clinical picture
Acute irritant dermatitis	Single exposure	Reaction usually restricted to exposed area, appears within minutes
	Strong irritant	Erythema, edema, blisters, bullae, pustules, later eschar formation
	Individual predisposition considered generally unimportant	Symptoms include burning, stinging, and pain
		Possible secondary infection
		Good prognosis
Irritant reaction	Follows repeated acute skin irritation	Repeated irritant reactions may develop into contact dermatitis
	Often occupational; hairdressers, wet workers	Good prognosis
Cumulative irritant dermatitis	Repeated exposures required	Initially subject may experience stinging
	Initial exposures cause invisible damage	Eventually erythema, edema, or scaling appears
	Exposure may be weeks, months, or years until dermatitis develops	Variable prognosis
	Individual variation is seen	
Delayed acute irritant contact dermatitis	Latent period of 12–24 hr between exposure and dermatitis	Clinically similar to acute irritant dermatitis
		Good prognosis
Subclinical irritation	Irritation detectable by bioengineering methods prior to development the of irritant dermatitis	
Subjective irritation	Subject complains of irritant symptoms with no clinically visible irritation	Perceived burning, stinging, or itching
Traumatic irritant dermatitis	Follows acute skin trauma, e.g., burn or laceration	Incomplete healing, followed by erythema, vesicles, vesicopapules, and scaling; may later resemble nummular (coin-shaped) dermatitis
Pusstular and acneiform dermatitis	Caused by metals, oils, greases, tar, asphalt, chlorinated napthalenes, polyhalogenated naphthalenes, cosmetics	Develops over weeks to months
		Variable prognosis
Friction dermatitis	Caused by friction trauma	Sometimes seen on hands and knees

Table 2 Draize-FHSA Model

Number of animals	Six albino rabbits (clipped)
Test sites	Two square inch sites on dorsum
	1 site intact, the other abraded, e.g., with hypodermic needle
Test materials	Applied undiluted to both test sites
	Liquids: 0.5 mL
	Solids/semisolids: 0.5 g
Occlusion	One square inch surgical gauze over each test site
	Rubberized cloth over entire trunk
Occlusion	24-hr period
Assessment	24 and 72 hr
	Visual scoring system

Abbreviation: FHSA, Federal Hazardous Substance Act.

in Tables 2 and 3 (3–5). Table 4 compares this method with some other modifications of the Draize model.

Draize used a scoring system to calculate the primary irritation index (PII). This is calculated by averaging the erythema scores and the edema scores of all sites (abraded and non-abraded). These two averages are then added together to give the PII value. A value of less than 2 was considered nonirritating, 2 to 5 mildly irritating, and greater than 5 severely

Table 3 Draize-FHSA Scoring System

	Source
Erythema and eschar formation	0
No erythema	
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar	4
Formation (injuries in depth)	
Edema formation	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised >1 mm)	3
Severe edema (raised >1 mm and extending beyond the area of exposure)	4

Abbreviation: FHSA, Federal Hazardous Substance Act.
Source: From Ref. 4.

Table 4 Examples of Modified Draize Irritation Method

	Draize	FHSA	DOT	FIFRA	OECD
Number of animals	3	6	6	6	6
Abrasion/intact	Both	Both	Intact 2 of each	Intact	
Dose liquids	0.5 mL undiluted	0.5 mL	0.5 mL undiluted	0.5 mL	
Dose solids in solvent	0.5 g	0.5 g moistened	0.5 g moistened	0.5 g	0.5 g
Exposure period (hr)	24	24	4	4	4
Examination (hr)	24, 72	24, 72	4, 48	0.5, 1, 24, 48, 72	0.5, 1, 24, 48, 72
Removal of test materials	Not specified	Not specified	Skin washed	Skin wiped	Skin washed
Excluded from testing	—	—	—	Toxic materials pH S2 or > 11.5	Toxic materials pH S2 or > 11.5

Abbreviations: FHSA, Federal Hazardous Substance Act; DOT, Department of Transportation; FIFRA, Federal Insecticide, Fungicide, and Rodenticide Act; OECD, Organization for Economic Cooperation and Development.
Source: From Ref. 4.

irritating. A value of 5 defines an irritant by Consumer Product Safety Commission standards. Subsequent laboratory and clinical experience that has shown the value judgments (i.e., nonirritating, mildly irritating, and severely irritating) proposed in 1944 requires clinical judgment and perspective and should not be viewed in an absolute sense. Many materials irritating to the rabbit may be well tolerated by human skin.

Although the Draize scoring system does not include vesiculation, ulceration, and severe eschar formation, all of the Draize-type tests are used to evaluate corrosion as well as irritation. When severe and potentially irreversible reactions occur, the test sites are further observed on days 7 and 14, or later if necessary.

Modifications to the Draize assay have attempted to improve its prediction of human experience. The model is criticized for inadequately differentiating between mild and moderate irritants. However, it serves well in hazard identification, often over-predicting the severity of human skin reactions (5). Therefore, Draize assays continue to be recommended by regulatory bodies for drugs and industrial chemicals.

Cumulative Irritation Assays

Several assays study the effects of cumulative exposure to a potential irritant. Justice et al. (6) administered seven applications of surfactant solutions at 10-minute intervals to the clipped dorsum of albino mice. The test site was occluded with a rubber dam to prevent evaporation, and the skin was examined microscopically for epidermal erosion.

Frosch et al. (7) described the guinea pig repeat irritation test (RIT) to evaluate protective creams against the chemical irritants, sodium lauryl sulfate (SLS), sodium hydroxide (NaOH), and toluene. The irritants were applied daily for two weeks to shaved back skin of young guinea pigs. Barrier creams were applied to the test animals two hours before and immediately after exposure to the irritant. Control animals were only treated with the irritant. Erythema was measured visually, and by bioengineering methods: laser Doppler flowmetry and transepidermal water loss (TEWL). One barrier cream was effective against SLS and toluene, whereas the other tested was not. In a follow-up study, another allegedly protective cream failed to inhibit irritation caused by SLS and toluene and exaggerated irritation to NaOH, contrary to its recommended use (8). The RIT is proposed as an animal model to test the efficacy of barrier creams, and a human version, described below, has also been proposed.

Repeat application patch tests have been developed to rank the irritant potential of products. Putative irritants are applied to the same site for 3 to 21 days, under occlusion. The degree of occlusion influences percutaneous penetration, which may in turn influence the sensitivity of the test. Patches used vary from Draize-type gauze dressings to metal chambers. Therefore, a reference irritant material is often included in the test to facilitate interpretation of the results. Various animal species have also been used, such as the guinea pig and the rabbit (9,10). Wahlberg measured skinfold thickness with Harpenden calipers to assess the edema-producing capacity of chemicals in guinea pigs. This model showed clear dose-response relationships and discriminating power, except for acids and alkalis where no change in skinfold thickness was found.

Open application assays are also used for repeat irritation testing. Marzulli and Maibach (11) described a cumulative irritation assay in rabbits that uses open applications and control reference compounds. The test substances are applied 16 times over a three-week period, and the results are measured with a visual score for erythema and skin thickness measurements. These two parameters are highly correlated. A significant correlation was also shown between the scores of 60 test substances in the rabbit and in man, suggesting that the rabbit assay is a powerful predictive model.

Anderson et al. (12) used an open application procedure in guinea pigs to rank weak irritants. A baseline response to SLS solution was obtained after three applications per day for three days to a 1 cm^2 test area. This baseline is used to compare other irritants, of which trichloroethane was the most irritant, similar to 2% SLS. Histology showed a mononuclear dermal inflammatory response.

Immersion Assay

The guinea pig immersion assay was developed to assess the irritant potential of aqueous surfactant-based solutions, but might be extended to other occupational settings such as aqueous cutting fluids. Restrained guinea pigs are immersed in the test solution while maintaining their head above water. The possibility of systemic absorption of a lethal dose restricts the study to products of limited toxic potential. Therefore, the test concentration is usually limited to 10%.

Ten guinea pigs are immersed in a 40°C solution for four hours daily for three days. A comparison group is immersed in a reference solution. Twenty-four hours after the final immersion, the animals' flanks are shaved and evaluated for erythema, edema, and fissures (13–16). Gupta et al. (17) concomitantly tested the dermatotoxic effects of detergents in guinea pigs and humans, using the immersion test and the patch test, respectively. Epidermal erosion and a 40% to 60% increase in the histamine content of the guinea pig skin were found, in addition to a positive patch test reaction in seven of eight subjects.

Mouse Ear Model

Uttley and Van Abbe (18) applied undiluted shampoos to one ear of mice daily for four days, visually quantifying the degree of inflammation as vessel dilatation, erythema, and edema. Patrick and Maibach (19) measured ear thickness to quantify the inflammatory response to surfactant-based products and other chemicals. This allowed quantification of dose-response relationships and comparison of chemicals. Inoue et al. (20) used this model to compare the mechanism of mustard oil-induced skin inflammation with the mechanism of capsaicin-induced inflammation. Mice were pretreated with various receptor antagonists, such as 5-HT2, HI, and tachykinin antagonists, showing that the tachykinin NK1 receptor was an important

mediator of inflammation induced by mustard oil. The mouse models provide simplicity and objective measurements. Relevance for man requires elucidation.

Other Methods

Several other assays of skin irritation have been suggested. Humphrey (21) quantified the amount of Evans blue dye recovered from rat skin after exposure to skin irritants. Trush et al. (22) used myeloperoxidase in polymorphonuclear leukocytes as a biomarker for cutaneous inflammation.

HUMAN MODELS

Human models for skin irritation testing are species relevant, thereby eliminating the precarious extrapolation of animal and in vitro data to the human setting. As the required test area is small, several products or concentrations can be tested simultaneously and compared. Inclusion of a reference irritant substance facilitates interpretation of the irritant potential of the test substances. Prior animal or in vitro studies, depending on model relevance and regulatory issue, can be used to exclude particularly toxic substances or concentrations before human exposure.

Single-Application Patch Testing

The National Academy of Sciences (NAS) (23) outlined a single-application patch test procedure determining skin irritation in humans. Occlusive patches may be applied to the intrascapular region of the back or the volar surface of the forearms, using a relatively non-occlusive tape for new or volatile materials. More occlusive tapes or chambers generally increase the severity of the responses. A reference material is included in each battery of patches.

The exposure time may vary to suit the study. NAS suggests a four-hour exposure period, although it may be desirable to test new or volatile materials for 30 minutes to 1 hour. Studies longer than 24 hours have been performed. Skin responses are evaluated 30 minutes to 1 hour after removal of the patch, using the animal Draize scale (Tabel 2) or similar. Kligman and Wooding (24) described statistical analysis on test data to calculate the IT50 (time to produce imitation in 50% of the subjects) and the ID50 (dose required to produce irritation in 50% of the subjects after a 24-hour exposure).

Robinson et al. (25) suggested a four-hour patch test as an alternative to animal testing. Assessing erythema by visual scoring, they tested a variety of irritants on Caucasians and Asians. A relative ranking of irritancy was obtained by using 20% SLS as a benchmark. Taking this model further, McFadden et al. (26) investigated the threshold of skin irritation in the six different skin types. Again using SLS as a benchmark, they defined the skin irritant threshold as the lowest concentration of SLS that would produce skin irritation under the four-hour occluded patch conditions. They found no significant difference in irritation between the skin types.

Cumulative Irritation Testing

Lanman et al. (27) and Phillips et al. (9) described a cumulative irritation assay, which has become known as the ''21-day'' cumulative irritation assay. The purpose of the test was to screen new formulas before marketing. A 1 in^2 of Webril® was saturated with a liquid of 0.5 g of viscous substances and applied to the surface of the pad to be applied to the skin. The patch was applied to the upper back and sealed with occlusive tape. The patch was removed after 24 hours, and then reapplied after examination of the test site. This was repeated for 21 days and the IT50 could then be calculated. Note that the interpretation of the data is best done by comparing the data to an internal standard for which human clinical experience exists.

Modifications have been made to this method. The chamber scarification test was developed to predict the effect of repeated applications of a potential irritant to damaged skin, rather than healthy skin. The cumulative patch test described above had failed to predict adverse reactions to skin damaged by acne or shaving, or sensitive areas such as the face (28).

Wigger-Alberti et al. (29) compared two cumulative models by testing skin reaction to metalworking fluids (MWF). Irritation was assessed by visual scoring, TEWL, and chromametry.

In the first method, MWF were applied with Finn Chambers® on the volunteers' mid-back, removed after one day of exposure, and reapplied further for two days. In the second method, cumulative irritant contact dermatitis was induced using a repetitive irritation test for two weeks (omitting weekends) for six hours per day. The three-day model was preferred because of its shorter duration and better discrimination of irritancy.

For low-irritancy materials in which discrimination is not defined with visual and palpatory scores, bioengineering methods (i.e., TEWL) may be helpful.

The Chamber Scarification Test

This test was developed (30,31) to test the irritant potential of products on damaged skin. Six to eight 1-mm sites on the volar forearm were scratched eight times with a 30-gauge needle, without causing bleeding. Four scratches were parallel, and the other four were perpendicular to these. Duhring chambers, containing 0.1 g of test materials (ointments, creams, or powders), were then placed over the test sites. For liquids, a saturated fitted pad (0.1 mL) may be used. Chambers containing fresh materials are reapplied daily for three days; the sites are evaluated by visual scoring 30 minutes after removal of the final set of chambers. A scarification index may be calculated if both normal and scarified skins are tested to reflect the relative degree of irritation between compromised and intact skins; this is the score of scarified sites divided by the score of intact sites. However, the relationship of this assay to routine use of substances on damaged skin remains to be established. Another compromised skin model, the arm immersion model of compromised skin, is described in the following immersion tests section.

The Soap Chamber Test

Frosch and Kligman (32) proposed a model to compare the potential of bar soaps to cause "chapping." Standard patch testing was able to predict erythema, but unable to predict the dryness, flaking, and fissuring seen clinically. In this method, Duhring chambers fitted with Webril pads were used to apply 0.1 mL of an 8% soap solution to the human forearm. The chambers were secured with porous tape, and applied for 24 hours on day 1. On days 2 to 5, fresh patches were applied for six hours. The skin is examined daily before patch application and on day 8, the final study day. No patches are applied after day 5. Applications were discontinued if severe erythema was noted at any point. Reactions were scored on a visual scale of erythema, scaling, and fissures. This test correlated well with skin-washing procedures, but tended to overpredict the irritancy of some substances (33).

Immersion Tests

These tests of soaps and detergents were developed to improve irritancy prediction by mimicking consumer use. Kooyman and Snyder (34) describe a method in which soap solutions of up to 3% are prepared in troughs. The temperature was maintained at 105°F while subjects immersed one hand and forearm in each trough, comparing different products (or concentrations). The exposure period ranged from 10 to 15 minutes, three times each day for five days, or until irritation was observed in both arms. The antecubital fossa was the first site to show irritation, followed by the hands (6,34). Therefore, antecubital wash tests and hand immersion assays were developed (5).

Clarys et al. (35) used a 30-minute/four-day immersion protocol to investigate the effects of temperature as well as anionic character on the degree of irritation caused by detergents. The irritation was quantified by assessing the stratum corneum (SC) barrier function (TEWL), skin redness (a^* color parameter), and skin dryness (capacitance method). Although both detergents tested significantly affected the integrity of the skin, higher anionic content and temperature increased the irritant response.

Allenby et al. (36) describe the arm immersion model of compromised skin, which is designed to test the irritant or allergic potential of substances on damaged skin. Such skin may show an increased response, which may be negligible or undetectable in normal skin. The test subject immersed one forearm in a solution of 0.5% sodium dodecyl sulfate for 10 minutes, twice daily until the degree of erythema reached 1 to 1+ on the visual scale. This degree of damage corresponded to a morning's wet domestic work. Patch tests of various irritants were applied to the dorsal and volar aspects of both the pre-treated and untreated forearms, and also to the back. Each irritant produced a greater degree of reaction on the compromised skin.

Wash Tests

Hannuksela and Hannuksela (37) compared the irritant effects of a detergent in use testing and patch testing. In this study of atopic and non-atopic medical students, each subject washed the outer aspect of the one forearm with liquid detergent for one minute, twice daily for one week. Concurrently, a 48-hour chamber patch test of five concentrations of the same detergent was performed on the upper back. The irritant response was quantified by bioengineering techniques: TEWL, electrical capacitance, and skin blood flow. In the wash test, atopies and non-atopics developed irritant contact dermatitis equally, whereas atopies reacted more readily to the detergent in chamber tests. The disadvantage of the chamber test is that, under occlusion, the detergent can cause stronger irritation than it would in normal use (38). Although the wash test simulates normal use of the product being tested, its drawback is a lack of standard guidelines for performing the test. Charbonnier et al. (39) included squamometry in their analysis of a hand-washing model of subclinical irritant dermatitis with SLS solutions. Squamometry showed a significant difference between 0.1% and 0.75% SLS solutions, whereas visual, subjective, capacitance, TEWL, and chromametry methods were unable to make the distinction. Charbonnier suggests squamometry as an adjunct to the other bioengineering methods. Frosch (33) describes an antecubital washing test to evaluate toilet soaps, using two washing procedures per day. Simple visual scoring of the reaction (erythema and edema) allows products to be compared. This comparison can be in terms of average score, or number of washes required to produce an effect.

Assessing Protective Barriers

Zhai et al. (40) proposed a model to evaluate skin protective materials. Ten subjects were exposed to the irritants, SLS and ammonium hydroxide (in urea), and Rhus allergen. The occluded test sites were on each forearm, with one control site on each. The irritant response was assessed visually using a 10-point scale, which included vesiculation and maceration unlike standard Draize scales. The scores were statistically analyzed for nonparametric data. Of the barrier creams studied, paraffin wax in cetyl alcohol was found to be the most effective in preventing irritation.

Wigger-Alberti and Eisner (41) investigated the potential of petrolatum to prevent epidermal barrier disruption induced by various irritants in a repetitive irritation test. White petrolatum was applied to the backs of 20 human subjects who were exposed to SLS, NaOH, toluene, and lactic acid. Irritation was assessed by TEWL and colorimetry in addition to visual scoring. It was concluded that petrolatum was an effective barrier cream against SLS, NaOH, and lactic acid, and moderately effective against toluene.

Frosch et al. (7) adapted the guinea pig RIT previously described for use in humans. Two barrier creams were evaluated for their ability to prevent irritation to SLS. In this repetitive model, the irritant was applied to the ventral forearm, using a glass cup, for 30 minutes daily for two weeks. One arm of each subject was pre-treated with a barrier cream. As in the animal model, erythema was assessed by visual scoring, laser Doppler flow, and TEWL. Skin color was also measured by colorimetry (La^* value). The barrier cream decreased skin irritation to SLS, the most differentiating parameter being TEWL and the least differentiating being colorimetry.

Bioengineering Methods in Model Development

Many of the models previously described do not use the modern bioengineering techniques available, and therefore data based on these models may be imprecise. Despite the skill in investigations, subjective assessment of erythema, edema, and other visual parameters may lead to confusion by inter-and intra-observer variation. Although the eye may be more sensitive than current spectroscopy and chromametric techniques, the reproducibility and increased statistical power of such data may provide greater benefit. A combination of techniques, such as TEWL, capacitance, ultrasound, laser Doppler flowmetry, spectroscopy, and chromametric analysis, in addition to skilled observation may increase the precision of the test. Andersen and Maibach (42) compared various bioengineering techniques, finding that clinically indistinguishable reactions induced significantly different changes in barrier function and vascular status. An outline of many of these techniques is provided by Patil et al. (5).

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45 Noninvasive Clinical Assessment of Skin Irritation/Inflammation

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INTRODUCTION

The study of skin biology has been undergoing a transformation over the past 20 years, owing to advances in technology that permit evaluation of parameters invisible to the naked eye. Many years ago, Kligman coined the term "invisible dermatoses" to emphasize that what appears visually normal can be quite abnormal under the skin surface (1). He even suggested that the future of dermatology would become so reliant on non-visual methods of diagnosis that the inability to see would not preclude one from a career in the field (2). Evolving techniques and instrumentation have facilitated the study of many of the skin's physiological and biophysical properties, including water content, barrier properties, tensile strength, and elasticity, and even estimates of melanin, hemoglobin, and collagen. For those interested in the immune and inflammatory response of the skin, however, instrumental methods have been less useful.

Surface assessment and grading of inflammatory skin reactions have long relied on visual methods (3–5). Certain instrumental methods, such as laser doppler flowmetry and colorimetry, provide some degree of numerical quantification of surface skin reactions that can supplement simple visual grades (6). However, the detailed study of the cells and molecular processes underlying skin inflammation has generally required use of highly invasive (e.g., biopsy), or moderately invasive (e.g., suction blister), techniques (7–10).

In the mid-1990s, new techniques were introduced to study skin inflammation by attempting to adsorb molecular mediators of inflammation from the skin surface or within layers of the stratum corneum. First introduced in the mid-1990s by Japanese investigators, the approach was to use a relatively mild cellophane tape-stripping method (11) and measure extracted constitutive cytokine levels (and ratios) in sun-exposed, unexposed, and UV-irradiated skin. At the time this work first appeared in print, our laboratory was independently developing and later presented (12,13) and published (14) an even less-invasive approach that used minimally adhesive Sebutape® for mediator adsorption. Over the next several years, both groups reported on the additional application of these procedures for the "targeted proteomic" detection of inflammatory mediators (cytokines) in various types of compromised skin conditions and diseases. Other investigators have more recently adopted these techniques, using somewhat more aggressive tape-stripping techniques to probe cytokine or mRNA expression patterns at deeper levels of stratum corneum.

CELLOPHANE TAPE ADSORPTION OF PROTEIN MEDIATORS OF SKIN INFLAMMATION

The original procedure for noninvasive tape adsorption of human skin for assessment of inflammatory mediators was that of Hirao and coworkers, who used cellophane tape to extract the constitutive cytokines interleukin-1 alpha (IL-1α) and its competitive inhibitor interleukin-1 receptor antagonist (IL-1ra) from the stratum corneum of sun (UV)-exposed and unexposed skin (11). Their basic procedure was to cleanse the sampling site with soap and water, tape strip once with cellophane tape (which was discarded), and re-strip the same site. The second tape was extracted (sonicated in buffer) and assayed for immunoreactive IL-1α and IL-1ra. The cytokines were measured directly by enzyme immunoassay as well as by immunoblotting and functional (induced cell proliferations) assays.

In comparing sun-exposed (face) and unexposed (inner arm) skin sites, they saw higher levels of IL-1α on the arm versus face and higher levels of IL-1ra on the face. Levels of both cytokines were normalized to total recovered protein. The ratio of IL-1ra/IL-1α was approximately 8 on the arm and >100 on the face. IL-1ra activity and the IL-1ra/IL-1α ratio were also increased on UV-unexposed back skin for one to four weeks after 2 MED irradiation of the skin. These results suggested that chronic inflammation due to UV from either natural sun exposure or UV lamp irradiation was associated with elevated IL-1ra production, perhaps a regulatory response to IL-1α-induced inflammation and an effort by the skin to quell this response and restore homeostatic balance.

Two years later, this same laboratory expanded on their initial findings by again demonstrating increased ratios of IL-1ra/IL-α in inflamed skin (15). In this second study, they examined involved versus uninvolved skin sites in subjects with psoriasis, atopic dermatitis, and senile xerosis. Their interest in psoriasis stemmed from earlier findings from Cooper's laboratory (using keratomed epidermal skin samples) that involved psoriatic skin had increased IL-1ra/IL-1α ratios compared with uninvolved skin from the same patients (16). The tape-stripping procedure they employed in the second study was a slight variation of the original method insofar as only a single tape application was used.

They confirmed increased IL-1ra/IL-1α ratios in sun-exposed versus unexposed skin and also observed increased ratios in all of the inflammatory skin conditions versus uninvolved skin from the same subjects. Because of intersubject variability in the measured cytokine amounts recovered, not all the comparisons were statistically significant; however, there were obvious directional changes even for those comparisons that were not significantly different. Their conclusion from these studies was that an increased IL-1ra/IL-1α ratio in the stratum corneum represents a nonspecific phenomenon in any inflammatory skin condition; likely reflecting a regulatory response against unchecked inflammation.

SEBUTAPE ADSORPTION OF PROTEIN MEDIATORS OF SKIN INFLAMMATION

The work of Hirao et al. was unknown to us as we began to investigate the possibility of using noninvasive tape adsorption methods to assess mediators of skin inflammation back in late 1994. We examined a variety of approaches to this problem, including a variety of tapes, extraction methods, and mediators of interest. We settled upon Sebutape as the adsorbent tape of choice for two main reasons. An acrylic polymer film manufactured by CuDerm, this tape was much less adhesive and adherent to skin than either cellophane tape or another CuDerm tape product, D-Squame® (a polyacrylate ester adhesive). Multiple one-minute applications of Sebutape to the same skin site did not strip off the stratum corneum, unlike the other two tapes. As noted in our first publications on the method, we obtained complete and quantitative recovery of spiked cytokine when the Sebutapes were sonicated and vortexed to extract the material (12,14).

Sebutape turned out to be a very flexible adsorbent tape for our purposes. It could be applied to overtly inflamed skin (including infant skin) without causing pain on removal. It could also be applied to mucosal surfaces and trimmed and applied to tight spaces with limited skin surface area (e.g., scalp skin between parted hairs). Proteins (cytokines) of interest were assayed by enzyme immunoassay and recoveries were normalized to total protein to reduce intersubject variability. Figure 1 shows some of the Sebutape application methods used in our studies.

Reapplication of up to 30 of these tapes to the same area of skin did not produce any erythema—common with cellophane tape stripping—and also tended to recover similar amounts of the cytokine IL-1α (Fig. 2) with each application, confirming historical findings that the stratum corneum acts as a reservoir (sink) for this cytokine (18). No inducible cytokine (IL-8) was detected even 24 hours after the initial tape collections—again indicating a lack of tape-induced irritation.

Consistent with the work of Hirao and coworkers, we found a reproducible elevation of IL-1ra and the IL-1ra/IL-1α ratio on sites of the body (face, lower leg, forearm) that were prone to sun exposure and lower ratios on generally sun-protected skin sites (upper leg, back, underarm) (Fig. 3). We also observed elevated IL-1ra/IL-1α ratios and evidence of induced IL-8 (Fig. 4) on infant skin associated with different types of diaper dermatitis. In contrast, an

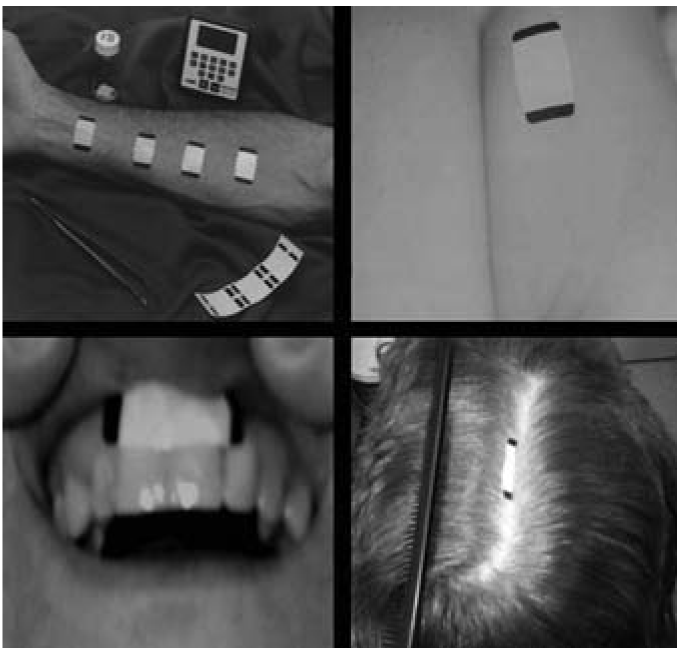


Figure 1 Photographs of Sebutape® application to various skin and mucosal surfaces. *Source*: From Ref. 17.

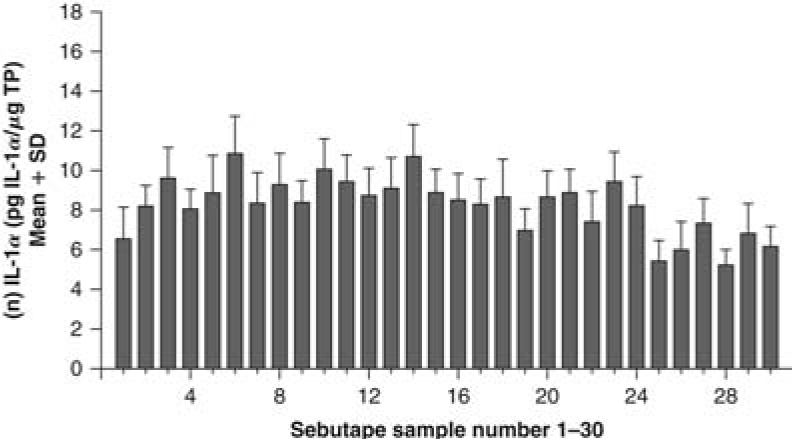


Figure 2 Recovery of IL-1α from Sebutape® samples collected from the same forearm skin site. Thirty successive Sebutape samples were collected from the same normal-appearing naive skin site on the lower volar forearm of four subjects ($n = 2$ sites/subject). Each bar represents the group mean IL-1α level (±SD; $n = 8$ samples) for each of the 30 Sebutape™ samples collected from each of the two skin sites. The IL-1α/total protein levels from samples 1 to 30 range between 5 and 15 pg IL-1α/mg total protein. There were no detectable levels of the inducible cytokine IL-8 (assay sensitivity 10 pg/ml) in any of the tape extracts, including samples collected 24 hours after the initial tape collection. *Source*: From Ref. 14.

acute (1 hour) exposure to a high concentration of the irritant surfactant, sodium dodecyl sulfate (SLS), which is sufficient to produce a weak erythematous response 24 hours after exposure (5), produced an opposite effect. In this situation, IL-1α (measured 24 hours after the SLS exposure) was elevated and the IL-1ra/IL-1α ratio was decreased. This indicated that IL-1α is induced and mobilized in the acute irritation response and that the IL-1ra levels increase later on; again supporting the role of elevated IL-1ra as a means to regulate more chronic inflammatory responses.

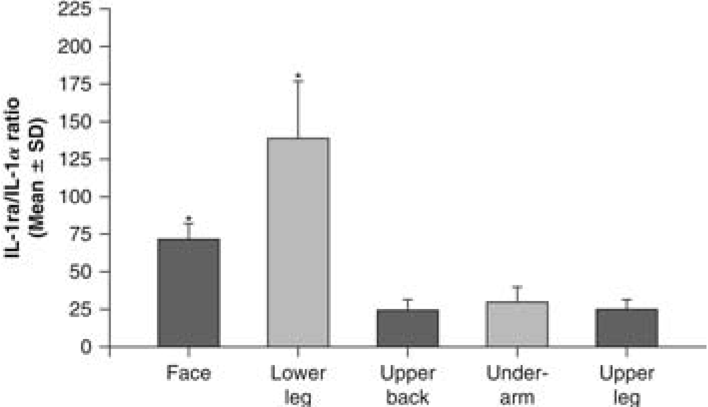
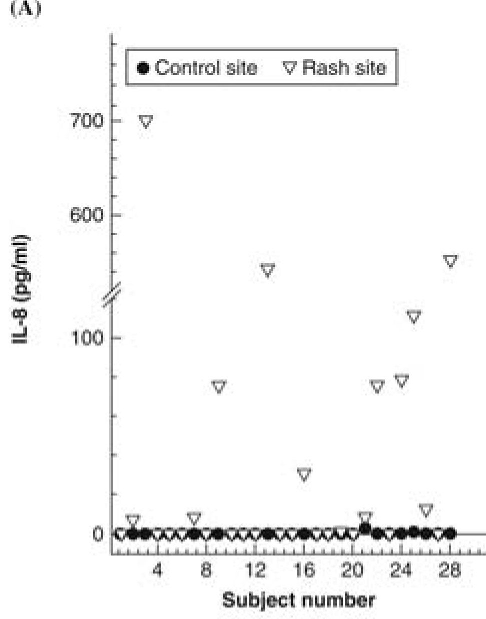


Figure 3 Cytokine levels in sun-exposed versus unexposed skin. Sebutape® samples were collected from different body sites of adult (aged 18–65 years) male and female subjects with normal-appearing unblemished skin. The ratio of IL-1ra/IL-1α for sun-exposed facial skin (mean ± SD of all sites) and lower leg were significantly higher (~3–6 times, respectively) than skin that was minimally sun-exposed (upper back, underarm, upper leg). *Source*: From Ref. 14.



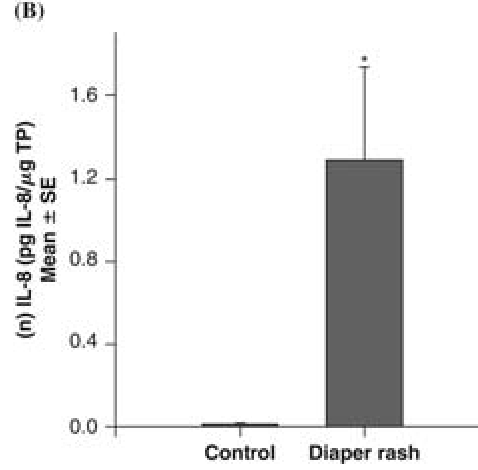


Figure 4 Correlation between skin reactions in diaper area and IL-8 recovery. Sebutape® samples were collected from infant skin sites with different rash severity and from control leg sites. Individual levels of IL-8 for each subject ($n = 28$) for control and diaper rash sites (all rash grades) are shown (**A**). The normalized IL-8 levels (**B**) were significantly higher in rash versus control sites (* $p \leq 0.05$; paired Student's t-test). *Source*: From Ref. 14.

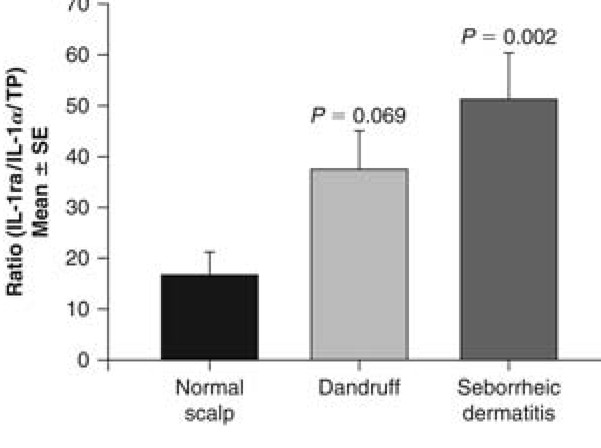


Figure 5 The ratio of IL-1ra/IL-1α normalized to total protein was significantly increased in the seborrheic dermatitis scalp group compared with normal scalp controls. The dandruff group approached significance when compared with the normal scalp group. *Source*: From Ref. 19.

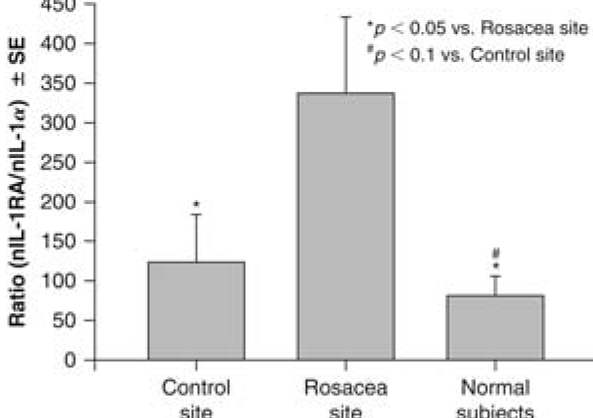


Figure 6 The ratios of normalized interleukin-1ra/interleukin-1α were determined for rosacea subjects (involved and noninvolved control sites) and normal subjects. *Source*: From Ref. 22.

The elevation of the ratio of IL-1ra/IL-1α was a hallmark indicator of every inflammatory condition (skin or mucosal) that we studied over a period of six years. In addition to our findings with sun-exposed skin and infant diaper dermatitis noted above, we saw directional or significant elevation in this ratio in dandruff and seborrheic scalp dermatitis (19) (Fig. 5). Seborrheic dermatitis and dandruff were also associated with elevated recovery of the inducible immune or inflammatory cytokines IL-2 and TNF-α, respectively. Rosacea, an inflammatory skin condition (20), with known cytokine involvement (21,22) was also shown to be associated with elevated IL-1ra/IL-1α ratios (23) (Fig. 6). Involved skin sites showed elevated ratios compared with uninvolved skin sites from the same subjects. However, even the uninvolved sites showed slightly elevated ratios compared with facial skin sites sampled from normal control subjects.

In addition to our work on skin and overt inflammatory conditions, we expanded this technique to other realms of the inflammatory response. A common technique for the study of oral inflammation (e.g, gingivitis) is to try to recover small amounts of gingival fluid for assay. As shown in Figure 1, we were able to apply Sebutape over the gum surface and adsorb cytokines as an alternative, a somewhat simpler method (24). The objective of the study was to examine the relationship between changes in cytokine levels and clinical inflammation. Subjects participated in a 14-day experimental gingivitis (EG) model, where five days following a dental prophylaxis subjects refrained from all oral hygiene measures for 14 days. A gingivitis index (GI) and gingival bleeding were assessed clinically by standard techniques. Sebutape samples of each subject's gingival surface were collected from the right posterior buccal quadrant at baseline (pre-EG) and day 14 (post-EG). The tapes were analyzed for both IL-1α and IL-1β. Over a 14-day EG period, statistically significant ($p < 0.05$) increases in GI, gingival bleeding, and IL-1α (Fig. 7) were observed. A directional increase in IL-1β was also observed.

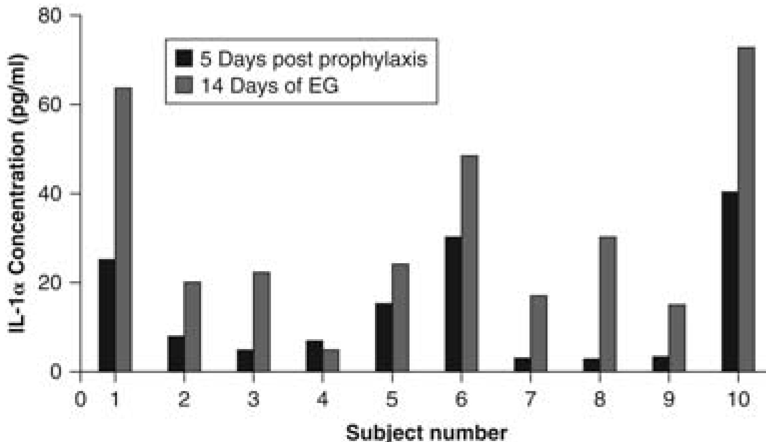


Figure 7 Sebutape samples were collected from 10 adult clinical subjects in an experimental gingivitis (EG) model. Baseline samples were collected 5 days after dental prophylaxis (*black bar*) and then after 14 days of EG (*gray bar*). Samples were extracted in saline and analyzed for IL-1α.

A final adaptation of this method was to try and identify skin surface biomarkers that could be associated with, and diagnostic of, neurosensory skin irritation. Because sensory irritation is purely symptomatic in nature, we applied this technique to see if a more objective index of the response could be developed through the Sebutape biomarker adsorption method. Though we were unable to completely investigate this hypothesis, we did have some early success in demonstrating reduced nitric oxide (NO) recoveries from the skin of test subjects experiencing weak-to-moderate stinging responses to lactic acid or capsaicin (25). Looking at cytokine recoveries, we also saw reduced IL-1ra levels in dandruff subjects after one and two weeks use of an antidandruff shampoos versus a placebo shampoo. This reduction was seen in parallel with a reduction in scalp itch symptoms (unpublished). Assuming that the antidandruff shampoo active was effective in helping to quell the inflammatory response in the scalp, this reduction in IL-1ra is consistent with its role in downmodulating the Il-1α triggered inflammatory response. A reduction in inflammation due to shampoo treatment would likely result in lower endogenous IL-1α and a resultant reduction in IL-1ra.

RECENT ADAPTATIONS OF SKIN TAPE APPLICATION METHODS FOR ASSESSMENT OF STRUCTURAL AND INFLAMMATORY PROTEINS AND GENE EXPRESSION PROFILING

A source of frustration surrounding our early work in this area was the need to run individual immunoassays on each protein of interest. This limited the number of analyses that could be run on each sample. More recently, multiplex immunoassays (e.g., Luminex beads) or assay services (e.g., Rules-Based Medicine, Austin, TX) have opened the door to more extensive analysis of adsorbed proteins or other biomarkers. One such application, looking at structural skin proteins and serological markers, was recently published by Hendrix et al. (26). They used D-Squame tapes to adsorb and quantify structural proteins (involucrin, fibronectin, keratins-1, -6, and -10) and plasma biomarkers (cortisol, human serum albumin) from healthy forearm skin. They used a multi-analyte-profiling method, SkinMAP™ (Linco Research, St. Charles, MO). This initial study defined study design and extraction procedures for future work. It is likely that cytokine analysis will also be forthcoming via this method.

A recent study from the Netherlands used a more aggressive tape-stripping method with several different types of tape (including D-Squame) and examined baseline and SLS-induced cytokine recovery at three different levels from the outer stratum corneum to the lower stratum corneum just above the viable epidermis (27). Similar to our findings, they reported increased IL-1ra (and IL-1ra/IL-1α ratios) in chronically irritated (SLS-exposed) skin. This increase was consistent across all levels of the stratum corneum. A slight increase in IL-8 was also observed.

Another application of tape stripping of skin has been to recover mRNA fragments that code for inflammatory or other proteins. Morhenn et al. first reported on this technique in 1999, showing differential recoveries of RNAs coding for the cytokines IL-4 and IL-8 and the enzyme-inducible nitric oxide synthase (28). They recovered the RNA from multiple (up to 23) D-Squame tape strips of the skin and used a ribonuclease protection assay for detection and quantification. They showed distinctly elevated recoveries of RNA for all three proteins from allergic contact dermatitis skin sites versus irritant contact dermatitis skin sites. Later, using a more limited four-repeated tape-stripping procedure, they recovered and amplified mRNA from normal and SLS-irritated skin and examined gene expression profiles via microarray analysis (29). They demonstrated significantly altered expression in over 1700 genes as the result of SLS-induced skin irritation. In similar fashion, Benson et al. (30) used four tape strips from psoriatic skin sites or non-lesion sites and measured cytokine and keratin protein recoveries via RT-PCR analysis. Using this approach, they detected changes (overexpression) in biomarkers that were distinct from those seen via biopsy procedures and felt that the more noninvasive approach was a useful adjunct for the study of this disease.

CONCLUSION

The ability to study the inflammatory response of the skin has traditionally relied on invasive techniques to collect cells and mediators of this complex response. Skin surface adsorption of biomarkers of inflammation certainly does not tell the entire story of the underlying inflammatory processes. By the simple fact that adsorption is from the surface of the nonviable stratum corneum, the molecules collected were derived from earlier synthetic processes in response to some stimulus. This may make it difficult to easily discern (from the absorbed molecule profiles) the exact nature or mechanisms of acute inflammatory responses. However, chronic conditions or diseases, for which the molecular responses are ongoing, are much more assessable by this approach. The consistent finding (from multiple skin conditions, diseases, and across several laboratories over the years) of elevated IL-1ra/IL-1α ratios is a testament to the validity and utility of the approach and the value of noninvasive tape adsorption of biomarkers as a means to detect, differentially diagnose, and evaluate inflammatory skin conditions.

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46 Detecting Skin Irritation Using Enhanced Visual Scoring: A Sensitive New Clinical Method[a]

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INTRODUCTION

Skin testing on panels of volunteer human subjects to confirm that a new material is nonirritating is a routine part of the overall product safety assessment. Tests to assess irritation range from standard occluded patch tests to tests that exaggerate the concentrations of actual expected exposures. Currently, these tests rely mainly on unaided visual scoring of erythema to determine the degree of irritation.

As with any other type of inflammatory reaction, skin irritation triggers a series of events involving subsurface dilatation of blood vessels with an influx of inflammatory cells. Subsequently, erythema (along with possible swelling and heat) appears on the skin surface. However, by the time consumer products being developed reach the stage when they can be safely and ethically tested on human volunteers, products and ingredients that may cause frank irritation have been eliminated. The resulting products are virtually nonirritating and produce, at most, very minor visual changes, even under conditions of highly exaggerated exposures. This presents a difficult challenge when trying to differentiate between the potential skin effects of two closely related products. [For a more complete discussion on irritation, see Chew and Maibach (1).]

Recently, we evaluated the effectiveness of a polarized light visualization system that uses both parallel- and cross-polarized light. Our goal was to determine if enhanced visual scoring using a polarized light visualization tool would enable us to detect subclinical irritation, i.e., changes that occur before irritation is obvious to the unaided eye, or changes that may still be present after visible changes have resolved. If successful, this would lead to (*i*) increased sensitivity of our testing program enabling us to differentiate between very mild test products, (*ii*) improved claims support, and (*iii*) better guidance in our product development efforts. It would also enable us to design tests that use fewer exposures of shorter duration, resulting in programs that are less costly and cause less discomfort to our panels of volunteer subjects.

PROCEDURE

Two basic test designs were used: the standard patch test and the behind-the-knee (BTK) test. Typically, these tests would be conducted as a part of routine evaluation of the potential irritant properties of a material. The patch test measures the potential for irritation due to the chemical structure of a material (i.e., chemical irritation). The BTK test measures both chemical irritation and the potential for mechanical irritation due to friction (2–4). Scoring was conducted to compare unaided visual scoring to enhance visual scoring with a polarized light visualization system.

The test materials consisted of low concentrations of Sodium Lauryl Sulfate (SLS) and two currently marketed brands of feminine hygiene pads. Pad A is a standard pad with an absorbent core and a fabric-like, polyethylene film top sheet. Pad B is a standard pad with an absorbent core and a nonwoven fabric top sheet. These products are similar in their potential to cause irritation, yet consumers prefer product B over product A due to the absence of adverse skin sensations.

[a]Same parts Adapted from Farage MA. Enhancement of visual scoring of skin irritant reactions using cross-polarized and parallel polarized light. Contact Dermatitis 2007; 57: 1–9. (With kind permission from Blackwell Publishing Group.)

For each study, the protocol was approved by the test facility's institutional review board. Participants in all the studies were healthy adult volunteers aged 18 to 65 years who had signed an informed consent. Subjects could withdraw from the study at any time. The studies were conducted in compliance with the Good Clinical Practices regulations as prescribed by the Food and Drug Administration (5).

All subjects had very sensitive to moderately sun-sensitive skin (types I–IV) according to Fitzpatrick's classifications (6). Subjects were excluded from participation if they (*i*) had sunburn, acne, scar tissue, dermatitis, or any other skin abnormality at the test sites, (*ii*) were taking anti-inflammatory corticosteroids or other medications that may interfere with test results, (*iii*) had participated in an arm patch test within the last four weeks, (*iv*) had diabetes or any medical condition that might compromise the immune system, or (*v*) were pregnant. Subjects were instructed to refrain from using lotions, creams, or any other skin preparation in the test area; to refrain from swimming and tanning/sun exposure; and to refrain from taking any anti-inflammatory, anti-histamine, and/or steroid medications while participating in the study.

Test materials were applied as follows.

Testing on the Upper Arms and Lower Forearms

Standard patch testing has been described previously (3). Samples were applied using an occlusive, nonwoven cotton pad (Webril patch, Professional Medical Products Company, Kendall LTP, Chicopee, Massachusetts). The adhesive was reinforced with an occlusive, hypoallergenic tape [Blenderm occlusive tape (3M Health Care, St. Paul, Minnesota)]. On the upper arm, patches were applied lengthwise midway between the shoulder and the elbow on the lateral surface, with a minimum of 2-cm space between patches. On the lower forearm, patches were applied lengthwise to the volar surface midway between the elbow and the wrist.

In the first experiment, samples were applied to the upper arm for 24 hr/day for three consecutive days. In the second experiment, patches were applied to both the upper arm and the lower forearm for either 2 or 6 hr/day for two consecutive days.

Patch sites for the different test materials were rotated among subjects. Separate patches were applied for sites that would be scored visually and sites that would be scored using the visualization instrument to avoid bias that might be introduced inadvertently by repeated grading of a specific site. Patch sites were marked with 0.5% gentian violet to aid in visual grading and to ensure that the patches were applied to identical sites each day for the duration of the test. Panelists were instructed to remove the patches at specific times and to return to the laboratory for grading and/or reapplication of test materials 30 to 60 minutes later. Further details of each experiment are included in the appropriate figure legend.

BTK Testing

The protocol was a modification of the BTK test described previously (2–4). The test material was placed horizontally and held in place behind the knee by an elastic knee band of appropriate size. Test materials were removed by the panelists 30 to 60 minutes prior to returning to the laboratory for grading and/or reapplication of materials. Samples were left in place for six hours each day, for four consecutive days.

Grading was conducted prior to the first patch application (i.e., baseline), and 30 to 60 minutes after removal of each patch (upper arm or lower forearm) or BTK application (i.e., "post patch"). In the BTK and short duration patches (2 or 6 hours on the upper arm or lower forearm), scoring was also conducted the following morning after sample removal, prior to the next sample application (i.e., "recovery").

Standard visual grading was done by a trained, expert grader under a 60 to 100 watt incandescent daylight blue bulb. Enhanced visual grading was conducted using a polarized light visualization system (Syris v600® Visualization System, Syris Scientific, LLC, Gray, Maine; available at: www.syrisscientific.com) with separate scores recorded for parallel-polarizing illumination (surface mode) followed by cross-polarizing illumination (subsurface mode). The subsurface mode allows visualization of the site at a depth of 1 mm beneath the surface.

Erythema was graded according to a previously described scale of "0" to "4", where 0 is no apparent cutaneous involvement and 4 is moderate-to-severe, spreading erythema and/or edema (2). The same grader was used throughout an experiment, and the grader was not

aware of the treatment assignments. If a test site exhibited a grade of "2" or greater, the test material was not reapplied at that site. However, the site was graded until completion of the test. Any test site showing a grade of 2 or more at the final grading timepoint was followed until the response regressed to a "1.5" or less.

For the BTK test, panelists kept a daily diary of skin problems experienced at the test sites, as described previously (7,8). Panelists were asked if they experienced any of the following sensations: the sample rubbing against the skin, the sample sticking to the skin, chafing, burning, itching, pain, edema, or any other discomfort. Results were evaluated for a higher occurrence of each individual skin problem with one treatment versus another.

Stratified CMH (Cochran-Mentel-Haenszel) comparisons were used to evaluate erythema scores, unless otherwise indicated. For sensory effects, treatment comparisons were evaluated using McNemar's test. Results of the statistical analyses conducted for each experiment are described in the appropriate figure legend.

SKIN IRRITATION ASSESSMENT

In the standard 24h-patch test: grades using polarized and cross-polarized light were similar to the grades resulting from standard visual assessment. The mean erythema grade after the first 24-hour patch application to the upper arm was statistically significantly different from the baseline grade for all three grading methods (visual, subsurface using cross-polarized light, and surface using parallel-polarized light) (Fig. 1). For all test concentrations of SLS (0%, 0.025%, 0.05%, and 0.1%), the mean erythema remained significantly elevated over baseline grade with subsequent patch applications. A repeat experiment using 24-hour patches of SLS at concentrations of 0.01% and 0.1% on the upper arm and 0.01% on the volar surface of the forearm showed similar results (data not shown).

Enhanced visual grading was more effective in detecting very minor irritation. Patch tests were conducted on the upper and lower arm under conditions that produce very low levels of irritation (e.g., concentrations of SLS of 0.01% and patch test exposures of 2 and 6 hours). Grading with cross-polarized light (subsurface grading) detected statistically significant increases in mean erythema compared with baseline after a single patch application for two hours or six hours on the forearm (Fig. 2A), and for six hours on the upper arm (Fig. 2B). With a patch exposure of two hours on the upper arm, the increase in subsurface erythema was not significant immediately after patch removal, but was significant after 22 hours of recovery (i.e., recovery 1).

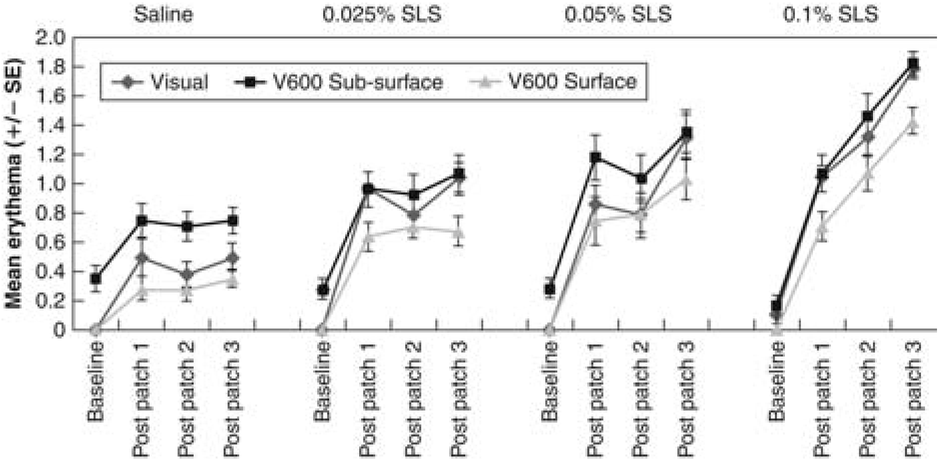
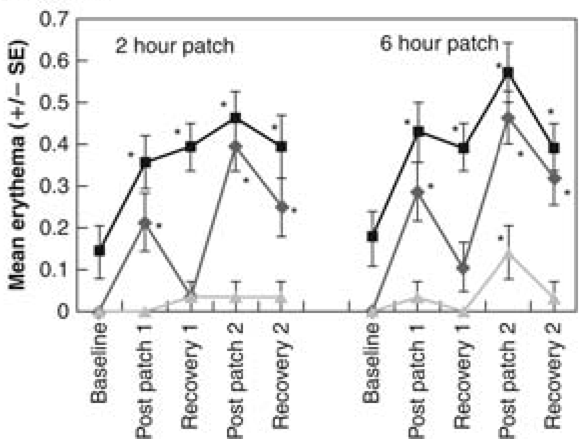


Figure 1 Standard visual and enhanced visual grading (24-hour patch test on the upper arm). Test samples in the 24-hour patch test consisted of saline, 0.025%, 0.05%, and 0.1% SLS. Panelists (13–14 per group) were patched on the upper arm for 24 hr/day for three consecutive days. Scoring was conducted at baseline (prior to treatment), and 30 to 60 minutes after removal of each patch (post patch 1–3). The graph plots mean erythema (±S.E.) at each scoring timepoint. Stratified CMH comparisons were used to evaluate visual scores for 0.05% and 0.1% SLS. ANCOVA was used for all other treatments. For all treatments, visual, subsurface and surface scores after patches 1 to 3 were significantly elevated over baseline ($p \leq 0.05$, not shown on graph). *Abbreviation*: CMH, Cochran-Mentel-Haenszel; ANCOVA, analysis of Co-variance.



(B) Upper arm

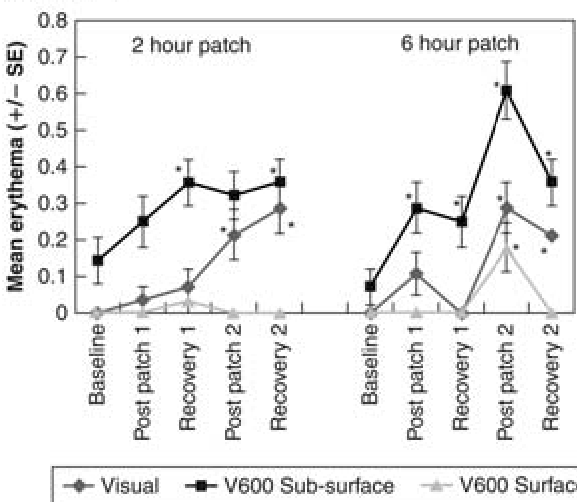


Figure 2 Standard visual and enhanced visual grading using very mild conditions in the patch test (lower forearm and upper arm). Multiple patches of the 0.01% SLS were applied to both (**A**) the forearm and (**B**) upper arm for two consecutive days (14 panelists per group). Patches were removed after two hours or six hours. Scoring was conducted at baseline, 30 to 60 minutes after removal of each patch (post patch 1–2), and the morning following each patch removal (recovery 1–2). The graph plots mean erythema (±S.E.) at each scoring timepoint. Results were evaluated using the stratified CMH test. (*Significant difference from baseline, $p \leq 0.05$). *Abbreviation*: CMH, Cochran-Mentel-Haenszel.

Similar results were obtained using 0.03% SLS, with patches applied for two and six hours (data not shown). Irritation was apparent earlier with the subsurface scoring (upper arm) and tended to remain elevated, even after overnight recovery.

Enhanced visual grading increased the ability to differentiate between two similar products in the BTK test. At the afternoon scoring, conducted immediately after removal of the first test sample (i.e., post–patch 1 scoring), the subsurface scores for the two products were significantly different and remained different for the subsequent post-patch scoring times. This difference was apparent using unaided visual scoring and surface scoring (Fig. 3A and C), and the subsurface assessment using cross-polarized light (Fig. 3B). However, at the scoring after the test sites recovered overnight (i.e., recovery scoring), significant differences between the two products were not apparent until after the third application for the visual scoring (recovery 3, Fig. 3D) and after second application for surface scoring (recovery 2, Fig. 3F). Subsurface scoring showed the products to be significantly different after one single application (Fig. 3E).

Enhanced visual grading results were consistent with sensory effects in the BTK test. Test panelists' diaries enabled evaluation of sensory effects. With every sample application, significantly more individuals reported burning sensations with pad A compared with pad B (Fig. 4). In addition, a significantly higher number of individuals reported pain with pad A during the third sample application and the sensation of the sample sticking to the skin during the second and third applications (data not shown).

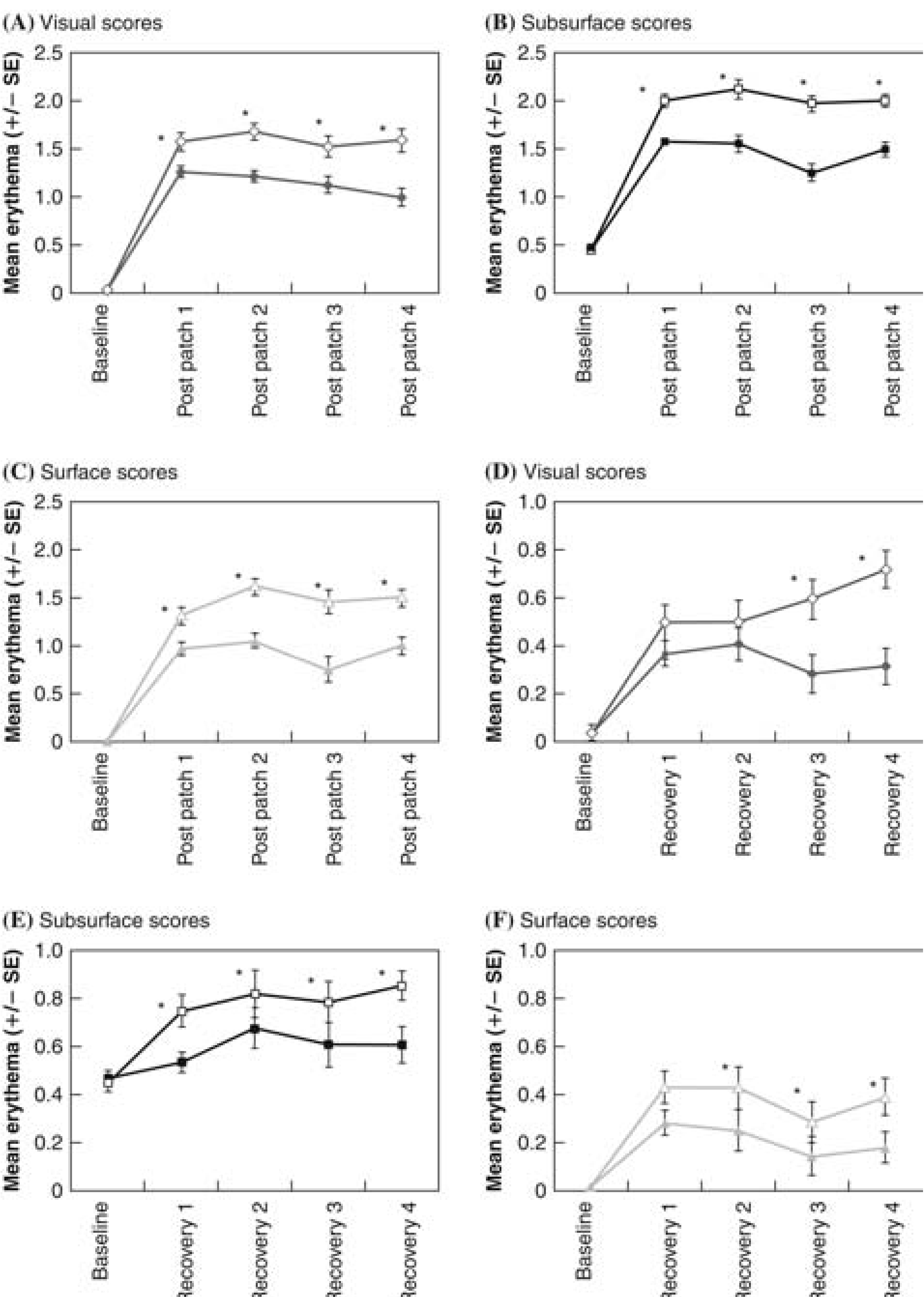


Figure 3 Standard visual and enhanced visual grading using two similar products in the BTK. Two feminine protection products (pad A and pad B) were evaluated in the BTK. Samples were applied for 6 hr/day for four consecutive days (14–16 panelists per group). Scoring was conducted at baseline, 30 to 60 minutes after removal of each patch (post patch 1-4, **A**–**C**), and the morning following each patch removal (recovery 1–4, **D**–**F**). The graph plots mean erythema (±S.E.) at each scoring timepoint. Treatment comparisons were evaluated using the stratified CMH test. (*Significant difference between pad A and pad B, $p \leq 0.05$). Pad A = open symbols. Pad B = closed symbols. *Abbreviations*: BTK, behind-the-knee; CMH, Cochran-Mentel-Haenszel.

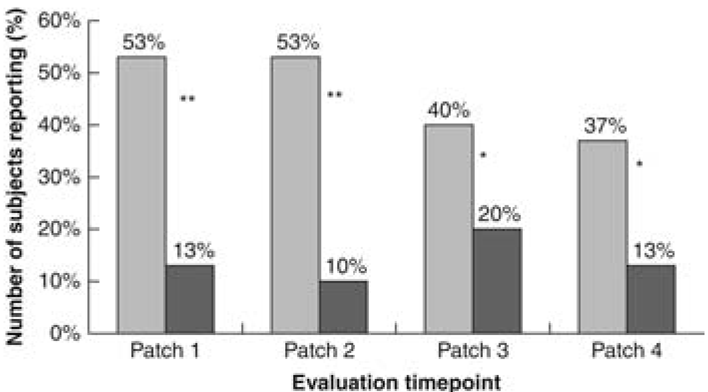


Figure 4 Reports of burning sensations in the BTK. In the BTK, each of the 30 panelists was asked to keep a daily diary of skin problems experienced at the test sites. Results were evaluated for a higher occurrence of each individual skin problem with one treatment versus another. The graph plots the number of subjects reporting sensations of burning at the test sites during each patch application. Treatment comparisons were evaluated using McNemar's test. (**Significant difference between pad A and pad B, $p < 0.001$). (*Significant difference between pad A and pad B, $p < 0.05$). Pad A = open symbols. Pad B = closed symbols. *Abbreviation*: BTK, behind-the-knee.

DISCUSSION

Evaluating the potential for skin irritation is an important step in assuring the safety of many consumer products. Visual grading of erythema has been used reliably for many years to detect skin irritation in a variety of test protocols and on various body sites. It requires no special equipment and is easily adaptable to large-scale testing, which is required to provide safety assurance for consumer products. Trained skin graders can accurately and reproducibly score test sites for erythema and dryness (9) and reliably detect evidence of irritation with equal or higher degrees of sensitivity to that of instrumental measures (10–14). However, enhanced visual scoring may increase the ability to differentiate very similar products without requiring other protocol modifications. In addition, by continuing to use erythema as a signal for skin irritation, we can compare data on new materials and products with a large, historical data set.

Current test methods were developed so a grader would see a reaction with the unaided eye. However, physiological changes that occur early in the process of irritation, such as changes in blood flow, moisture content, and pH, would be expected to occur before any reaction is visible. In other words, by the time the reaction is visible, it may be too late to measure the early changes in skin physiology. These early changes may be key to our ability to distinguish subtle skin effects and, therefore, support future product development efforts.

When skin reactions are scored visually, the grader is seeing a combination of endpoints: the surface changes, which provide information about the shape and texture of the skin surface, and the subsurface changes, which provide information about internal components such as erythema, pigmentation, and the vasculature (15,16). Polarized light sources can enable the observer to selectively examine either the surface or subsurface components.

Authors have described the use of polarized light as an aid in visualizing various skin conditions, including acne vulgaris, rosacea, photoaging, lentigo simplex, and basal cell carcinoma (17–19). Kollias et al. (20) studied irritation reactions in individuals' patch tested with various concentrations of SLS. After 24 hours of patch testing, test sites were photographed with standard and perpendicular polarized (or cross polarized) light 20 minutes, 24, and 48 hours after patch removal. Visible erythema was evaluated on both sets of photographs. These authors found that erythema was apparent at the 20-minute photograph and persisted for the next 48 hours.

We conducted these studies to determine if the use of cross-polarized light would increase the sensitivity of our scoring and allow us to detect reactions that were not apparent with unaided visual grading. In the first experiment, the standard, 24-hour patch test to the upper arm was used (Fig. 1). The low concentrations of SLS produced very low levels of irritation, as indicated by the relatively low mean erythema scores. All concentrations,

including saline alone, were sufficient to produce enough erythema so that the results were significantly different from the pre-patch (baseline) scores after the first 24-hour patch.

Evaluation of irritation reactions by expert graders has been used very effectively for decades in protocols designed to investigate skin effects (10,13,21–25). In an earlier publication, we conducted direct comparisons between visual scoring and instrumental scoring methods [TEWL using a ServoMed Evaporimeter EP1®(Servomed AB, Stockholm, Sweden), and redness using a Minolta Chromameter CR-200®(Minolta Corp., U.S.)] (26). The results of the visual scoring method were very similar to those of the instrumental scoring methods. Significant differences between treatments and scoring timepoints of visual scores were very consistent with those observed with the instrumental scores. The results presented in Figure 1 provide a comparison with an instrument designed to enhance visual scoring (Syris v600® Visualization System). Once again, results are very consistent and confirm that an expert grader can detect very low levels of irritation. Results of the experiment presented in Figure 1 indicated that, although the test conditions were mild, the 24-hour patch on the upper arm produced irritation reactions that were already visually apparent, i.e., the reactions were not subclinical. Therefore, a subsequent experiment was designed using even milder conditions in an attempt to produce subclinical changes.

Results of the milder patch conditions (Fig. 2) demonstrated that, using enhanced visual scoring, irritant effects were apparent at certain timepoints in the study when standard visual scoring showed no significant effect. A low concentration of SLS (0.01%) was patched for very brief periods (2 hours and 6 hours). On the upper arm, the test conditions did not produce significant visible erythema until after the second patch. However, grading the subsurface reactions using cross-polarized light demonstrated significant erythema after removal of the first 6-hour patch, or 24 hours earlier (Fig. 2B).

When 0.01% SLS was patched on the volar forearms, the mean erythema was significantly different from baseline after a single patch using both standard visual scoring and cross-polarized light (subsurface) scoring. However, using standard visual scoring, the erythema disappeared with an overnight recovery (Fig. 2A, recovery 1). Yet, using cross-polarized light showed that subsurface changes in erythema were still present under the skin surface. The ability of the enhanced visual scoring to detect subclinical irritation may indicate that the use of this tool has the potential to increase the sensitivity of standard tests.

The results shown in Figure 3 demonstrate the challenge in detecting differences in the potential for irritant effects between two very similar products and the potential usefulness of the enhanced visual scoring. Pads A and B produce indistinguishable irritant reactions when tested in the standard 24-hour patch test on the upper arm. However, consumers and panelists who participate in skin effects' studies with these two products consistently report a higher number of unpleasant sensations, i.e., itching, sticking, etc., associated with pad A (7,8). The BTK test, which was developed to evaluate the potential for both chemical and mechanical irritation, has been the only test method to consistently detect a difference in the irritation potential of these two products (3,7).

In this study, we also demonstrated a significant difference between these two products after the first six-hour application in the BTK test (Fig. 3A–C). However, after an overnight recovery period, the difference between erythema using the standard visual scoring method was no longer significant (Fig. 3D). Using cross-polarized light to grade the subsurface reactions, the changes in erythema produced by pads A and B remained statistically significantly different, even after overnight recovery.

Enhanced visual scoring enables detection of subclinical physiological changes that are not apparent using standard visual scoring. We have reported previously that subjective consumer comments indicate that consumers can detect differences in skin effects caused by the use of two similar products. For example, consumers have consistently indicated that pad B is seen as less irritating than pad A in "real use" situations; however, most test protocols repeatedly fail to differentiate between these two products (3). The BTK test, which was developed to further exaggerate exposure due to the mechanical irritation component, is the only protocol that consistently differentiates between these two products.

Sensory effects have been shown to be consistent with BTK test results and to differentiate between pad A and pad B reliably (8). This current investigation confirms that sensory effects correlate with visual scoring in the BTK test and confirms that sensory effects enable the differentiation between two very similar products (Fig. 4). With enhanced visual

scoring, we can begin to bridge the gap in sensitivity between sensory effects and physiological changes that can be evaluated by an external observer.

The ability of enhanced visual scoring to detect irritant-related changes, even when standard visual scoring indicates recovery of the skin, has important implications for testing protocols. Currently, standard protocols on potential skin effects do not focus on recovery. It is assumed that a resolution of the erythema and other visual changes indicate an end to the irritant reaction. However, with enhanced visual scoring, subclinical changes are still evident, even with the mild reactions seen in this series of experiments. Future protocols can be designed to follow the subsurface changes to their resolution more effectively and to provide a clearer understanding of the process of skin healing.

CONCLUSIONS

We have developed a new innovative clinical method with several advantages:

- Subclinical skin irritation can be detected using enhanced visual scoring.
- Enhanced visual scoring indicates that subsurface changes to the skin persist, even when standard visual scoring indicates recovery.
- Enhanced visual scoring has the potential to increase the sensitivity of our clinical studies by detecting changes that are not apparent with standard visual scoring.
- Skin irritation is apparent with fewer applications of the test material. This may allow development of shorter protocols for evaluating the skin effects of consumer products.
- Enhanced visual scoring has the potential to bridge the gap in sensitivity between sensory effects experienced by the consumer and physiological changes that can be evaluated by an external observer.

This new method has the potential to increase the sensitivity of all clinical dermatological studies.

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47 Sodium Lauryl Sulfate–Induced Irritation in the Human Face: Regional and Age-Related Differences

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Although extensively studied (1), sodium lauryl sulfate (SLS) has been rarely used on the face to investigate mechanisms of irritation (2). Because of the particular skin sensitivity of the face and the neck and because of the regional variability in the face reactivity to water-soluble irritant compounds (3) and to compounds inducing contact urticaria (4,5), we conducted this study with SLS 2% under occlusion for one hour.

Because baseline transepidermal water loss (TEWL) has been speculated as a predictive parameter to skin susceptibility to SLS (6) and changes in hydration of superficial epidermis suspected to be responsible for the seasonal variability of skin irritation induced by SLS (7), we measured the baseline TEWL and capacitance before SLS application and studied their correlation with changes in TEWL (∂TEWL), 1 hour and 23 hours after patch removal (1).

INVESTIGATIONAL STUDY

Two age groups were examined: 10 young subjects, aged 25.2 ± 4.7 years ranging from 19 to 30 years and 10 older subjects aged 73.7 ± 3.9 years ranging from 70 to 81 years. Twelve volunteers were Caucasians and eight Hispanics.

Eight areas of the skin (forehead, nose, cheek, nasolabial and perioral areas, chin, neck, and volar forearm) were studied.

After 15 minutes of rest, necessary to suppress excess water evaporation, baseline TEWL was measured using an evaporimeter, Tewameter® TM 210* (Courage + Khazaka, Cologne, Germany) and baseline capacitance was measured with a Corneometer® CM 820 PC (Courage + Khazaka, Cologne, Germany).

SLS (Sigma, St. Louis, Missouri, U.S.) 2% (w/v) in water was then applied to each of the eight areas for one hour under occlusion, using a saturated absorbent filter paper disc (0.8 cm diameter) in small Finn Chamber aluminum discs (Epitest Ltd. Oy, Finland). On the contralateral side, water was applied in the same conditions as control.

To assess skin irritation, TEWL was measured 1 hour and 23 hours after patch removal.

TEWL values of the areas tested were corrected according to the changes in the control areas:

$$\text{TEWL} = \text{TEWL measured} - \text{TEWL } H_2O,$$

where TEWL measured is that in the tested area at 1hour or 23 hours, and ∂TEWL H_2O = TEWL control – baseline TEWL H_2O, where TEWL control is the measured TEWL value in the control area at 1 hour or 23 hours.

The skin reactivity to SLS was assessed by the changes in TEWL (∂TEWL = TEWL – baseline TEWL).

To compare the skin reactivity (∂TEWL) of the regions within each group, the two-tailed Student *t* test for paired data was used. The two-tailed Student *t* test for unpaired data was used to compare the two age groups.

Simple linear regression and correlation analysis between basal TEWL and skin irritation (∂TEWL) and between baseline capacitance and ∂TEWL for each skin location combining the data of the two age groups were used. ∂TEWL was considered as the dependent variable.

Table 1 Reactivity of Regions in the Young and Old Group

Area	∂TEWL (Mean ± SD) g/m^2 hr		p value
	Young group	Old group	
Cheek	15.1 ± 12.8	6.8 ± 7.3	0.093
Chin[a]	13.5 ± 9.9	6.0 ± 3.3	0.035
Forearm	1.9 ± 2.1	1.1 ± 1.5	0.354
Forehead	10.4 ± 13.9	2.3 ± 2.3	0.086
Neck	6.8 ± 6.0	3.6 ± 3.7	0.165
Nasolabial area[a]	12.4 ± 6.3	4.4 ± 4.8	0.005
Nose	8.6 ± 7.6	5.0 ± 6.0	0.251
Perioral area	10.7 ± 10.0	4.2 ± 4.1	0.074

Note: ∂TEWL = TEWL 23 hours after patch test removal corrected to the control – baseline TEWL
[a]Difference between the young and old group statistically significant ($p < 0.05$).

COMPARISON OF REACTIVITY BETWEEN AGE GROUPS AND FACE AREA

SLS 2% under occlusion for one hour induced in most of the cases a subclinical irritation and sometimes minimal erythema. The absolute TEWL values taken after 1 hour and 23 hours did not show significant differences. Since the 23-hour measurements demonstrated lower standard deviation values, only the irritation assessed at 23 hours was considered.

Comparison Between the Regions

In the young group, all areas except the forearm reacted to SLS. Skin irritation induced by SLS and assessed by ∂TEWL was greater in the cheek and chin when compared with that the neck and forearm ($p < 0.05$). The highest ∂TEWL mean values were found in the cheek and chin (Table 1), but no statistically significant differences with the remaining regions of the face were detected. All the other regions except the forehead showed a significantly higher irritation than the forearm.

In the old group, all regions reacted to SLS except the nose, perioral area, and forearm. The cheek and chin showed the highest ∂TEWL mean values (Table 1).

Significantly ($p < 0.05$) higher reactivity of these two areas was found when compared with that in the forearm, and when the reactivity in the chin was compared with that in the forehead.

Comparison Between the Two Age Groups

In all the areas studied, the mean ∂TEWL values were higher in the young than in the older group (Table 1). Only in the chin ($p = 0.035$) and nasolabial area ($p = 0.005$) were the differences significant.

CORRELATION STUDY BETWEEN MEASUREMENTS

Correlation Between Baseline TEWL and ∂TEWL

Table 2 summarizes the correlations in each area between baseline TEWL and ∂TEWL 23 hours after patch removal.

The forehead and the neck showed the strongest correlations ($r = 0.6474$, $p = 0.002$ in the forehead; $r = 0.6273$, $p = 0.003$ in the neck).

The nose and chin did not demonstrate a significant correlation between basal TEWL and TEWL changes induced by SLS. The forearm was not studied since this area did not react to the surfactant in the same test conditions.

Correlation Between Baseline Capacitance and ∂TEWL

The baseline capacitance was not correlated to the skin irritation induced by SLS in any area studied.

Table 2 Correlations in Each Area Between Baseline TEWL (BTEWL) and Reactivity of the Skin to SLS, 23 hours after Patch Removal (∂TEWL)

	BTEWL (mean ± SD)	TEWL 23 hr (mean ± SD)	∂TEWL (mean ± SD)	*r*	*p*
Cheek[a]	15.63 ± 6.70	26.63 ± 15.30	10.96 ± 11.01	0.4616	0.040
Chin[a]	20.87 ± 6.37	30.47 ± 12.08	9.77 ± 8.13	0.3535	0.126
Forearm	8.64 ± 3.97	9.70 ± 4.92	1.51 ± 1.83	–	–
Forehead[a]	14.10 ± 5.71	20.40 ± 14.96	6.39 ± 10.53	0.6474	0.002
Neck[a]	11.55 ± 4.35	16.63 ± 8.54	5.18 ± 5.12	0.6273	0.003
Nasolabial[a]	28.74 ± 8.56	36.93 ± 13.44	8.40 ± 6.78	0.4831	0.031
Nose[a]	19.04 ± 6.03	25.27 ± 11.15	6.77 ± 6.92	0.3218	0.166
Perioral	24.25 ± 8.93	29.98 ± 14.6	7.47 ± 8.17	0.4547	0.044

Note: *r* coefficient of correlation
p significance (significant correlation when $p < 0.05$)
[a]Areas that reacted to SLS: statistically significant ($p < 0.05$) difference baseline TEWL and TEWL 23 hours after patch removal.

CONCLUSIONS

SLS, an anionic surfactant is widely used to study the sensitivity of the skin to irritants. Little information on the susceptibility of the face to SLS is available (2).

In this study, the influence of age and regional variability on SLS irritation was investigated with a focus on the skin of the face.

Only TEWL 23 hours after patch removal was taken into consideration because of the lower standard deviation (SD) when compared with the one-hour values. This difference in SD might be explained by the "transient damage to the water barrier of the skin" described by Agner and Serup (8) and induced by exposure to water. This transient increase of TEWL not related to SLS or to the evaporation of additional water lasts between 1 and 3 hours after patch removal.

Considering the increase of TEWL after SLS exposure (∂TEWL), the young group had a higher irritant response than the old group in the chin and nasolabial area. In the remaining regions including the neck, ∂TEWL mean values were higher in the young group, although the differences were not significant. This lack of significant differences might be explained by the high SD values (Table 1) in these regions. Previous studies (2,9) investigated the influence of age on the susceptibility to SLS and reported a decrease in sensitivity in the elderly, which is in concordance with our results.

Various protocols (concentrations, application time) use SLS in water solution to induce skin irritation (10,11). In our study, since the face was suspected to be more sensitive than the remaining regions of the body, and for a practical purpose, SLS 2% was applied only for one hour under occlusion. This protocol was sufficient to induce subclinical irritation in most of the areas of the face but not in the forearm, confirming that the face is more sensitive than the forearm.

Although the cheek and chin showed the highest ∂TEWL mean values, no regional variations were detected between the various regions of the face in both age groups, but the cheek and chin were more sensitive than the neck in the young group. This lack of significant differences between regions might be explained by the high SD observed in ∂TEWL values.

To see whether significant differences in skin irritation induced by surfactant exist between the regions of the face, higher SLS concentrations as well as repeated open applications should be tested.

Skin sensitivity to water-soluble irritants has also been explored by the stinging test (3,12). Marked regional variation in the intensity of stinging was found (3): nasolabial fold > cheek > chin > retroauricular area > forehead. However, stinging test expresses the percutaneous penetration of the irritant compound and the sensory nerve response that depends upon nerve density in the skin. Marked regional variability of the nerve density has been reported (13).

Skin irritation expressed by ∂TEWL is the result of percutaneous penetration of the compound and the changes made to the skin barrier. This could explain that both methods (stinging test and SLS-induced irritation) might not show the same sensitivity regional

variation in the human face, although the cheek and the chin were among the most sensitive areas demonstrated by both methods.

The correlation study showed a significant correlation between basal TEWL and ∂TEWL in five of the seven areas that have reacted to SLS (Table 2).

The correlations between baseline TEWL and TEWL 23 hours after patch removal were more obvious. All the areas that reacted to SLS (all the areas studied except the forearm) showed a strong correlation coefficient varying between 0.76 and 0.88, with a highly significant p value <0.001.

However, we think that the correlation between basal TEWL and the absolute TEWL values after irritation does not imply that higher basal TEWL values predispose to higher skin sensitivity, but only the correlation between baseline TEWL and the changes in TEWL after irritation (∂TEWL) may have this significance. Even if for different basal TEWL values, the changes in TEWL are the same, a positive correlation could be found because TEWL is considered as a stable parameter (14).

Conflicting results have been published with regard to this aspect. Some authors correlated the absolute TEWL values before and after irritation (6,15–17). Others (18) used basal TEWL and ∂TEWL. Agner (18), studying healthy and atopic subjects, reported a positive correlation between baseline TEWL and the increase in TEWL induced by SLS only in the healthy group. Although in the atopic group, basal TEWL was significantly higher than the normal subjects, the changes after SLS exposure were not significantly different between the two groups.

These findings are in concordance with our study, where some areas of the face (nasolabial area) showed higher basal TEWL values than others (the cheek) but failed to demonstrate higher sensitivity (Table 2). So, each region of the face has probably its own characteristics influencing the skin sensitivity to irritants, probably independent, from basal TEWL.

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48 Irritation Differences Between Genital and Upper Arm Skin and the Effects of Emollient Application[a]

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The vulva comprises variable, specialized tissues that differ in structure, morphology, and embryonic derivation. The cutaneous epithelium of vulvar structures, including the labia major and labia minora, is keratinized. The epidermis of the labia majora contains sweat glands, sebaceous glands, and hair follicles; these structures are absent in the labia minora.

The labia majora has greater cutaneous thickness and keratinization than the labia minora.

The vulvar structures are highly vascularized, with the labia majora exhibiting more than twice the blood flow as in forearm skin (1).

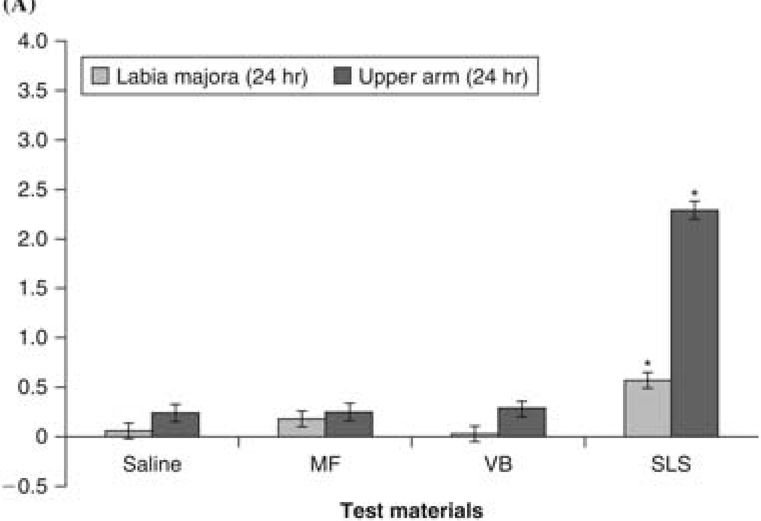
Menses and venous blood differ in many factors of their composition. Approximately 50% of menstrual fluid is blood; the remainder of the menses fluid is made up of desquamated endometrial tissue and vaginal epithelial cells, cervicovaginal secretions, and endogenous vaginal microbes. The hemoglobin and iron content of menses varies throughout the menstrual cycle and is generally higher than in venous blood, whereas white blood cell and platelet counts are lower in menses than in venous blood (2).

The tissues of the labia majora and minora are more permeable than tissues at other anatomical sites, such as the forearm. To assess whether menses exposure contributes to vulvar irritation, we performed a four-day skin patch test of menses and venous blood on the labia majora and on the upper arm. To our knowledge, the potential contribution of menses to vulvar irritation has not been examined previously by skin patch test methods. In the study presented here, menses and venous blood have been compared to determine whether components unique to menses fluid (e.g., the matrix metalloproteinases, enzymes that catalyze endometrial breakdown) (3,4), might contribute to skin irritation. Although patch testing is performed routinely on the back or the arm, we chose to assess both the arm and the vulva because anatomical differences in irritant susceptibility can affect the erythema response (5,6).

The study that has been conducted was approved by an Institutional Review Board. All 20 women volunteers provided written informed consent. Physiologic saline (nonirritant control), aqueous sodium lauryl sulfate (SLS, 0.6% w/v, irritant control), and each volunteer's own venous blood and menses collected overnight with an intravaginal cup (Instead Softcup®, Ultrafem Inc., Missoula, Montana, U.S.) (0.3 mL each) were applied for two consecutive 24-hour periods to the lateral labia majora (randomized across 2 clipped sites on each labium) and to the upper arm (randomized across 5 sites per arm, see below). Occlusive patches (Webril® cotton pad, Professional Medical Products Company, Kendall LTP, Chicopee, Massachusetts, U.S.) secured with Blenderm® occlusive tape (3 M Health Care, St. Paul, Minnesota, U.S.) were applied to the labia and to one upper arm; semi-occlusive patches (Finn Chamber®, Epitest, Hyria, Finland) secured with Tegaderm® tape (3 M Health Care, St. Paul, Minnesota, U.S.) were applied to the alternate arm. The fifth site on each arm was pretreated with a proprietary, petrolatum-based emollient prior to menses application. A standard five-point erythema scale was used to score skin irritation (7,8).

This study showed that the labia majora were less responsive than the upper arm to all applied materials (Fig. 1A and B). Notably, menses and venous blood elicited no significant

[a]Some parts adapted from Farage M, Warren R, Wang-Weigand S. The vulva is relatively insensitive to menses-induced irritation. Cutan Ocul Toxicol 2005; 24(4):243–246. With kind permission from Taylor and Francis Group.



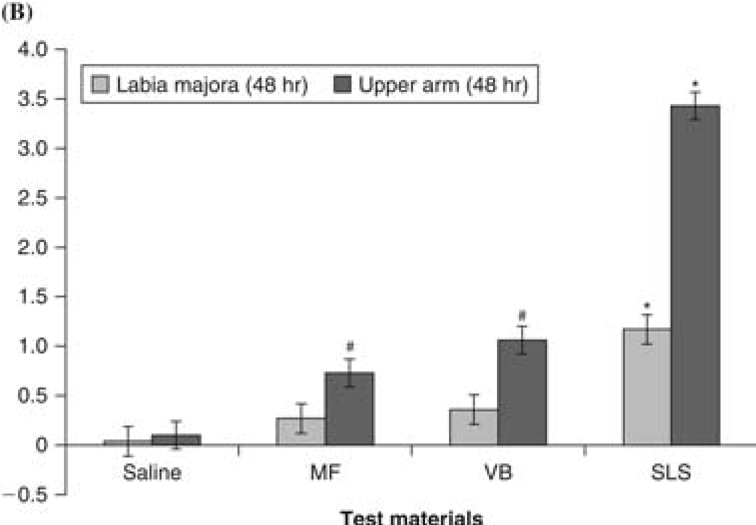


Figure 1 Skin erythema of the labia majora and upper arm following test materials application under occlusive patch for 24 and 48 hours, respectively. *Abbreviations*: Test materials: Saline, nonirritant control; MF, menses fluid; VB, venous blood; SLS, 0.6% aqueous sodium lauryl sulfate (irritant control). (**A**) 24-hour exposure. *: Significantly different ($p \leq 0.05$) from other test materials applied to that anatomical site. (**B**) 48-hour exposure. #: Significantly different ($p \leq 0.05$) from the nonirritant control (saline) applied to that anatomical site. *: Significantly different ($p \leq 0.05$) from other test materials applied to that anatomical site.

erythema on the labia majora at either time point; SLS, the irritant control, elicited significant, mild erythema (0.6 ± 0.08 and 1.2 ± 0.15 at 24 and 48 hours, respectively). The limited response to menses and venous blood on the labia could not have been predicted a priori. Indeed, some polar substances (e.g., benzalkonium chloride, maleic anhydride) elicit heightened irritant reactions on the labia relative to the arm (5).

On the upper arm, menses and venous blood elicited mild erythema at the 48-hour time point only (Fig. 1B: 0.7 ± 0.14 and 1.1 ± 0.14, respectively). No discernible difference in skin irritation to these fluids was observed at this site, despite differences in composition between menses and venous blood. Sodium lauryl sulfate elicited moderate to severe erythema on the arm at both the 24- and 48-hour time points (Fig. 1A and B: 2.3 ± 0.09 and 3.4 ± 0.14, respectively). Mean scores to SLS application on the arm were three- to fourfold higher than

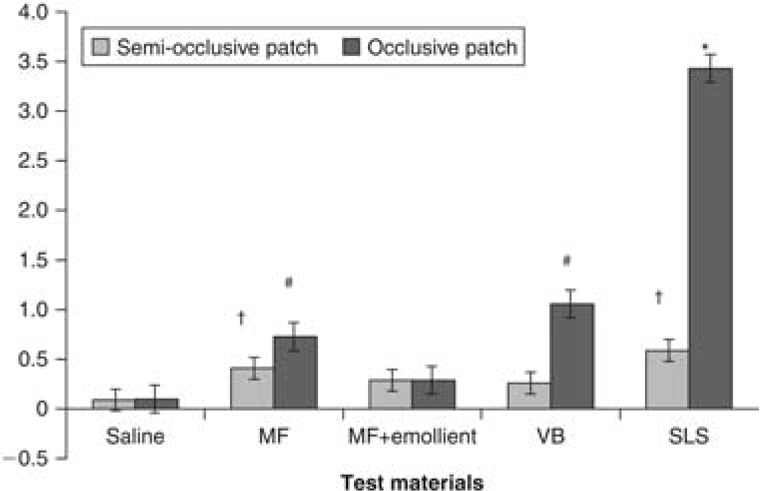


Figure 2 Skin erythema of the upper arm induced by test materials applied for 48 cumulative hours under semi-occlusive or occlusive patch. *Abbreviations*: Test materials: Saline, nonirritant control; MF, menses fluid; MF + emollient, menses fluid applied to emollient treated skin; VB, venous blood; SLS, 0.6% aqueous sodium lauryl sulfate (irritant control). †: Significantly different ($p \leq 0.05$) from the nonirritant control (saline) under semi-occlusive conditions. #: Significantly different ($p \leq 0.05$) from the nonirritant control (saline) under occlusive conditions. *: Significantly different ($p \leq 0.05$) from other test materials under occlusive conditions.

those observed on the labia; this is consistent with prior reports that the arm is more susceptible to SLS-induced skin irritation than the labia (6,9).

Semi-occlusive conditions attenuated the erythematous response to all materials (Fig. 2, upper arm, 48 hours). Notably, SLS-induced erythema was reduced almost sixfold (mean scores of 0.6 ± 0.1 vs. 3.4 ± 0.14, semi- and full-occlusion, respectively). Pretreatment of the upper arm with emollient prevented menses-induced skin irritation, regardless of the degree of occlusion (10).

CONCLUSIONS

To our knowledge, the potential contribution of menses to vulvar irritation has not been examined previously by skin patch test methods. Our preliminary observations suggest that the vulva (labia majora) is adapted to be less sensitive to menses-induced skin irritation. This adaptation is not universal, as other irritants have elicited heightened responses on the vulva (5).

We also found that pretreatment with a petrolatum-based emollient attenuates potential skin irritation from menses. If the latter observation can be extended to other biological fluids, it may be clinically relevant to limiting skin irritation under wound dressings, sanitary pads, and incontinence garments.

One caveat to interpreting visually scored skin erythema at these anatomical sites: heightened vulvar pigmentation as compared with the arm may mask inflammation, and utilizing bioengineering technology may clarify this issue. Future studies should also examine whether the menstrual cycle has an impact on the irritant response. Skin barrier function and reactivity to irritants at other sites exhibit cyclical variability (11,12), but an effect of the menstrual cycle on vulvar skin reactions has not been documented.

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49 Ethnicity as a Possible Endogenous Factor in Irritant Contact Dermatitis: Comparing the Irritant Response Among Caucasians, Blacks, and Asians

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INTRODUCTION

Irritant contact dermatitis (ICD) is a common and potentially serious dermatological disorder (1–3). It is also the second most common occupational illness (4). Since contact dermatitis can develop into chronic skin disease, understanding the underlying factors of its etiology is clinically important.

This condition is divided into several forms, depending on the nature of exposure and the resulting clinical presentation. Two common entities are acute and cumulative dermatitis. Acute contact dermatitis presents the classic symptoms of irritation such as localized and superficial erythema, edema, and chemosis. It occurs as a result of single exposure to an acute irritant (5). Cumulative irritant dermatitis presents similar symptoms but occurs when exposure to a less potent irritant is repeated until signs and symptoms develop over weeks, years, or decades.

The ability of the offending irritant to cause dermatitis depends on both the nature of the irritant agent and the initial skin condition. The severity of symptoms depends on exogenous and endogenous factors (6–8). Exogenous factors include the irritant's chemical and physical properties and the vehicle and frequency of application. Endogenous factors have been speculated to be age, sex, preexisting skin diseases, skin sensitivity, genetic background, and—the subject of this review—race (6), or, in today's parlance, ethnicity.

Ethnic differences in skin physiology and pathophysiology exist (9–11), and so whether ethnicity, is in fact, an endogenous factor affecting ICD is an important question in dermatotoxicology. Ethnic predisposition to ICD has been studied by comparing the irritant responses of blacks and Asians to those of Caucasians as a benchmark. We review these studies to evaluate if ethnic differences in susceptibility to ICD do exist.

The answer to the question of ethnicity as a factor in ICD has clinical and practical research consequences. Premarket testing of topical products (soaps, detergents, perfumes, and cosmetics), risk assessment for occupational hazards, and subject-inclusion requirements for product safety studies require knowledge about ethnic differences in irritation (12).

BLACK VS. CAUCASIAN IRRITATION RESPONSE

Using erythema as the parameter to quantify irritation, early studies note that blacks display less redness than Caucasians. In a hallmark paper, Marshall et al. (13) showed that while 59% of Caucasians exhibit acute irritant dermatitis as defined by erythema from 1% dichlorethylsulphide (DCES), only 15% of blacks do. Later, Weigand and Mershon (14) performed a 24-hour patch test using orthochlorobenzylidene malononitrile as an irritant, which confirmed that blacks are less susceptible than Caucasians to ICD as defined by erythema (Table 1, item A). Further studies, also using erythema as a measure of irritation, showed that blacks are less reactive than Caucasians to irritants (160 and 1280 mM/L methacoline) (19,20).

Weigand and Gaylor (21) showed that if the stratum corneum (SC) of black and Caucasian subjects is removed, there is no significant difference in irritation as measured by erythema between the two groups. They concluded that there might be structural differences

Table 1 Black Vs. Caucasian Irritation Response

Interference	Endpoint	Comment	Reference
A. Findings that show a statistically significant difference in the irritation response between blacks and Caucasians:			
1% Dichlorethylsulfide	Erythema	Untreated	Marshall et al. (13)
Orthochorobenzylidene	Erythema	Untreated	Weigand et al. (14)
100-mM methyl nicotinate	PPG	Untreated	Guy et al. (15)
0.05% Clobetasol	LDV	Pre-occluded	Berardesca and Maibach (16)
0.5–2.0% SLS	TEWL	Pre-occluded	Berardesca and Maibach (17)
B. Findings that do not show a statistically significant difference in the irritation response between blacks and Caucasians:			
0.5–2.0% SLS	LDV and WC	Untreated, pre-occluded, and pre-delipidized	Berardesca and Maibach (17)
100-mM methyl nicotinate	LDV	Untreated	Guy et al. (15)
0.1-, 0.3-, and 1.0-M methyl nicotninate	LDV and Erythema	Untreated	Gean et al. (18)

Abbreviations: PPG, photoplethysmography; LDV, laser Doppler velocimetry; TEWL, transepidermal water loss; WC, water content.

in the SC that provide more protection from chemical irritation to black skin than Caucasian skin. Indeed, while the SC thickness is the same in both races (22), the SC of black skin has more cellular layers and stronger cells (12), more casual lipids (23), increased desquamation (24), decreased ceramides (25), and higher electrical resistance (26) than Caucasian skin. Wesley and Maibach also found significant evidence that innate differences exist in skin properties between black and Caucasian skin (3). They found that blacks had higher TEWL (transepidermal water loss) values, decreased skin surface pH, variable blood vessel reactivity, and large mast cell granules. These variables, they concluded, may play a role in the differences observed in dermatologic skin disorders between blacks and Caucasians.

It is difficult, however, to conclude that blacks are less susceptible to cutaneous irritation only on the basis of studies using visual scoring. Erythema is notoriously difficult to measure in darker skin. Perhaps the difference in skin irritation between the two test groups is simply a result of the difficulty of assessing erythema in black subjects.

To better understand this issue, it is necessary to analyze studies that use alternative accurate detection methods (27) to assess the level of induced cutaneous irritation. Berardesca and Maibach (17) performed such a study to determine the difference in irritation between young Caucasian and young black skin. They applied 0.5% and 2.0% sodium lauryl sulfate (SLS) to untreated, pre-occluded, and pre-delipidized skin. Then they quantified the resulting level of irritation using objective techniques: laser Doppler velocimetry (LDV), TEWL, and water content (WC) of the SC. They found no statistical difference in irritation between the two groups as measured by LDV and WC, but they did find a statistical difference in the TEWL results of the pre-occluded test with 0.5% SLS. In that test, blacks had higher TEWL levels than Caucasians, suggesting that in the pre-occluded state, blacks are more susceptible to irritation than Caucasians. The finding of this study contradicts the hypothesis that blacks are less reactive than Caucasians. Further, Wesley and Maibach observed that six out of eight studies demonstrated higher TEWL values in black skin (Table 1, item B) (18).

Gean et al. (18) found no statistically significant difference in the maximum LDV response between black and Caucasian subject groups when they challenged skin with topical methyl nicotinate (0.1, 0.3, and 1.0 M). Further, unlike the earlier studies, they found no difference in the blood flow and erythema responses between the two groups.

Guy et al. (15) supports the results finding that LDV measurements of induced blood flow after application of 100-mM methyl nicotinate reveal no significant differences between black and Caucasian subject groups; however, a significant difference was found using photoplethysmography (PPG). Caucasians had a greater PPG value than blacks did, suggesting that Caucasians may be more susceptible to irritation. The authors did not explain why blood flow measurements using PPG showed a statistically significant difference between the groups when LDV did not.

Berardesca and Maibach (16) also found decreased reactivity in blood vessels in the black test group than in the Caucasian test group. They measured the post-occlusive cutaneous

reactive hyperaemia—temporary increase in blood flow after vascular occlusion—after an application of a potent corticoid, and measured vasoconstriction using LDV; the black subject group had several significantly different parameters of the hyperaemic reaction. They found a decreased area under the LDV curve response, a decreased LDV peak response, and a decreased decay slope after peak blood flow, showing that blacks have a decreased level of irritation-induced reactivity of blood vessels. These results are consistent with their previous work.

In conclusion, older studies using erythema as the only indicator for irritation show that Blacks have less irritable skin than Caucasians, but more recent studies using objective bioengineering techniques suggest that the eye may have misled us to an incorrect interpretation.

ASIAN VS. CAUCASIAN IRRITATION RESPONSE

An early study comparing Caucasian and Japanese susceptibility to cutaneous irritation was conducted by Rapaport (28). He performed a standard 21-day patch test protocol on Caucasian and Japanese females in the Los Angeles area, in which 15 irritants (different types or concentrations of cleansers, sunscreen, and SLS) were tested. The results were reported according to the cumulative readings of all subjects in an ethnicity group for each irritant. Japanese women had higher cumulative irritation scores for 13 of the 15 irritants tested; Rapaport interpreted these findings to confirm the common impression that Japanese are more sensitive to irritants than Caucasians are. Also, this sensitivity was independent on the concentration or exact chemical formulation of the substance tested, suggesting that Japanese are in general more sensitive than Caucasians.

While these findings are important, it is difficult to interpret the data. First, as also noted by Robinson (12), Rapaport provides little experimental detail and data. For example, while the study required 21 separate days of irritation readings, only the end cumulative irritation scores are reported. Had he reported daily irritation readings, we would have been able to note the time pattern of response. Further, no statistical tests were conducted to ascertain if the differences between the Japanese and Caucasian subjects were statistically significant. Note, too, that the cumulative irritation test score does not distinguish between the intensity of a subject's response and the number of subjects responding. Thus it is possible, e.g., for a few extremely sensitive Japanese subjects to inflate the overall irritation score. Therefore, at the minimum, it would be helpful to provide standard deviations to rule out such problems.

What at first seems surprising, Basketter et al. (29) found that Germans are more sensitive than Chinese subjects. Subjects in Germany, China, and the United Kingdom were exposed to varying concentrations (0.1–20%) of sodium dodecyl sulfate (SDS) for four hours on the upper outer arm, and the resulting dose-response irritation was measured on the basis of erythema. They concluded that the German subjects tend to be more sensitive than the Chinese, and the Chinese to be slightly more sensitive than the British. This conclusion is the opposite of popular belief and of the Rapaport study, which indicated that Asians are more likely to develop ICD than are Caucasians.

There are, however, inherent flaws in this study, some of which the authors acknowledged. First and foremost, this study does not control the variables of time and location. The German and Chinese studies were performed in three to six weeks in the winter, while the U.K. study was spread over 15 months. Also, in particular, German winters are colder and drier than Chinese winters, which in turn tend to be colder than English winters. These variables will distort the results in a predictable way if we assume that an individual becomes more sensitive to ICD in colder and drier climates (2). We would then expect, on the basis of climatic conditions, that the German subjects would be more reactive than the Chinese, and the Chinese more reactive than the British. As these are the actual results, we cannot necessarily contribute the differences in irritant response to ethnicity, as it is reasonably likely that the differences are possibly due to weather conditions. Also, they mention that 15% of the U.K. volunteers were black. While they account for this by showing that the black irritant response was similar to the overall U.K. group response, it is scientifically problematic to mix racial groups in a study testing for racial differences. Furthermore, they supplied no statistical tests for their conclusion that Germans are slightly more sensitive than the other ethnic groups.

Table 2 Statistical Analysis of the Basketter et al. (29) study

	0.1% SDS	0.25% SDS	0.5% SDS	1.0% SDS	2.5% SDS	5.0% SDS	10% SDS	20% SDS
Germany	0.03	0.09	0.23	0.50	0.65	0.72	0.76	ND
China	0	0	0.01	0.21	0.45	0.61	0.79	0.90
U.K.	0.01	0.01	0.06	0.15	0.33	0.41	0.49	0.76
N	100	100	100	100	100	100	100	100
Z (Germany-China)	1.75	3.07*	4.79*	4.29*	2.84*	1.65	−0.51	NA
Z (U.K.-China)	1.00	1.00	1.92	−1.10	−1.74	−2.83*	−4.42*	−2.64*
Z (U.K.-Germany)	−1.01	−2.60*	−3.41*	−5.28*	−4.53*	−4.42*	−3.94*	NA

The numbers in the first 3 rows are the decimal value of the percentage of the group that developed a positive irritant reaction at a specific SDS concentration. The numbers in the last 3 rows are the *Z*-values. We applied the binomial test to ascertain the differences in the percentage response of the subject groups:

$$Z = \frac{r_1 - r_2}{[2r(1-r)/100]^{50}}$$

where r_1 and r_2 are the ratios for the 2 ethnic groups and r is the weighted average. Since the sample sizes for different groups are equal, r becomes the simple average. An asterisk indicates that the ratios are significant at the 5% level.
Note that all the U.K.-Germany differences, except 1, are statistically significant; however, more than half of the U.K.-China and almost half of Germany-China differences are not statistically significant. This indicates a larger statistically significant difference between the 2 Caucasian groups than that between the Caucasian and Asian groups.
Abbreviations: SDS, sodium dodecyl sulfate; Z, *Z*-values.

To shed more light on the results, we conducted simple binomial tests of the differences in the percentage response of the subject groups. Using the resulting statistics, we found a larger statistically significant difference between the two predominately Caucasian groups than between each of the Caucasian and the Chinese groups (Table 2). These results indicate that race may not be the predominant factor affecting susceptibility to ICD in this study; other uncontrolled variables may dominate the results.

Variables such as time and location were eliminated by the Goh and Chia (30) study that tested the susceptibility to acute irritant dermatitis in Chinese, Malay, and Indian subjects. These subjects were exposed to 2% SLS in the right scapular region, and resulting irritation was measured using TEWL. This technique is an objective way to indirectly quantify irritation—the higher the TEWL value, the greater the implicit irritation. There was no significant difference in the TEWL level of irritant skin in a three-way statistical test of the three racial groups. There was a significant difference, however, between the TEWL values of Chinese and Malay subjects so that Chinese subjects were more susceptible to contact dermatitis. While this test does not contribute to the discussion of the difference in predisposition of irritation in Caucasian skin versus Asian skin, it does add to the overall question of whether race can be a predisposition to irritant dermatitis.

Foy et al. (31) clearly added to our knowledge of the difference in the acute and cumulative irritation response in Japanese and Caucasian female skin. They reduced some variables that compromised other studies; location, time, season, and scores were the same for both study populations. Eleven different materials were tested in the acute test; they were applied to the upper arms for 24 hours, and irritation was measured on the basis of erythema. The cumulative test consisted of testing five irritants using a four-exposure cumulative patch protocol.

In the acute test, while there is a slight tendency toward greater susceptibility to irritation among Japanese subjects, only 4 out of the 11 irritants caused a significant difference in reactivity between the two groups—these were the most concentrated irritants used. This shows that perhaps for more concentrated irritants, there is indeed a statistical difference in the acute contact dermatitis response; of course, this study needs to be interpreted in context with others to follow. For the cumulative study, the skin irritation scores between the two test groups are close, but the Japanese tended to have slightly higher numbers. The differences, however, only reached statistical significance in two instances. And as the authors noted, it is

difficult to interpret the importance of those two instances since the statistical significant differences are not maintained at later points in the timeline. It is safe to conclude, therefore, that while the acute irritant response to highly concentrated irritants was significantly different between the Japanese and Caucasian subjects, the cumulative irritant response rarely reaches a statistical difference.

Studies that include both acute and cumulative irritant tests, like the one above, are more informative than single tests since they give a more complete view of differences in skin irritation between groups. Robinson (32) conducted a series of studies that tested racial differences in acute and cumulative skin irritation responses between Caucasian and Asian populations. In the first acute tests, Caucasian and Japanese groups were exposed on the upper outer arm to five irritants under occlusion for up to four hours. The resulting erythema was scored on an arbitrary visual scale. The results are represented as the cumulative percentage incidence of positive test reactions to the different irritants.

It is curious to note that while Japanese subjects tend to be more susceptible to acute irritation than Caucasians, neither one irritant nor one test time caused a significant response difference between the two groups. Further, note that for three of the five irritants only Caucasians reacted at early test times, contradicting the hypothesis that Japanese are more reactive to irritants. But even this trend-breaking difference is not considered statistically significant. The acute irritation response data were then reanalyzed in terms of possible differences in temporal response. The analysis showed that Japanese subjects generally react faster than their Caucasian counterparts, as indicated by their shorter TR50 values (the time it takes for the cumulative irritation score to reach 50%). While this result is interesting and adds the new dimension of temporal differences in reactivity between the two groups, hard data were not provided and statistical analysis was not conducted to see if this temporal pattern difference is indeed statistically significant.

The cumulative irritation test was conducted concurrently and on the same Japanese and Caucasian subjects. Four concentrations of SDS (0.025%, 0.05%, 0.1%, and 0.3%) were applied on the subjects' upper backs for 24 hours for 14 days. The resulting skin grades were summed for all subjects for all test days. For the two lower SDS concentrations the Japanese subjects reacted only slightly more than the Caucasian subjects, but only the difference in skin grades for 0.025% SDS reached statistical significance. When this data were analyzed in terms of temporal response, for the two lowest concentrations, the Japanese reacted only slightly faster than their Caucasian counterparts. Whether the difference in reaction time is statistically significant is not known.

In the same study, Robinson then applied both the acute and cumulative irritation protocols to compare three new subject groups—Chinese, Japanese, and Caucasian—with each other. The cumulative irritation study found no statistically significant differences between the different groups. In the acute test, he found that, in most cases, the Chinese subjects were more reactive to irritants than Caucasians, but that this difference significant in only one case, and he stated that most likely this was an anomaly. There was no discernable difference between the Japanese and Chinese groups. And surprisingly, when the Japanese subjects were again compared with the Caucasian subjects, as they were in the beginning of his study, the results showed no significant difference between the two groups.

While Robinson's first two-way irritation response comparison test between Japanese and Caucasian subjects did show some statistical differences, the fact that they could not be confirmed in the second half of the study emphasizes the difficulty in obtaining repeatable results in this type of study. For one, in the statistical sense, Robinson's sample sizes (approximately 20 people) were small, combined with the variability between human skin within an ethnic group; this makes it difficult to make concrete conclusions. His study showed, however, that there were essentially no significant differences between the Asian and Caucasian groups, at least none that could be repeated.

Robinson et al. (33) had similar results. Using the four-hour occlusion patch method, they compared the relative acute skin reactivity of Asian and Caucasian subjects using the irritation temporal response to measure the difference in reactivity between the test groups. They tested five chemicals, including 20% SDS and 100% decanol. Unlike the previously described study, they failed to find a statistical difference between the reactivity to multiple irritants between the two groups even at the four-hour mark. Then they did something new: They separated the

racial subpopulations into "sensitive" and "normal" groups to test any differences in percentage cumulative scores and temporal responses within these new groups but across race (i.e., he compared sensitive Asians with sensitive Caucasians). There were no statistically significant differences between subjects of the same skin type in different racial groups. This further contraindicates the hypothesis that Asians are more reactive to irritants than Caucasians.

Recently Robinson (34) compiled five years of his previous data and compared the acute reactivity differences between Caucasian and Asian (combined Japanese and Chinese) subgroups using the four-hour human patch method. The data were represented in terms of the time it took for subjects to have a positive response to the irritant chemical. Again, as in most experiments, Asians displayed a greater irritation response score than Caucasians. However, this difference only reached statistical difference at the four-hour mark, with SDS and decanol as the irritants. Note that while these results of this study are probably more representative of the population at large because of the relatively large sample size (200 plus), the data from this study were compiled from three different testing centers over five years. This could have potentially added uncontrolled and unaccounted-for variables.

In support of the long-held belief that Asians are more susceptible to ICD, several studies do indeed demonstrate this tendency (31,33,34). Rarely, however, is this trend statistically significant, and even more rarely can the statistical significance be repeated in another study. Therefore, it can be concluded from these studies that there is no fundamental difference between Asian and Caucasian cutaneous irritant reactivity—the overall irritant response and the time to reach that response is similar in both subgroups.

But the lack of comparable studies, small sample sizes, external variability, and intravariability within the subgroups make it difficult to completely dismiss Rapaport's original findings that Asians are more reactive than Caucasians. For example, different studies apply the irritant test material on different parts of the body, which might have different reaction responses. This makes it difficult to compare the results of one study with another and therefore raises the question of whether a more solid trend among studies would exist if the irritants were applied to the same anatomical site. Further, with regard to skin properties, Wesley and Maibach found that the data remain largely discordant and poorly characterized when discussing Asian skin (3). For the time being, however, in terms of topical product safety, risk assessment for occupational hazards, and global product marketing it would be practical to assume that few statistical differences between Asian and Caucasian cutaneous reactivity exist.

CONCLUSION

In Table 3 are summarized some potent factors that might influence the refinement of interpretation in future investigations. These studies demonstrate that there is little evidence of statistically significant differences in the irritant response between Caucasian and black or Asian groups. We can see no consensus on whether race is indeed an endogenous factor in ICD. Intuitively, we suspect that ethnic differences exist in skin function and may have evolved as have those in hair and other differences. Basically, the studies suggesting differences in skin (15,17) are "stress" in nature (pre-occluded). Presumably new insights into physiology, pharmacology, and toxicology may clarify this situation.

Also, it is possible that the well-known, divergent response to irritants is due to intraindividual variations in the skin irritation response (35–37). This is a relatively new idea, and therefore further studies need to be conducted in this area before a definitive statement

Table 3 Potent Factors that Might Influence Refinement of Interpretation in Future Investigations

Experimental design
Baseline versus "stress" test differences
Anatomic site
Open versus occluded irritant stresses
Ethnic groups in the same versus varying geography
Comparable climatic conditions
Presentation of hard data and statistical analysis

can be made linking intraindividual variation to ethnic differences in the intensity of an irritation response.

The above discussion is clearly limited in scope when considering the wide array of ethnic groups present globally. Considerable work remains to be done before the role of ethnicity in ICD is fully appreciated. Future investigations, such as that conducted by Peters et al. on Punjabi and Tamil subjects (38), is required before the interplay between skin and ethnicity can be completely defined.

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50 In Vitro Skin Irritation Testing on SkinEthic™-Reconstituted Human Epidermis: Reproducibility for 50 Chemicals Tested with Two Protocols

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INTRODUCTION

Evaluation of the irritancy potential to human skin of any chemical or formulation used in the chemical, pharmaceutical, and cosmetics industries is a necessity. Several in vivo and in vitro tests aim to determine the risk of irritation resulting from the contact between these compounds and human skin. The most commonly used test is the rabbit skin irritation test described in the OECD test guideline 404 and in the European Chemicals Bureau Annex V part B.4 (http://ecb.jrc.it/testing-methods/) and initially described by Draize et al. (1). This animal test consists in topically applying substances, which are raw materials or, depending on regulations, formulations (i.e., finished products), on the rabbit's shaved skin. A score is attributed according to physiological observations on the animals, which allows the classification of each tested product.

However, the Draize test presents several major disadvantages. The first is due to the fact that rabbit skin and human skin have different physiological properties and responses to environmental and chemical agents (2–5). Unfortunately, the biological basis for the variability of skin irritation among species remains unknown (6). Rabbit data have often been taken as reference to determine the irritant potential of chemicals although, to the exception of rare publications (2,4), few studies have compared data obtained both on animals and humans. Some compounds are more toxic for rabbits than for humans and vice versa (2,4,7). Moreover, the Draize test lacks reproducibility (8,9). The third major inconvenience concerns animal suffering and discomfort since eschar formation can be observed with severe irritants.

Few experiments were done on humans because of direct risk of lesion and intoxication for the subject, resulting from the application of potentially dangerous compounds. Among the available human data on chemical toxicity, some derive from chemical insults with severe irritants due to accidents at home or work or due to repeated skin exposure to moderate irritants. The other human data for skin irritation testing were obtained by patch testing performed on relatively high numbers of volunteers (4,10–16). Compounds were tested pure or diluted, for different application times, but the experiments were stopped when moderate to severe reactions to the test compound were observed. Many parameters influence the reproducibility of this type of tests. First, variability is observed in function of the patches used. York et al. (17) showed that generally the "Webril" and the "Hill Top" patches produced greater reactivity than the "Van der Bend" and the "Finn" patches. Some other parameters responsible for the variability of test results are directly correlated with the choice of the volunteers: the interindividual variability of reactivity is the principal factor (18). Moreover, interethnic differences have been observed (19,20). The reactivity of human skin changes also with the anatomical site (21) and decreases with age (20). Even abiotic factors must be considered since the seasonal variability plays a role in skin reactivity (22,23). The seasonal effect was particularly evident in the experiment described by Basketter et al. (18) when a four-hour patch test with SDS 20% provoked skin irritation in 45% of the volunteers in summer, but increased to 91% in winter.

The development of in vitro alternative methods for testing skin irritation has been the aim of an increasing number of scientists. This can be explained by their ethical advantage, and in several cases, also by their enhanced convenience. The skin irritation function test (SIFT) (24) and the pig ear test (25) are two of these in vitro methods. These tests are thus performed on ex vivo animal tissues (mouse and pig, respectively).

To permit the testing on human tissues without the disadvantages of performing tests directly on humans, the development of cell and tissue culture appeared promising. Several models are now commercially available for testing skin irritation (26–30). Using reconstructed epidermis, all classical methods determining cell viability (such as MTT reduction, resazurin reduction, LDH release) are easy to perform. Moreover, the SkinEthic™ model allows the measurement of additional endpoints such as the release of IL-1α and IL-8 (28,31–33). Reconstructed epidermis consists of human epidermal cells cultured in chemically defined medium by using semiautomatic production procedures, producing human epidermis standardized in terms of thickness, terminal differentiation, and reactivity to test compounds.

The repeated experiments described here are performed on different batches of human reconstructed epidermis. They correspond to different production cycles using epidermal cells of different donors. In vitro experimentation allows testing compounds on human tissues whatever the age, gender, and race of the donor. Each of the 50 chemicals has, at least, been tested in triplicate in two different experiments using two protocols: an in vitro patch test and a direct topical application test. The in vitro patch test protocol mimics closely the human in vivo patch test protocol (14). We applied the compounds on 0.95-cm^2 polypropylen Hill Top chambers® (Cincinnati, Ohio, U.S.) for four hours. The quantity of chemicals applied is proportional to the size of the patches (0.95 cm^2) used. This technique allows the containment of the product on a determined surface in the center of the 4 cm^2 epidermis. In parallel, our direct topical application test is performed by applying 100 μL of the test compound directly onto the epidermal surface of 0.63 cm^2 for four hours. Among the 50 chosen chemicals, 20 chemicals were previously tested in the ECVAM pre-validation study (PVS chemicals) on acute skin irritation (25) and 30 chemicals were previously tested in the in vivo human patch test (HPT) described by Basketter et al. (14) (HPT chemicals). After test compound application, tissues were incubated at 37°C, 5% CO_2 for four hours in both protocols. The 20 PVS chemicals were tested in two additional separate experiments using the direct topical application protocol (4 times in total). Multiple endpoint analysis including cell viability (MTT reduction), histology, and IL-1α release measurements was performed. Absence of direct interaction between test chemicals and the MTT solution or nonspecifically on frozen-killed tissues was verified. Our goal was to study the reproducibility of reference chemical testing on epidermis with two convenient protocols, and to compare the results with available in vitro, as well as animal and human in vivo data.

SKIN MODELS AND TESTS

Reconstituted Human Epidermis

Tissues (SkinEthic™, Nice, France) used were fully differentiated three-dimensional reconstituted human epidermal cultures grown on the air-liquid interface for 17 days in defined growth medium (27,34). Each experiment was performed in triplicate on one single tissue production batch, but different batches (different production cycles and/or donor cells) were used for each repeated experiment.

Selection and Coding of Test Chemicals

We chose reference chemicals upon two criteria: their irritation status should have been defined in the European Community classification, and, furthermore, they should have been tested either on other three-dimensional models (25) or by the human in vivo patch test (14). The present study includes irritant and nonirritant compounds. Details of the 50 chemicals tested are in Table 1. The experiment performed on run B was realized as a blind test. The 20 HPT chemicals were coded by H. Maibach, UCSF, United States.

Table 1 Chemicals tested and corresponding skin irritation data

	Compound	CAS no.	Supplier	EU class.	OECD class.	Human patch class.	S/L
1	Sodium lauryl sulphate (50%)	151-21-3	Sigma	I[a]	I[a]		L
2	1,1,1-Trichloroethane	71-55-6	Aldrich	I[a]	I[a]		L
3	Potassium hydroxide (5%)	1310-58-3	JT Baker	I[a]	I[a]		L
4	Heptanal	111-71-7	Aldrich	I[a]	I[a]		L
5	Methyl palmitate	112-39-0	Aldrich	I[a]/NC [b]	I[a]	NC[b]	L
6	Lilestralis / Lilial	80-54-6	Aroma & Fine Chemical	I[a]	I[a]		L
7	1-Bromopentane	110-53-2	Aldrich	I[a]	I[a]		L
8	dl-Citronellol	106-22-9	Aldrich	I[a]	SLI[a]		L
9	d-Limonene	5989-27-5	Aldrich	I[a]	SLI[a]		L
10	10-Undecenoic acid	112-38-9	Aldrich	I[a]	SLI[a]		L
11	Dimethyl disulphide	624-92-0	Lancaster	NI[a]	SLI[a]		L
12	Soap from 20/80 coconut oil/tallow		Quimasso	NI[a]	NI[a]		S
13	cis-Cyclooctene	931-87-3	Aldrich	NI[a]	SLI[a]		L
14	2-Methyl-4-phenyl-2-butanol	103-05-9	Aldrich	NI[a]	NI[a]		L
15	2,4-Xylidine	95-68-1	Aldrich	NI[a]	NI[a]		L
16	Hydroxycitronellal	107-75-5	Astier-Demarest-Leroux	NI[a]	NI[a]		L
17	3,3'-Dithiodipropionic acid	1119-62-6	Aldrich	NI[a]	NI[a]		S
18	4,4-Methylene bis-(2,6-ditert-butyl)phenol	118-82-1	Aldrich	NI[a]	NI[a]		S
19	4-Amino-1,2,4-triazole	584-13-4	Aldrich	NI[a]	NI[a]		S
20	3-Chloronitrobenzene	121-73-3	Aldrich	NI[a]	NI[a]		S
21	1-Decanol	112-30-1	Aldrich	R38[b]		NC[b]	L
22	2-Propanol	67-63-0	Aldrich	NC[b]		NC[b]	L
23	Isopropyl palmitate	142-91-6	Aldrich	NC[b]		NC[b]	L
24	Octanoic acid	124-07-2	Aldrich	R34[b]		R38[b]	L
25	Methyl caproate	106-70-7	Aldrich	NC[b]		NC[b]	L
26	Methyl laurate	111-82-0	Aldrich	R38[b]		NC[b]	L
27	Decanoic acid	334-48-5	Aldrich	R38[b]		R38[b]	L
28	Dodecanoic acid	143-07-7	Aldrich	R38[b]		NC[b]	L
29	N,N-Dimethyl-N-dodecyl aminobetaine (20%)		Albright & Wilson	R38[b]		R38[b]	L
30	Benzalkonium chloride (10%)	8001-54-5	Sigma	R38[b]		R38[b]	L
31	Dimethyl sulphoxide	67-68-5	Sigma	NC[b]		R38[b]	L
32	Polyethylene glycol 400	25322-68-3	Aldrich	NC[b]		NC[b]	L
33	Acetic acid (10%)	64-19-7	Sigma	R38[b]		NC[b]	L
34	Hydrochloric acid (10%)	7647-01-0	Prolabo	R38[b]		NC[b]	L
35	Sodium hydroxide (0.5%)	1310-73-2	Sigma	R38[b]		R38[b]	L
36	Heptanoic acid	111-14-8	Aldrich	R34[b]		R38[b]	L

(*Continued*)

Table 1 Chemicals tested and corresponding skin irritation data (*Continued*)

	Compound	CAS no.	Supplier	EU class.	OECD class.	Human patch class.	S/L
37	Lactic acid	50-21-5	Aldrich	NC[b]		R38[b]	L
38	Benzyl alcohol	100-51-6	Aldrich	NC[b]		NC[b]	L
39	Triethanolamine	102-71-6	Aldrich	NC[b]		NC[b]	L
40	Dodecanol	112-53-8	Aldrich	NC[b]		NC[b]	L
41	Tween 80	9005-65-6	Sigma	NC[b]		NC[b]	L
42	Benzalkonium chloride (7.5%)	8001-54-5	Sigma	R38[b]		R38[b]	L
43	Propylene glycol	4254-14-2	Fluka	NC[b]		NC[b]	L
44	Octanol	111-87-5	Aldrich	R38[b]		NC[b]	L
45	Eugenol	97-53-0	Sigma	R38[b]		NC[b]	L
46	Geraniol	106-24-1	Sigma	R38[b]		NC[b]	L
47	Linalyl acetate	115-95-7	Aldrich	R38[b]		NC[b]	L
48	Hexanol	111-27-3	Aldrich	R38[b]		NC[b]	L
49	α-Terpineol	10482-56-1	Aldrich	R38[b]		NC[b]	L
50	Ethanol	64-17-5	Merck	NC[b]		NC[b]	L

I = Irritant, NI = Non Irritant, SLI = Slight Irritant, S = Solid, L = Liquid.
[a]Fentem et al., 2001.
[b]Basketter et al., 1999.

In Vitro Direct Topical Test Protocol

Three reconstituted epidermal tissues of 0.63 cm^2 on 0.3-mL-defined maintenance medium in a 24-well plate were used per control or tested compound. Test compounds of 100 μL or 100 mg were homogeneously displayed on the total surface of the reconstructed epidermis. Negative controls and positive controls were run in parallel for each experiment. Cultures were incubated for four hours at 37°C, 5% CO_2. The three cultures were then transferred into new wells of the same 24-well plate containing 0.3 mL of maintenance medium. Tissues were washed three times with 0.5-mL saline solution A. With solids (powders or crystals), the insert was turned upside down before washing and—maintained in this position with forceps—knocked two- to threefold on the inner wall of a beaker to mechanically remove most of the applied compound. Histology, MTT reduction, and IL-1α release endpoints were measured as described below. Untreated tissues and H_2O-treated tissues were used as negative controls, while SDS 20% (14,25) and nonanoic acid–treated tissues (35) were used as positive controls. Negative controls were considered satisfactory if three criteria were met: a high cell viability measured by MTT reduction ($\geq$ 85% of untreated epidermis), a normal histology (score $\geq$ 75) (see histology scoring below), and no release of large amounts of IL-1α (< 30 pg/mL). Positive controls were considered satisfactory when a low cell viability was measured by MTT reduction (< 50%) and when a necrosed histology (score < 75) and an increase of the amount of secreted IL-1α ($\geq$ 30 pg/mL) were observed.

In Vitro Patch Test Protocol

Three reconstituted epidermal tissues of 4 cm^2, placed on 1-mL-defined maintenance medium in a 6-well plate, were used per control or test compound. A measure of 75 μL of the compound was homogeneously displayed on a 0.95 cm^2 Hill Top chamber (Cincinnati, Ohio, U.S.), which was immediately applied, carefully, to the center of a 4 cm^2 culture. In case of solid compounds, 75 mg of the powder or crystals was spread on 0.95 cm^2 (same surface as for liquids) on the center of the culture and covered immediately by a Hill Top chamber. A 5-mm large brush was used to improve the contact between the compound/patch and the epidermal tissue. The patches were homogeneously applied with delicacy; strong pressure was avoided. Negative controls and positive controls were performed in parallel for each experiment. The Hill Top chamber was removed after a four-hour incubation at 37°C, 5% CO_2. No washing step was included in this protocol because most liquid compounds were absorbed by the patch. With solids, the culture was turned upside down, and—maintained in this position with forceps—knocked two- to threefold on the inner wall of a beaker to mechanically remove most of the applied compound. Histology, MTT reduction, and IL-1α release endpoints were performed as described below. Untreated tissues and H_2O-treated tissues were used as negative controls, while SDS 20% (14,25) and nonanoic acid–treated tissues (35) were used as positive controls. Negative controls were considered satisfactory if three criteria were met: a high cell viability measured by MTT reduction ($\geq$ 85% of untreated epidermis), a normal histology ($\geq$ 75) (see histology scoring below) and no release of large amounts of IL-1α (< 105 pg/mL). Positive controls were considered satisfactory when a low cell viability was measured by MTT reduction (< 50%) and when a necrosed histology (< 75) and an increase of the amount of secreted IL-1α ($\geq$ 105 pg/mL) were observed.

Histology

Per test condition, and whatever the protocol, one of three tissues was harvested for histology. The tissues were fixed in a balanced 10% formalin solution and embedded in paraffin. Vertical sections measuring 4 μm were stained with hematoxylin/eosin and photographed under a microscope.

Scoring of histology sections was performed as follows:

- No or minor epidermal changes: 100
- Slight epidermal changes (stratum corneum thickening and/or dissociation and/or parakeratosis; slight edema and/or cellular alterations in the viable layers): 75
- Severe epidermal changes (marked edema and/or less viable cell layers and/or cellular alterations and/or partial tissue necrosis and/or partial tissue disintegration): 25
- Total tissue necrosis and/or tissue disintegration: 0

Cell Viability Measurement by MTT Reduction

The MTT test was used to measure the viability of living cells via mitochondrial dehydrogenase activity (36). The ring of 3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT), yellow, is cleaved by dehydrogenases, yielding blue/purple MTT crystals, which are insoluble in culture medium. An intense purple color is observed when the tissue is healthy, while the culture remains white when necrosis occurred.

Per test condition, the two remaining tissues were incubated in a 0.5 mg/mL MTT solution (0.3 mL MTT for 0.63 cm^2 cultures, and in 1 mL MTT for 4 cm^2 cultures) for a three-hour incubation at 37°C, 5% CO_2. MTT crystals of 0.63 cm^2 inserts were dissolved in 2 mL isopropanol. In the case of the 4 cm^2 inserts, a 0.5 cm^2 biopsy from the treated center of the culture was harvested using a 8-mm diameter biopsy punch (Stiefel) and plunged in 1 mL isopropanol. After an overnight extraction at room temperature, the quantification of cell viability was obtained by comparing the optical density of the extracts measured at 570 nm (reference filter 690 nm) in percentage to the negative H_2O-treated controls.

IL-1α Release

Conditioned media (3 per test condition) underneath the epidermal cultures were collected after the four-hour incubation with the chemicals and kept frozen at –20°C. Inflammatory mediator IL-1α was measured quantitatively using ELISA kits (R&D Systems, U.K., Catalogue number DLA50) (31,32). Results were expressed in picogram of mediator released per milliliter of conditioned medium.

Direct Interaction Between MTT and Chemicals

A measure of 100 μL or 100 mg of each compound was incubated in 1 mL MTT solution (0.5 mg/mL) for three hours at 37°C, 5% CO_2. The interaction was quantified by measuring the MTT/compound mixture OD value (200 μL in triplicate) at 570 nm (reference filter 690 nm).

MTT Interaction with Chemicals on Frozen-Killed Controls

The same procedure as for the direct topical application test protocol was applied onto frozen-killed tissues (–20°C, overnight). The results were expressed as the percentage of fold increase compared with the corresponding value of nontreated living control tissues.

Prediction Models

To classify the chemicals as irritants or nonirritants, we propose a prediction model based on the three endpoints described above. A chemical was classified as nonirritant when two or three of the endpoints led to the following results: cell viability measured by MTT reduction over 50% compared with that of the H_2O-treated control, normal histology (score $\geq$ 75), and a release of IL-1α comparable to that observed for the H_2O-treated control ($<$ 30 pg/mL for the direct topical application test and $\geq$ 105 pg/mL for the in vitro patch test).

On the contrary, a chemical was classified as irritant since two or three of the endpoints measured corresponded to the following criteria: cell viability lower than 50% compared with that of the H_2O-treated control, partial or total necrosis of the epidermal tissues (score $<$ 75), and an amount of secreted IL-1α higher than the IL-1α release induced by the H_2O-treated control ($\geq$ 30 pg/mL for the direct topical application test and $\geq$ 105 pg/mL for the in vitro patch test).

In parallel, a single endpoint prediction model (viability by MTT reduction only) was used for comparison.

Statistical Analysis

Specificity corresponds to the percentage of nonirritant chemicals (according to the EU classification) identified as nonirritants in our test.

Sensitivity represents the percentage of irritant chemicals (according to the EU classification) identified as irritants in our test.

Accuracy corresponds to the overall percentage of correct classification.

Pearson correlations, slope, and variation coefficient were calculated by Dr. Els Adriaens (University of Gent, Belgium).

RESULTS OF THE TESTS

MTT Interaction with Chemicals

Most of the compounds did not present significant interaction with MTT, nor directly, nor on frozen-killed controls. However, these two additional control experiments have shown that the interaction was significant for three chemicals (eugenol, potassium hydroxide 5%, and heptanal) for at least one of the two experiments. First, a direct contact between eugenol and MTT solution quickly produced a dark blue/purple color (OD = 1.4), while, in parallel, the MTT solution alone remained yellow (OD = 0.0). Moreover, eugenol on frozen-killed epidermal tissues induced the change of color from white to dark blue/purple after a four-hour incubation. The dissolution of the formazan blue crystals in isopropanol exhibited an OD value, which represented 82.2% of the OD value obtained for living untreated tissues (with the other 47 compounds, the epidermal tissues remained uncolored, and the OD values were negligible). Nevertheless, a strong direct interaction between a compound and MTT solution was not always correlated with an increased MTT value during the tests on living tissues or even on killed tissues. For example, 5% potassium hydroxide strongly reduced the MTT solution after direct contact, leading to a dark blue/purple mixture (OD = 1.6). On the contrary, 5% potassium hydroxide on frozen-killed tissues led to a light blue color of the culture. The corresponding OD value represented only 14.4% of the living untreated culture. Finally, in the case of heptanal, direct contact with the MTT solution provoked a change of color to blue (OD = 0.2). The application of heptanal on frozen-killed tissues led also to a blue color, with an OD value of 35% of the living untreated tissues.

In Vitro Direct Topical Test

Results of the multiple endpoint analysis of the repeated experiments are in Fig. 1 (PVS chemicals) and Fig. 2 (HPT chemicals). The percentage of cell viability (MTT reduction) is expressed in comparison to the H_2O-treated control. All controls of each experiment were satisfactory according to our criteria. Moreover, results obtained with the blind test were completely comparable to the normal test.

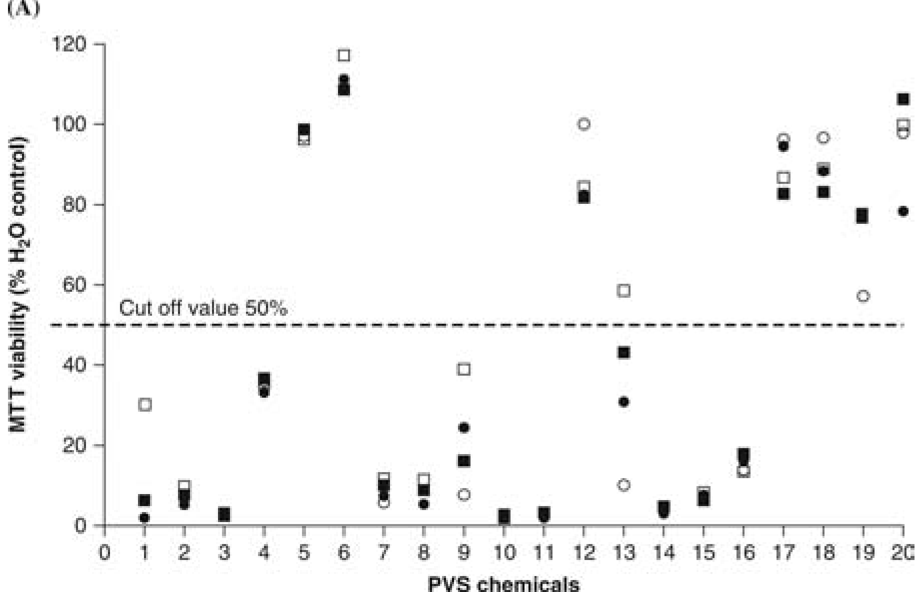
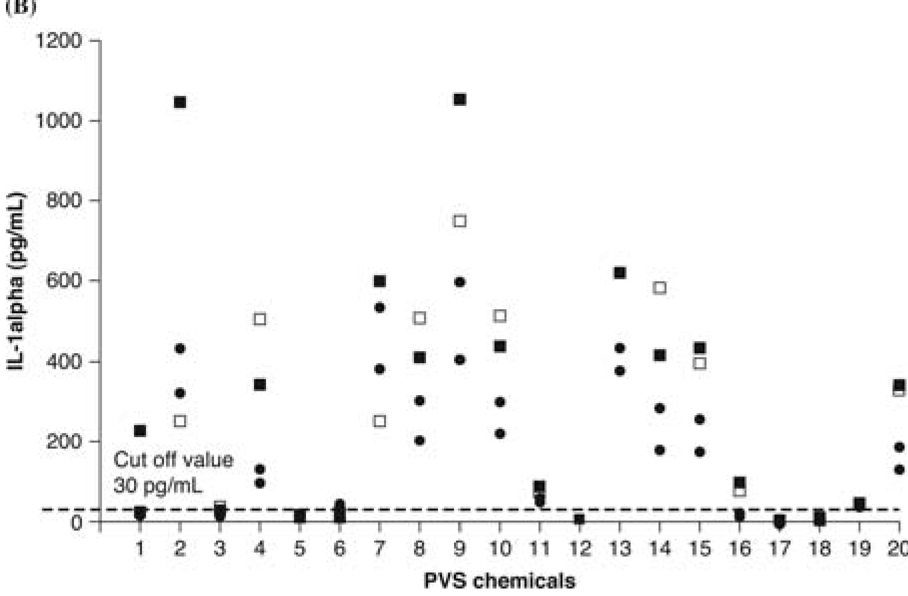


Figure 1 Multiple endpoint analysis for the PVS chemicals with the direct topical application test: (□) run D, (■) run E, (○) run G, (●) run H. (A) Viability (MTT reduction assay). (B) IL-1alpha release. (C) Histological observations. (See next page for Parts B and C.)



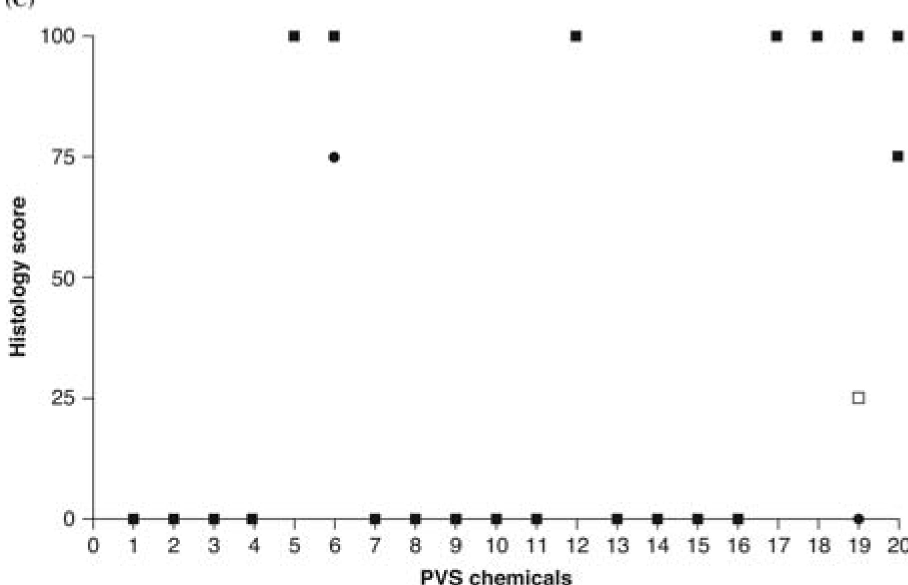
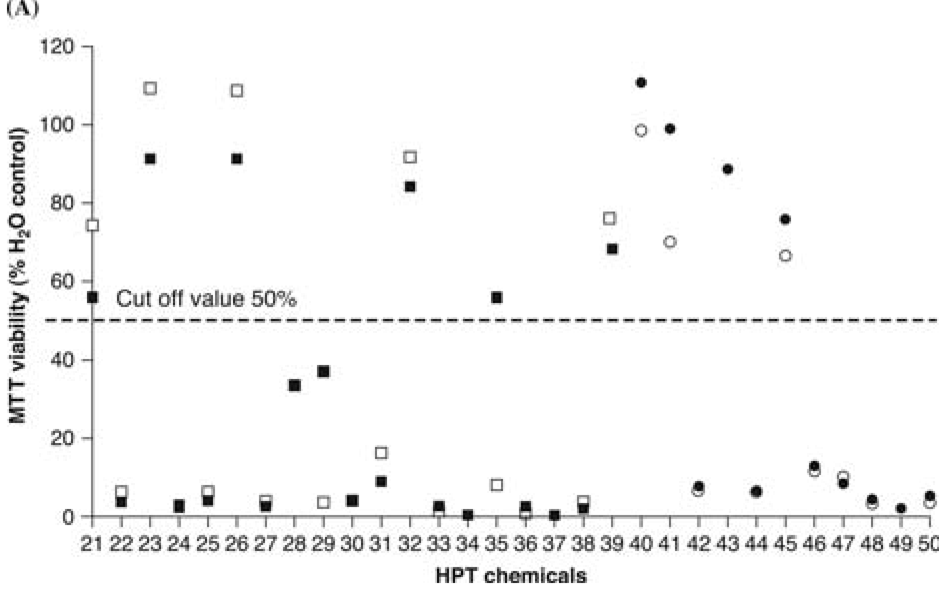


Figure 1 (*Continued*)

The response of the epidermal tissues to the chemicals could be classified in five families (Tables 2 and 3). The first is composed of compounds that induced a response comparable to the negative controls. The second family consists of the chemicals, which allowed a high cell viability, a normal histology (score ≥ 75), but provoked an increase of IL-1α release (≥ 30 pg/mL). The third family is represented by chemicals, which allowed a cell viability higher than 50%, but necrosis was visible on corresponding histological sections (score < 75), and an increase of the amount of IL-1α (≥ 30 pg/mL) release was measured. The fourth family includes chemicals, which induced low cell viability, tissue necrosis (score < 75), but no release of large amounts of IL-1α (< 30 pg/mL). All remaining chemicals belong to the fifth and last family. This family is composed of the chemicals, which were responsible for a low tissue viability, tissue necrosis (score < 75), and a significant increase of secreted IL-1α (≥ 30 pg/mL). Whatever the family, we observed that in most cases the MTT values were of the same range for a given chemical. For example, among the 20 PVS chemicals, for which four independent experiments were



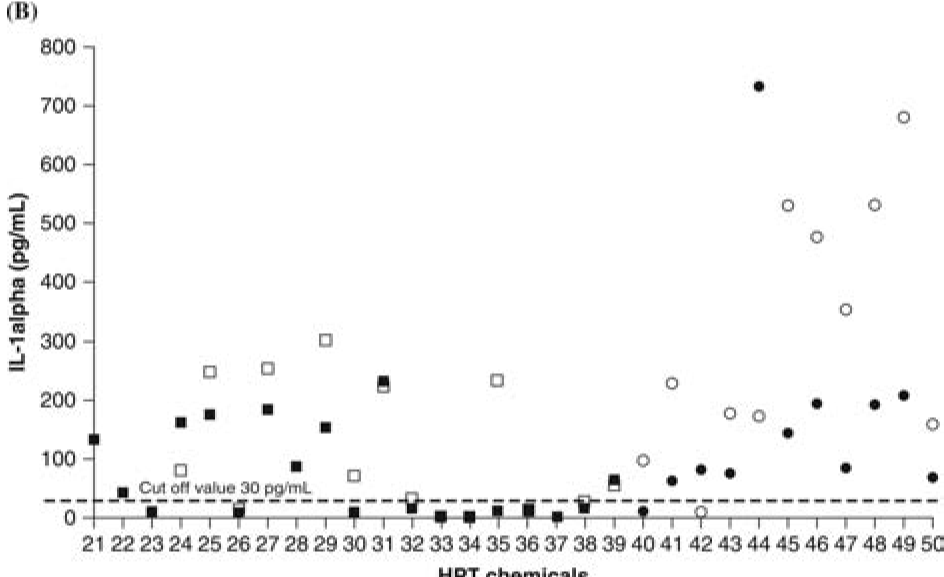


Figure 2 Multiple endpoint analysis for the HPT chemicals with the direct topical application test: (□) run A, (■) run B, (○) run F, (●) run I. (A) Viability (MTT reduction assay). (B) IL-1alpha release. (C) Histological observations. (See next page for Part C.)

performed, dimethyl disulfide–induced MTT values contained between 2.0% and 3.4% of negative control values; for heptanal they were contained between 33.1% and 36.0%, and for 3,3′-dithiodipropionic acid between 82.6% and 96.2%. Similarly, the histological appreciations were highly reproducible, especially when a given chemical was responsible for specific histological effects. Regarding the IL-1α release values, a reproducible effect was obtained. The chemicals induced two kinds of effects: on one hand, some compounds were responsible for an IL-1α release comparable to those of negative controls (e.g., methyl palmitate) and, on the other hand, some compounds provoked a significant increase of IL-1α release compared with negative controls ($\geq$ 30 pg/mL), although the absolute values presented high variations. For example, d-limonene provoked large amounts of released IL-1α (404.2–1055.9 pg/mL), and these amounts were higher than those of the 20% SDS-treated positive control tissues.

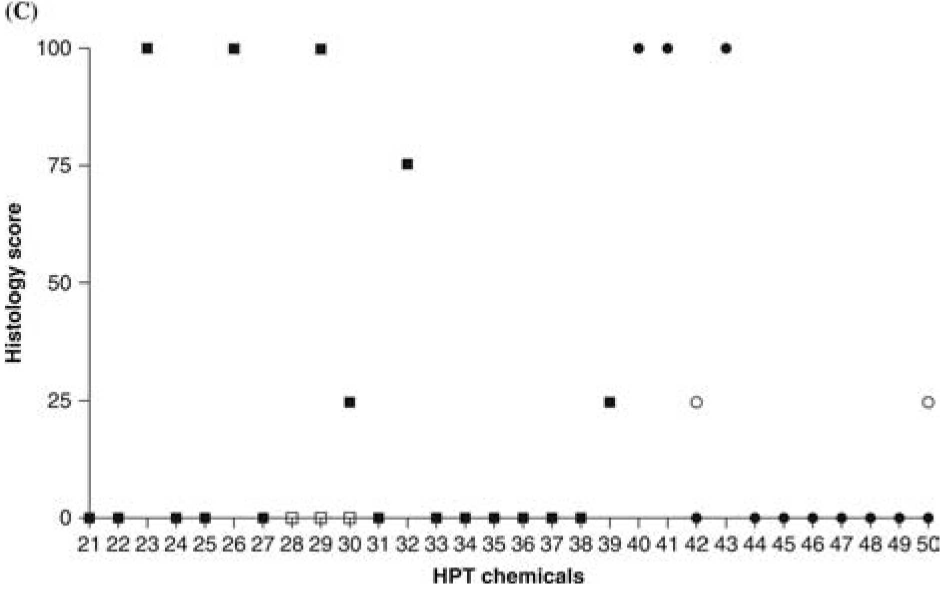


Figure 2 (*Continued*)

Table 2 Comparison of the results obtained with the 20 ECVAM chemicals

	Compound	EU class.	OECD class.	Epiderm class.[a]	Episkin class.[a]	SkinEthic dir. appl. (family) class.	SkinEthic patch (family) class.
1	Sodium lauryl sulphate (50%)	I[a]	I[a]	I	I	(4) I	(5) I
2	1,1,1-Trichloroethane	I[a]	I[a]	NI	I	(5) I	(5) I
3	Potassium hydroxide (5%)	I[a]	I[a]	I	I	(4) I	(5) I
4	Heptanal	I[a]	I[a]	I	I	(5) I	(5) I
5	Methyl palmitate	I[a]/NC[b]	I[a]	NI	NI	(1) NI	(1) NI
6	Lilestralis/Lilial	I[a]	I[a]	I	I	(2) NI	(1) NI
7	1-Bromopentane	I[a]	I[a]	I	I	(5) I	(3) I
8	dl-Citronellol	I[a]	SLI[a]	I	I	(5) I	(3) I
9	d-Limonene	I[a]	SLI[a]	I	I	(5) I	(3) I
10	10-Undecenoic acid	I[a]	SLI[a]	NI	NI	(5) I	(5) I
11	Dimethyl disulphide	NI[a]	SLI[a]	I	I	(5) I	(4) I
12	Soap from 20/80 coconut oil/ tallow	NI[a]	NI[a]	NI	NI	(1) NI	(1) NI
13	cis-Cyclooctene	NI[a]	SLI[a]	I	I	(5) I	(3) I
14	2-Methyl-4-phenyl-2-butanol	NI[a]	NI[a]	I	I	(5) I	(5) I
15	2,4-Xylidine	NI[a]	NI[a]	I	I	(5) I	(5) I
16	Hydroxycitronellal	NI[a]	NI[a]	I	I	(5) I	(6) NI
17	3,3'-Dithiodipropionic acid	NI[a]	NI[a]	NI	NI	(1) NI	(1) NI
18	4,4-Methylene bis-(2,6-ditert-butyl)phenol	NI[a]	NI[a]	NI	NI	(1) NI	(1) NI
19	4-Amino-1,2,4-triazole	NI[a]	NI[a]	NI	NI	(3) NI/I	(2) NI
20	3-Chloronitrobenzene	NI[a]	NI[a]	I	I	(2) NI	(1) NI

I = Irritant, NI = Non Irritant, SLI = Slight Irritant, NC = Non Classified.
Family 1: high cell viability, normal histology, no release of large amounts of IL-1α release.
Family 2: high cell viability, normal histology, increase of the amount of IL-1α.
Family 3: high cell viability, necrosed histology, increase of the amount of IL-1α.
Family 4: low cell viability, necrosed histology, no release of large amounts of IL-1α release.
Family 5: low cell viability, necrosed histology, increase of the amount of IL-1α.
Family 6: high cell viability, necrosed histology, no release of large amounts of IL-1α release.
[a]Fentem et al., 2001.
[b]Basketter et al., 1999.

Table 3 Comparison of the results obtained with the 30 chemicals tested in human patch test

	Compound	EU class.[a]	Human patch class.[a]	SkinEthic direct appl. (family) class.	SkinEthic patch (family) class.
21	1-Decanol	R38	NC	(3) I	(3) I
22	2-Propanol	NC	NC	(5) I	(6) NI
23	Isopropyl palmitate	NC	NC	(1) NI	(1) NI
24	Octanoic acid	R34	R38	(5) I	(5) I
25	Methyl caproate	NC	NC	(5) I	(5) I
26	Methyl laurate	R38	NC	(1) NI	(2) NI
27	Decanoic acid	R38	R38	(5) I	(4) I
28	Dodecanoic acid	R38	NC	(5) I	(3/6) I
29	N,N-dimethyl-N-dodecyl aminobetaine	R38	R38	(5) I	(1/3) NI / I
30	Benzalkonium chloride (10%)	R38	R38	(5) I	(5) I
31	Dimethyl sulphoxide	NC	R38	(5) I	(3/6) NI / I
32	Polyethylene glycol 400	NC	NC	(2) NI	(1) NI
33	Acetic acid (10%)	R38	NC	(4) I	(4) I
34	Hydrochloric acid (10%)	R38	NC	(4) I	(6) NI
35	Sodium hydroxide (0.5%)	R38	R38	(5) I	(1) NI
36	Heptanoic acid	R34	R38	(4) I	(5) I
37	Lactic acid	NC	R38	(4) I	(4) I
38	Benzyl alcohol	NC	NC	(4) I	(5) I
39	Triethanolamine	NC	NC	(3) I	(1) NI
40	Dodecanol	NC	NC	(2) NI	(1) NI
41	Tween 80	NC	NC	(2) NI	(1) NI
42	Benzalkonium chloride (7.5%)	R38	R38	(5) I	(5) I
43	Propylene glycol	NC	NC	(2) NI	(1) NI
44	Octanol	R38	NC	(5) I	(5) I
45	Eugenol	R38	NC	(3) I	(5) I
46	Geraniol	R38	NC	(5) I	(5) I
47	Linalyl acetate	R38	NC	(5) I	(3) I
48	Hexanol	R38	NC	(5) I	(5) I
49	α-Terpineol	R38	NC	(5) I	(5) I
50	Ethanol	NC	NC	(5) I	(1) NI

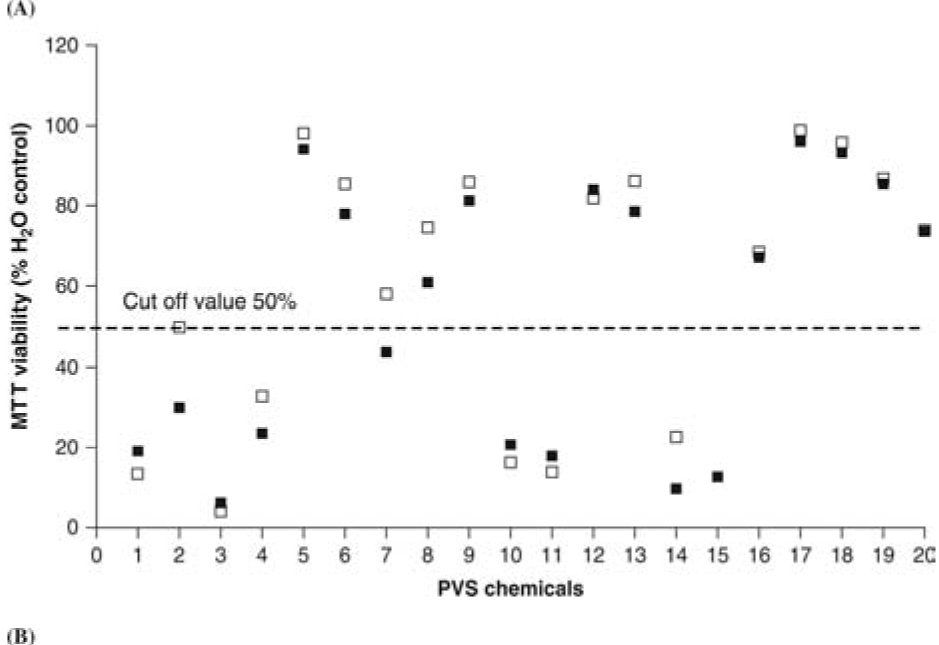
R38 / I = Irritant, NC = Non Classified, R34 = corrosive, NI = Non Irritant.
Family 1: high cell viability, normal histology, no release of large amounts of IL-1α release.
Family 2: high cell viability, normal histology, increase of the amount of IL-1α.
Family 3: high cell viability, necrosed histology, increase of the amount of IL-1α.
Family 4: low cell viability, necrosed histology, no release of large amounts of IL-1α release.
Family 5: low cell viability, necrosed histology, increase of the amount of IL-1α.
Family 6: high cell viability, necrosed histology, no release of large amounts of IL-1α release.
[a]Basketter et al., 1999.

According to our prediction of a model based on multiple endpoint analysis, the first and second families contained nonirritants, and the third, fourth, and fifth families contained irritants. The resulting classification is shown in Tables 2 and 3. A strong reproducibility was obtained between separate experiments. The one single exception for the 50 chemicals tested with this protocol was 4-amino-1,2,4-triazole (irritant in two and nonirritant in the two other experiments).

In Vitro Patch Test

Fig. 3 (PVS chemicals) and Fig. 4 (HPT chemicals) show the results obtained by multiple endpoint analysis with the in vitro patch test. As for the in vitro direct topical application test results (described above), both negative and positive controls were satisfactory. However, note that the amount of released IL-1α was four- to fivefold higher for the H_2O-treated control compared with that of the direct topical test. The ratio of medium volume to tissue surface was 0.48 mL/cm^2 for 0.63 cm^2 tissues, compared with 0.25 mL/cm^2 for 4 cm^2 tissues. Also, the topical application of an empty patch induces a slight increase of basal IL-1α secretion. We thus considered that the level of released IL-1α had increased significantly when it was over 105 pg/mL. Moreover, the cell viability of the positive controls of the patch test was

(A)



(B)

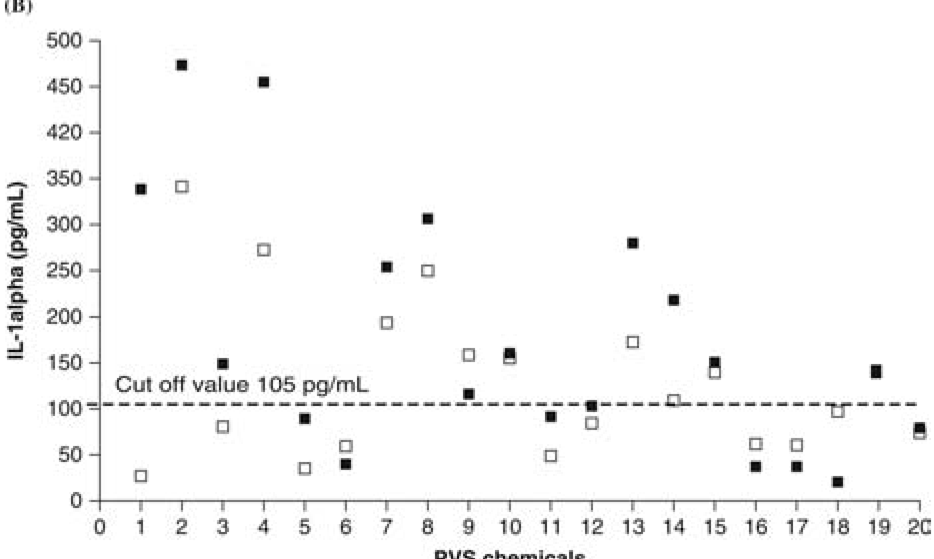


Figure 3 Multiple endpoint analysis for the PVS chemicals with the in vitro patch test: (□) run D, (■) run E. (A) Viability (MTT reduction assay). (B) IL-1alpha release. (C) Histological observations.

higher compared with those of the direct topical application test. More generally in this protocol, the percentage of cell viability was increased in most test conditions compared with the direct topical application protocol; this was probably due to the lower amounts (50%) of test chemicals, which, moreover, were applied to, and partially absorbed by the patches.

The same classification in families as for the direct topical test was applied to the experiments performed with this patch test protocol. However, a new sixth family has been created for hydroxycitronellal, 2-propanol, and 10% hydrochloric acid, which allowed a high cell viability and a small amount of IL-1α release, but provoked tissue necrosis as shown in histology sections.

The same prediction model as for the direct topical test was applied to the patch test results (Tables 2 and 3). A high intra-laboratory reproducibility could also be observed with this protocol to the exception of 20% *N*,*N*-dimethyl-*N*-dodecyl aminobetaine, dimethyl

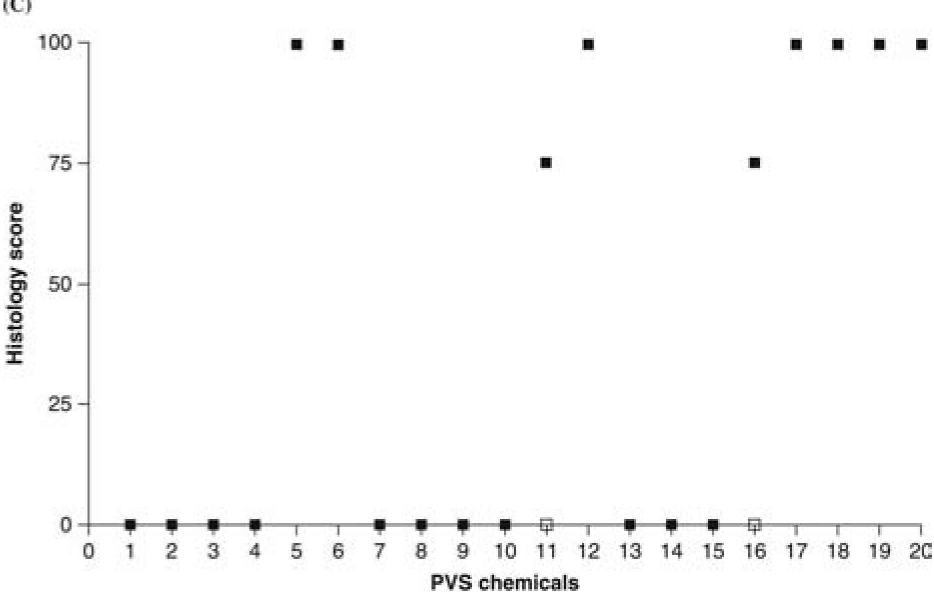


Figure 3 (*Continued*)

sulfoxide, and dodecanoic acid, which were classified as irritant in one experiment and as nonirritant in the other.

Comparison Between the Two Test Protocols

The comparison (Tables 2 and 3) between the predictions of the direct topical application test, and those of the in vitro patch test, shows that even when the experiments were performed using two protocols, the final results were similar for most chemicals. However, the direct topical application test seemed more sensitive, since hydroxycitronellal, 2-propanol, 20% *N*,*N*-dimethyl-*N*-dodecyl aminobetaine, 10% hydrochloric acid, 0.5% sodium hydroxide,

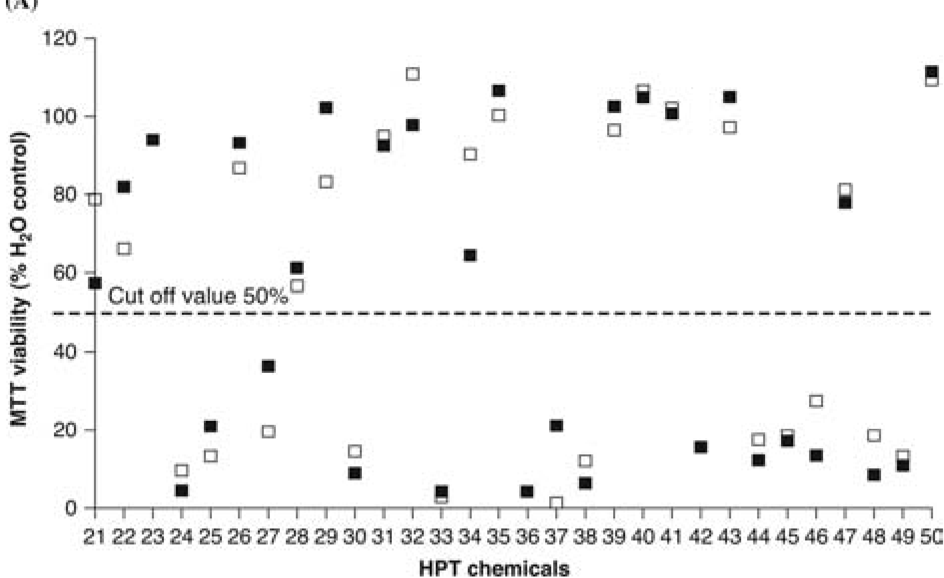


Figure 4 Multiple endpoint analysis for the HPT chemicals with the in vitro patch test: (□) run C, (■) run J. (A) Viability (MTT reduction assay). (B) IL-1alpha release. (C) Histological observations. (See next page for Parts B and C.)

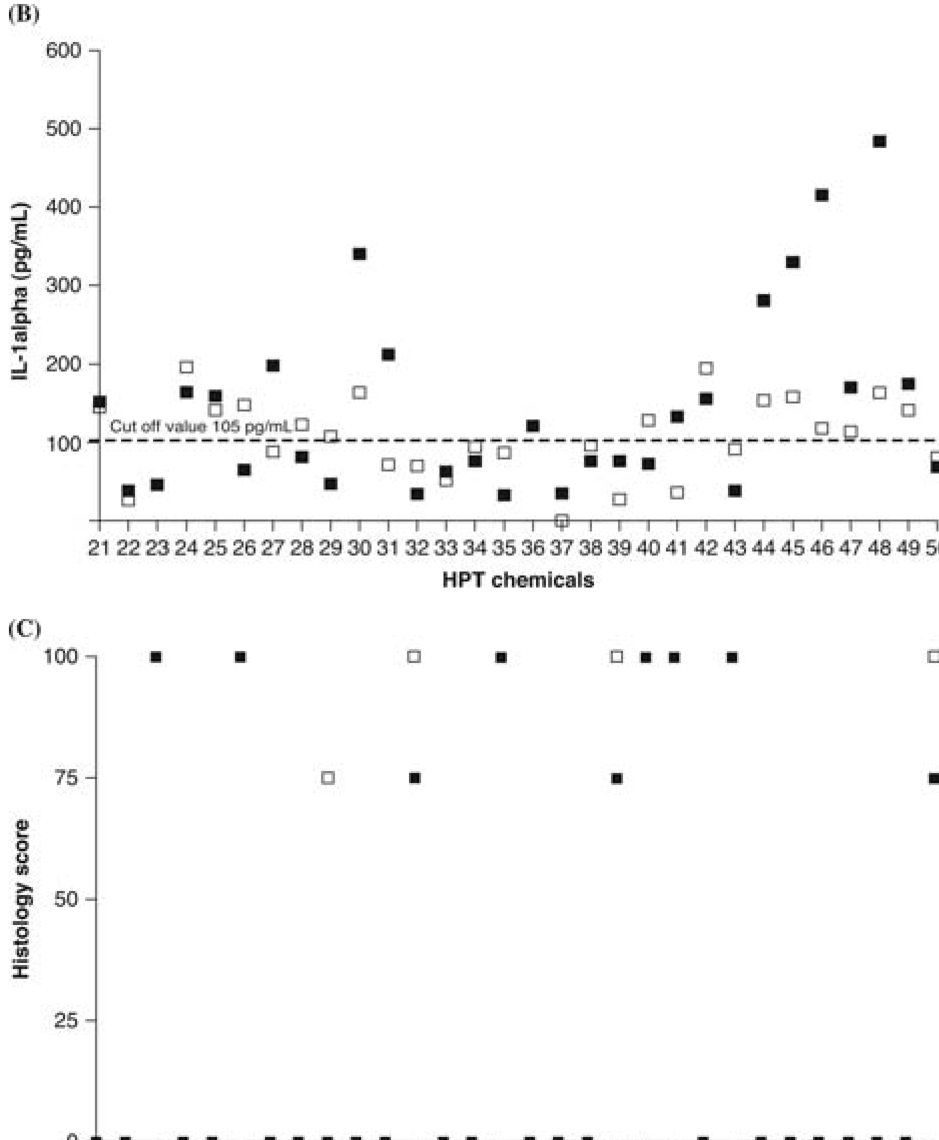


Figure 4 (*Continued*)

triethanolamine, and ethanol were detected as irritants with this protocol, while they appeared to be nonirritant with the in vitro patch test. These differences are discussed below.

Table 2 presents a summary of test results for the 20 PVS chemicals, including existing EU and OECD classifications, based on rabbit test results, as well as in vitro test results obtained with other tissue models [SkinEthic™ direct topical application test, SkinEthic™ in vitro patch test, Episkin™, Lyon, France, and EpiDerm™, MatTek Corp., Massachusetts, U.S. (25)]. All in vitro tissue models showed similar classifications of compounds whatever the tissue supplier. On the opposite, the comparison between the rabbit data and the human in vitro data revealed differences. In particular, this was true for the following compounds: dimethyl disulfide, cis-cyclooctene, 2-methyl-4-phenyl-2-butanol, and 2,4-xylidine. These compounds were classified as irritants by all the in vitro tests, and as nonirritants or slightly irritants according to the EU and OECD classifications.

Hydroxycitronellal was the one single PVS compound with an opposite classification using our two protocols: in the in vitro patch test, it was found nonirritant, like in the European and OECD classifications, while it was classified as irritant with the SkinEthic™ direct topical application test, similar to EpiDerm™ and Episkin™ classifications. Moreover, 4-amino-1,2,4-triazole (nonirritant in two experiments and irritant in the two others, with the topical application test) was classified unambiguously as nonirritant by the in vitro patch test.

A comparison between the EU classification, the human in vivo patch test, our in vitro patch test, and our in vitro direct topical application test is in Table 3. Among these 30 HPT chemicals, only 17 compounds were classified identically in the Draize test (EU classification) and in the human in vivo patch test (15). Among these 17 chemicals, 12 were also classified similarly with the in vitro direct topical application test; 13 were also classified similarly with the in vitro patch test; 10 of them are shared in common with the 12 cited above: isopropyl palmitate, octanoic acid, decanoic acid, benzalkonium chloride (10%), polyethylene glycol 400, heptanoic acid, dodecanol, tween 80, benzalkonium chloride (7.5%), and propylene glycol. Among the seven other chemicals (of the 17), methyl caproate and benzyl alcohol were found irritant with both in vitro protocols, while they were classified as nonirritant by the EU and OECD classifications. For the five remaining compounds, the in vitro direct topical application test led to the most severe classification, while 20% *N*,*N*-dimethyl-*N*-dodecyl aminobetaine was classified ambiguously, and 2-propanol, 0.5% sodium hydroxide, triethanolamine, and ethanol were classified as nonirritants with the in vitro patch test.

Among the 13 remaining chemicals, which were not classified similarly by the rabbit test and the human in vivo test, 12 compounds were found irritant with the in vitro direct topical application test. Thus, to the exception of the methyl laurate, which was classified as nonirritant, the in vitro topical application test also led to the most severe classification for these chemicals. In parallel, 10 of these 13 compounds were found irritant by the in vitro patch test, methyl laurate and 10% hydrochloric acid were classified as nonirritant, and dimethyl sulfoxide's classification was ambiguous.

Statistical Reproducibility

When the classification of a tested compound was unclear (I/NI), the most pessimistic prediction (irritant) was chosen, according to the principle of precaution.

Statistical analysis (including Pearson correlation and slope and variation coefficient) was performed for the different endpoints of both the direct topical application test and the in vitro patch test protocol. This study revealed an excellent reproducibility from one batch to another. Details are presented in Tables 4 and 5. Upon the batches D, E, G, and H, the Pearson correlation of MTT values of the repeated experiments was contained between 0.94 and 0.98, and the Pearson correlation of IL-1α values of the repeated experiments varied from 0.92 to 0.99.

The Pearson correlation was also calculated for the 50 compounds tested with both protocols. Concerning the direct topical application test, runs D and E were taken into account for the PVS chemicals. An MTT Pearson correlation of 0.96 was obtained for this protocol, and

Table 4 Correlations between MTT-values of repeated experiments

Pearson Correlation	D	E	G	H
D	1	0.98	0.94	0.97
E		1	0.97	0.98
G			1	0.98
H				1

Table 5 Correlations between IL-1α-values of repeated experiments

Pearson Correlation	D	E	G	H
D	1	0.92	0.92	0.94
E		1	0.92	0.93
G			1	0.99
H				1

Table 6 Summary of the results applied to both protocols for the 50 tested chemicals

	Dir. Top. Appl. Test	In vitro Patch Test
% Specificity	40.9	63.6
% Sensitivity	89.3	82.1
% Accuracy	68.0	74.0

the value was of 0.97 for the in vitro patch test protocol. The corresponding measures of variation (mean standard deviation) are 5.2% and 2.7%, respectively. In parallel, the IL-1α Pearson correlation for the 50 chemicals was evaluated as 0.75 and 0.65, respectively. Moreover, the mean standard deviation for IL-1α release corresponds to 4.3% of the mean nonanoic acid–treated positive control for the direct topical application test. For the in vitro patch test, the mean standard deviation value for IL-1α release is 15.6%.

The predictive capacity of both protocols for the testing of the 50 tested chemicals obtained in our laboratory is shown on Table 6. For the in vitro patch test particularly, multiple endpoint analysis allows an improvement of accuracy and sensitivity, but a decrease in specificity (specificity = 63.6%, sensitivity = 82.1%, and accuracy = 74.0%), compared with the single endpoint approach (MTT reduction only) (specificity = 72.7%, sensitivity = 55.6%, and accuracy = 64.0%). For the direct topical application test, multiple endpoint analysis allows an improvement of specificity, but a decrease in accuracy and sensitivity (specificity = 40.9%, sensitivity = 89.3%, and accuracy = 68.0%), compared with the single endpoint approach (MTT reduction only) (specificity = 50.0%, sensitivity = 82.2%, and accuracy = 68.0%).

VALIDITY OF THE EPIDERMIS MODEL TO PREDICT SKIN IRRITATION IN HUMANS

A multiple endpoint analysis, including percentage of cell viability (MTT reduction), histology, and IL-1α release, has been elaborated in an attempt to ensure the relevance and improve the quality of the test results. Among the families of compounds described above, the chemicals of the first family mimic the negative controls, and on the opposite, the chemicals of the fifth react like the positive controls. The classification of these chemicals as nonirritant or irritant, respectively, is therefore unambiguous since all three endpoints lead to the same conclusion. If all compounds reacted similarly, one single endpoint would have been enough to classify chemicals. However, a multiple endpoint analysis is not only a reassuring method, which permits multiple information, but it also reveals its usefulness for the chemicals of families two, three, and four. The second comprises chemicals, which allow high epidermal cell viability, a normal histology, but provoke an increase of IL-1α release. According to our prediction model, chemicals belonging to this second family are classified as nonirritants. However, the increase of epidermal IL-1α release could be an early sign of skin irritation. These compounds may be irritant over a prolonged or repeated application, or on weakened epidermis.

The chemicals of the third family provoke tissue necrosis, a higher amount of released IL-1α compared with H_2O-treated negative control; however, cell viability remains higher than 50%. This class contains, therefore, irritants. MTT reduction is an efficient cell viability test when the whole tissue is necrosed. Then, no or little mitochondrial activity is observed leading to a strong decrease of the amount of formazan blue crystals in comparison to the negative controls. However, when suprabasal cell layers are necrosed while the basal cell layer remains viable, a normal MTT reduction takes place, resulting in a percentage of cell viability comparable with negative controls (37). Moreover, some other false test results can be due to interactions between MTT and chemicals. This was the case for eugenol in the four repeated experiments on (living) cultures using the direct topical application protocol, the MTT values were contained between 66.5% and 75.9% of the H_2O-treated control, while histology showed necrosis, and the level of released IL-1α was high ($\geq$ 143.9 pg/mL). This elevated MTT value was due to the interaction between eugenol and the MTT solution, since the application of eugenol on killed cultures induces an OD value, which is 82.8% compared with the viable

untreated control tissue. On the opposite, we observed that the MTT values from eugenol-treated cultures in the patch test protocol are contained between 12.0% and 17.5% of the H_2O-treated control. This could seem paradoxal, but it is necessary to remember that a smaller quantity of compound is applied in this protocol and, moreover, that the patch partially absorbs the compound. In the case of eugenol, in patch testing, our hypothesis is that a four-hour incubation is sufficient to allow complete necrosis of the tissues and increased IL-1α release, but it did not allow eugenol to reach the MTT solution and interact with it. On the contrary, 5% potassium hydroxide did not provoke any interaction with the MTT, nor on living tissues nor on frozen-killed controls, although its direct contact with MTT solution provokes an OD value of 1.6. Concerning heptanal, the third and last compound, which showed interaction with MTT, MTT values of 35% of the H_2O-treated control were observed during the experiments on living tissues. This is completely comparable to the results obtained on frozen-killed controls. Although it is cautious to proceed to additional controls such as MTT interaction with chemicals, multiple endpoint analysis also allows detection of false viability measurements. Moreover, the classification according to our prediction model is not modified, even when the interaction with MTT is significant. In particular, it allowed to classify eugenol as irritant (with the direct topical application test protocol), while it would have been impossible to conclude with a single MTT endpoint approach.

The fourth family includes chemicals, which induced low cell viability and tissue necrosis, but did not provoke any significant increase of the IL-1α release. Therefore, most of the compounds of this class are highly irritant or corrosive. The small amount of released IL-1α could be due to the fast and massive destruction of the tissues, which did not have time to release cytokines. Another explanation could be the direct destruction of the cytokines by the chemical. Most of these compounds belong to the strong acid/base family. The epidermal tissues are severely damaged, and necrosis is provoked by these compounds. They can thus penetrate the epidermal tissue more easily and dissolve in the defined nutrient medium. This passive diffusion of the compound through the tissue to the medium was visually detected by the modification of the color of the medium.

Furthermore, some differences are observed between test results of the two in vitro protocols described. First, MTT values are in most cases higher with the patch test protocol. Accordingly, histology sections present a less severe necrosis. One possible reason for this is the reduced amount of chemical applied in the patch test in comparison with the direct topical test. A double quantity per centimeter square is applied on the tissues in this latter test. The quantity applied in the direct topical application test has been chosen to mimic published in vitro test protocols (25) and for its capability to cover uniformly the whole surface of the epidermis, whatever the texture of the compound to be tested. In the in vitro patch test, the quantity of chemical applied was defined proportionally to the human patch test described by Basketter et al. (14). Moreover, the structure of the Hill Top chamber itself is responsible for a partial absorption of some chemicals, reducing even more the amount of chemical that is in contact with the epidermis. It seems that the tissues necrosed more slowly in the case of irritant chemicals than in the direct topical application test. False negatives may result from these two parameters in the patch test protocol. In particular, in the case of the compounds of the sixth class, the increased MTT values may represent overestimations since high percentages of cell viability are observed, although the histology sections show necrosis.

However, although the chemical's effects seem to be less severe with the patch test protocol, the application of an empty patch alone is responsible for a four- to fivefold increase of the basal level of IL-1α release. This increase is probably due to the ratio of tissue surface to medium volume that was double for 0.63 cm^2 tissues compared with the 4 cm^2 tissues. Also, the topical application of an empty patch as well as some occlusive effects due to the patch induce a slight increase of basal IL-1α secretion.

The principal interest of in vitro experiments is not only to obtain reproducible data using more convenient and more ethical test protocols but also to produce useful indications on the human skin irritation potential of raw materials and finished products. The result of the comparison between in vitro and in vivo data is heterogeneous (Tables 2 and 3). Several chemicals were classified differently. On one hand, there are the compounds for which all in vitro and in vivo data corroborate, and, on the other hand, the chemicals for which in vitro classifications are in conflict with those obtained in vivo; or even more, chemicals for which in vivo rabbit data are in opposition to human in vivo data. Concerning the PVS chemicals, we

observed that our results resembled the results of those performed with other epidermal models in most cases. Notably, dimethyl disulfide, cis-cyclooctene, 2-methyl-4-phenyl-2-butanol, and 2,4-xylidine were classified irritant in vitro and nonirritant by the rabbit test. Lilestralis has been classified nonirritant with our two in vitro test protocols, although the EU and the OECD data filed it as an irritant. However, the documentation available from its suppliers does not mention any irritant properties for this compound, but only sensitizing effects. All results shown here are relative to the sample of chemicals obtained. Therefore, the comparisons made with in vivo and in vitro test results are only indicative, as other batches of chemicals were tested. Moreover, methyl palmitate, which was tested both on rabbits (25) and humans (14), is classified as irritant and nonirritant, respectively. All in vitro tests, like the in vivo patch test, classified this compound as nonirritant. Interspecies differences could be the explanation.

In vitro reconstructed human epidermal tissues mimic the biophysical properties of in vivo human epidermis. However, the reconstructed epidermis seems more sensitive to some families of compounds. Although 17 days of airlifted tissue cultures feature a fully differentiated stratum corneum (34,38), and a normal lipid composition (39), their barrier function seems to be less efficient (40,41) compared with adult skin samples, leading to a higher sensitivity to chemicals. This higher relative permeability may correspond to the epidermis of a newly (17 days) re-epidermized wound. This increased sensibility is considered as an advantage by Jones et al. (42) and Garcia et al. (41). Thus, when no toxicity is observed for a compound tested on reconstituted epidermis in vitro, the toxicologist can be confident about its safety regarding human use. Reconstituted epidermis could therefore be used according to the principle of precaution. Furthermore, in vitro tests using reconstructed epidermis present reproducible results leading to an unambiguous classification for almost all the tested chemicals.

Although the identification of the chemical's potential hazard is of the highest importance for both industries and consumers, its classification is difficult. Human data are certainly the most informative, but they are available for only few chemicals, and it is dependent on the protocol used, the age (20), the anatomical site (21), and the seasonal variability (18,22,23). On the contrary, animal data are more easily available, but the protocol used, the organism, and even the laboratory may provide different results as shown by Weil and Scala (8). Today, the relevance of the animal tests to assess human potential hazard is discussed, and the lack of reproducibility makes them even more questionable.

We cannot assure that our current protocols using three-dimensional reconstituted human epidermis is perfect to predict human skin irritation, but in addition to the classical advantages of in vitro methods, such as a great convenience and reduced costs, reproducibility is strongly increased compared with other methods. This reproducibility is not only seen for a given product on repeated experiments, but by individual endpoint measured for each tested compound. In our experiments using the PVS chemicals with the direct topical application protocol, the Pearson correlation is contained between 0.94 and 0.98. The MTT values show that they are almost always of the same magnitude, not just under or over 50% of viability. In the same way, we could make very similar histological observations for a chosen chemical. The statistical comparison of the IL-1α results shows that even if the amount of released IL-1α is not always of the same range for the irritant compounds (Pearson correlation for 50 compounds of 0.75 for the direct topical application test and of 0.65 for the in vitro patch test), chemicals can be classified in two classes. One contains the compounds, which always present an amount comparable to that of the negative control, and the other exhibits a significant increase of the amount of IL-1α, compared with the negative control. Such a reproducibility has never been shown with the Draize test or human patch test. Because of this strong reproducibility, the human in vitro epidermis already represents the tool of choice for screening compounds for their skin irritation potential.

Interestingly, note that the results obtained in our laboratory with the in vitro patch test protocol met the specificity, sensitivity, and overall accuracy performance criteria ($> 60\%$) defined for the ECVAM pre-validation study described by Fentem et al. (25) (Table 6). Moreover, a recent study performed by Kandárová et al. (43) revealed that dimethyl disulfide had been improperly tested in vivo. Consequently, the real classification of this compound is unknown. In parallel, methyl palmitate presents an ambiguous in vivo classification according to the literature (Table 1). If we remove these two chemicals, specificity obtained with the in

vitro patch test increases to 71.4%, sensitivity to 85.2%, and accuracy to 79.2%. Consequently, our in vitro patch test should be accepted for formal pre-validation by ECVAM.

However, all these test results are relative to the samples and lot numbers of the tested compound. We observed important variability in test results when certain compounds (lilestralis, hydroxycitronellal) came from different suppliers (unpublished data). For official validation studies, great care should be taken to control the quality of the reference compounds tested. Transferability being one of the parameters for ECVAM validation, it is indisputable that our encouraging intra-laboratory results should be followed by an inter-laboratory study. On the opposite, performance of the direct topical application protocol was disappointing compared with the in vitro patch test protocol and other published data. Taken together, this data set provides a platform for further mechanistic and validation studies. We do not wish to overgeneralize these data; judgment will continue to be required when extrapolating such information for new chemicals in terms of their complex uses in biology. Moreover, SkinEthic^{TM} epidermis is also involved in the current ECVAM skin irritation validation studies using the 15-minute direct application time followed by a washing step and a 42-hour incubation (44).

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51 Reconstructed Corneal and Skin Models

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INTRODUCTION

During the last decade tissue engineering became a progressing field in biotechnological research. The vision of medical treatment of burnt patients, the treatment of ulcera, and the idea of reconstructing damaged organs revealed very rapidly further possibilities. Tissues resembled not only morphologically the situation in vivo but also revealed comparable physiology. This made artificial tissues interesting for testing efficacy of pharmaceutical and cosmetics products. The development of the tissue models was paralleled by an increasing demand in using alternative methods for the identification of toxicological hazards inherent to raw material with the vision to replace animal testing for human safety assessment in toto.

Increasing efforts were made to validate such alternatives against the existing animal tests. Some of those are already successful, others, though promising, need further refinement.

Since the major field of applications for the cosmetic industry is doubtlessly the surface epithelial lining of humans, i.e., the skin and the mucous membranes of mouth and eye, progress in reconstructing such models is followed thoroughly and applications for these alternatives are evaluated extensively.

This chapter deals with the comparability of the reconstructed human corneal and skin models with the in vivo situation and shows up some areas of application in cosmetic science.

RECONSTRUCTED CORNEAL MODELS

Corneal Tissues

The epithelium of the eye surface distinguishes three regions: centrally the cornea, the limbus as transitional zone, and the peripheral conjunctiva. As a mucous membrane, it is a squamous epithelium, not keratinized but stratified. In contrast to the conjunctiva, the central cornea and the limbus are devoid of other cells such as Langerhans cells, melanocytes, and endothelial capillary cells.

The cornea itself is formed by three layers: an epithelium, a stroma, and an endothelial lining.

The epithelium consists of four to five cell layers with changing morphology. The cells of the basal layer are polygonal in shape. While proliferating, they produce lateral extensions to form so called wing cells. Apically they become flattened and compose a superficial cell lining. The outermost cells are strongly interlocked, building tight junctions to form a non-keratinized barrier. All cells contain a nucleus.

The stroma below the basal cells constitutes the majority of the corneal thickness. Directly below the basal cells of the epithelium, there is an acellular region called the Bowman's membrane, going over into the stroma. The stroma itself is a highly organized tissue made up by paralleled lamellae of collagen fibrils. Elongated fibroblast-like keratocytes are found throughout the stroma, running in parallel to the collagen lamellae. Posterior, a single layer of endothelial cells lines the stroma separated from it by the Descemet's membrane, a true basement membrane.

Damage to the Cornea

Irritation of the eye is a local and reversible response to external stimuli. Corneal and conjunctival cells are involved in this response. When evaluating damaging effects in animal testing like the rabbit Draize test, the majority derives from damage to the cornea (1). Studies on pathological changes after application of surfactants in standard animal irritation assays revealed that at early timepoints of three hours and one day after application, the potency of

the compounds could be differentiated. Innocuous and slight irritants affected superficial cells, mild to moderate compounds affected the epithelium and the superficial stroma, whereas severely irritating substances deteriorated deep stroma down to the endothelium (2).

Studies using non-surfactant compounds widened the insight into irritation, indicating that compounds may differentially injure cornea and conjunctivae. Furthermore, timepoints of evaluation played a critical role since damage at three hours could not predict severity of damage at one hour. Examples were given that, though in many cases damage by compounds is a progressive event first affecting the epithelium, some compounds have stronger effects on the stroma without involving the epithelium.

Thus, aspects of penetration, cytotoxicity, as well as time-related effects have to be considered when addressing hazard assessment by alternative methods. Therefore, as injury is a three-dimensional process, alternatives should focus on three-dimensional models.

Reconstructed Cornea Models

Corneal tissues produced by methods of tissue engineering resemble the in vivo situation more and more with respect to morphology, physiology, and biochemistry.

Tissue constructs exist on the basis of cells of different animal origin, i.e., human, rabbit, or bovine. They differ in their complexity. While some models are simply made up by epithelial cells (3), others comprise an outer epithelium grown on a stromal equivalent (4). The most complex equivalents even contain a posterior endothelial lining (5,6).

In all these models, the in vivo situation is closely mimicked. Depending on the origin of corneal cells, the epithelium is composed by 5 to 6 or 9 to 10 cell layers. Basal cells and wing cells might not be differentiated as clearly as in vivo, but stratification is obvious from flattened superficial cells being tightly packed and interlocked and joint by tight junctions forming a barrier. Slight differences in the morphology of this superficial cell lining are discussed to be due to the absence of lacrimal fluid and eyelid blinking (6). Basal layers express hemidesmosomes. Their internal placodes are connected to the cytoskeleton (3).

A functional expression of extracellular matrix plays an important role in the integrity and function of a tissue. A major component in the basement membrane, i.e., laminin, is detectable in the basal layers of the reconstructed skin model. In vivo fibronectin is localized in the basement membrane and promotes corneal migration and re-epithelialisation. In the reconstructed cornea models it is mostly detected at the epithelial-stromal junction as well as the collagen matrix making up the stromal equivalent (4,6). Integrins are expressed differentially throughout the epithelium according to their location of action. Their expression resembles the situation in vivo (4).

Permeation studies with pharmacologically active compounds underline the close resemblance and functionality of the described models. Permeation coefficients evaluated by penetration studies with organotypic cornea models differed form the in vivo situation in factors only smaller than two indicating the functionality of the epithelial barrier (6).

Use of Reconstructed Corneal Epithelia in Safety Assessment

For the prediction of irritating effects, mainly reconstructed epithelial models are used. In contrast to the more complex models, these are commercially available. The use comprises testing of pure compounds as well as formulations.

The main endpoint evaluated in such prediction models is cytotoxicity based on the conversion of MTT in the vital layers of the cornea (7,8). After application of cosmetic formulations for different time durations to the corneal equivalents, ET50 values are calculated estimating a time at which 50% of the tissues are mortal. On the basis of internal benchmarks, such models are used internally for product development (7). In a study with 68 tested products, irritant effects were overestimated by 10% compared with modified maximum average scores (MMAS) data, while there was only an underestimation of 1.5% (8). Therefore, it can be argued that they have a predictive ability to identify nonirritants from irritant products.

Testing pure compounds showed that prediction models from formulation testing with cytotoxicity as the only endpoint could not be transferred as such (8). Cytotoxicity testing focuses on the conversion of MTT in the viable layers of the equivalent not picking necrotic effects in the suprabasal layers. Additional parameters for evaluation of toxicity such as of histological parameters should be taken into account as well. A pre-validation study analyzing the viability of corneal tissues by the ability to reduce MTT after treatment with 20 pure chemicals, irritants and nonirritants, revealed an overall concordance of 80%. While all irritants

were predicted correctly (sensitivity = 100%), the prediction model produced a number of false positives (specificity = 56%) (9). Officially validated prediction models by European Center for the Validation of Alternative Methods (ECVAM) based on human reconstructed cornea do not exist yet.

Further endpoints such as release of cytokines and chemokines often used in other epithelial models are not subject to prediction models for eye irritation, though they are released after damage of the cornea (10), are detected in tears (11,12), and play a vital role in its regeneration (13). Studies have shown that cytokines can be produced after stimulation of corneal epithelial rat cell lines (14) and immortalized human cell lines (15). The evaluation of these parameters together with the already established multi-endpoint analyses might give us the chance to have a validated and internationally accepted alternative method to the Draize test.

RECONSTRUCTED SKIN EQUIVALENTS

Skin Equivalents

It has been 20 years since epidermal equivalents have been produced successfully for clinical applications (16). Since this time several equivalents of different complexity have been produced for scientific investigations as well as for commercial use. Today the most common equivalents are made up of keratinocytes. Biopsies from clinical surgery of adults and foreskin of young boys are sources of keratinocytes. In the simplest models, these cells are grown in culture under submerged condition for approximately 14 days until a multilayered tissue equivalent is formed. This equivalent is lifted to the so-called air-liquid interface in culture dishes to become stratified and cornified. Thus, such models distinguish several layers resembling in morphological characteristics to native skin: a stratum basale, a stratum spinosum, a stratum granulosum, and a stratum corneum.

Epidermal equivalents consist of 5 to 14 layers of viable cells depending on the model. Their thickness varies between 23 and 100 μm. Overall, they do not seem to be as thick as native skin with 80 to 90 μm.

The cells of the stratum corneum are of columnar to round shape. In contrast to native skin, they regularly contain intracellular lipid droplets. Keratinocytes of this layer are involved in the formation of the basement membrane. A lamina densa and a lamina lucida are produced by all equivalents in a patchy instead of continuous manner. Hemidesmosomes as anchoring structures between cells and basement membrane indicate the functionality of the stratum basale.

The stratum spinosum is characterized in the upper layers in vivo as in vitro by flattened cells. Unlike in native skin, few intracellular lipid droplets are recognized in cells of this layer.

The stratum granulosum produces numerous lamellar bodies of normal appearance thus correlating with the in vivo situation. Depending on the skin equivalent, they are more or less rapidly extruded at the interface to the stratum corneum. The unique organization of the alternating electron-dense and electron-lucent lipid lamellar sheets in the intercellular space between the corneocytes indicates a physiological processing of extracellular lipids of the stratum corneum.

The stratum corneum is made up of 14 to 25 cell layers compared with 15 to 20 in native skin. Its thickness ranges from 12 to 37 μm instead of 10 to 12 μm. In equivalents used for penetration studies, this number increases to 100 μm due to 100 layers of cells.

A detailed comparison of the characteristics of the different epidermal equivalents is given by Ponec et al. (17).

In concordance with the morphological resemblance of the reconstructed skin equivalents to native skin, expression and localization of differentiation markers correlate to the in vivo situation. Keratin 1 and 10 as indicators for early differentiation are present in all suprabasal layers of nearly all skin equivalents. The same holds true for loricrin and SPRR2, markers only present in the stratum granulosum. The localization of other differentiation markers like involucrin and transglutaminase does not resemble native skin as they are not restricted only to the stratum granulosum and found in all suprabasal layers. SKALP and SPRR3 are expressed in some epidermal tissue equivalents though absent in vivo.

Studies on comparative gene expression uncovered similarities between equivalents and native skin and showed up differences to monolayer cultures.

Monolayer cultures lack differentiation markers expressed in the upper layers of the epidermis, such as filaggrin, loricrin, involucrin, and keratins K1 and K10. Furthermore they

overexpress actin-associated cytoskeletal proteins and different integrins, reflecting their motility and adherence to the culture dishes, respectively. Proteins related to cell cycle and DNA replication are expressed in cultured keratinocyte monolayers while repressed in native skin. Together with a high expression of nucleoskeletal proteins, they reflect requirements in rapidly proliferating cells. The expression of corresponding genes identifies skin equivalents as metabolic active tissues somewhere in between monolayer cultures and native skin.

Similarities to native skin are found in the expression of cell-to-cell signalling molecules, as secreted proteins and cell surface receptors. This difference to monolayer cultures might indicate that cell-cell communication is important in the organization and maintenance of a stratified epidermis (18).

Recently, Poumay et al. (19) published a protocol that allows any experienced laboratory to produce its own epidermal equivalent. According to the protocol, one obtains a fully stratified epidermis within 14 days that reveals all the characteristic markers of differentiation (keratins 14 and 10, involucrin, and filaggrin).

Full-thickness skin models are of a higher complexity. They comprise a dermal and an epidermal compartment. Fibroblasts have to be cultivated in a dermal compartment and have to be given enough time to populate the space before keratinocytes can be seeded on top. Fibroblasts need a matrix that offers an environment facilitating the cells to exert their physiological characteristics. The use of collagen without any further treatment for its use as a dermal equivalent bears problems. The physiological characteristic of fibroblasts to exert traction forces leads to a contraction of the gel. Thus a matrix is required to be rigid enough to resist the contraction forces of the fibroblast while offering them the right physiological environment. Fibroblasts seeded to such a lattice produce their own extracellular material: collagen, elastin, fibrillin, fibronectin, and fibulin to mention a few. They organize the orientation of the fibers in the extracellular matrix as to be found in vivo. Short fibers are in close vicinity to the dermo-epidermal junction (DEJ) oriented perpendicularly, and long fibers oriented along with the DEJ in deeper parts of the dermis. This organization corresponds to the situation in vivo where similar differences can be seen between the papillary dermis and the reticular dermis. A proper DEJ is formed between the fibroblasts and the keratinocytes comprising the epidermis. Typical markers are expressed like laminin and collagen IV and VII. The basal layers of the epidermis express strong integrins, while in the suprabasal layers, one can find the differentiation markers transglutaminase and different cytokeratins (20).

Barrier Function and Penetration

One of the major functions of the human skin is the protection of the body against the loss of water. This function is fulfilled by a barrier in the upper layers of the stratum corneum being produced during the process of keratinization. In epidermal equivalents, this terminal differentiation is induced by culturing tissue equivalents at the air-liquid interface. Its development can be followed by the cutaneous permeability of caffeine in epidermal equivalents, which decreases with time at the air-liquid interface, finally reaching a plateau. After approximately 16 days in culture, no further improvement of the penetration characteristics can be observed (21).

The barrier is made up of three major components: the multiple lipid lamellae filling the extracellular space between the corneocytes, an impermeable cornified envelope made of proteins produced during terminal differentiation and coating corneocytes internally, and a corneocyte lipid envelope of ω-OH-ceramides, ω-OH-hydroxy acids, and free fatty acids situated externally to the cornified envelope. A proper composition and a structural organization of the lipids in the stratum corneum are required for a functional barrier (17,22). Though skin equivalents contain all major lipid classes, differences are noticed in content and profile either between the models or in native skin. None of the models resembled native skin in terms of lipid composition and ceramide profiling. With respect to ceramides, content of ceramide 2 is much higher in the epidermal equivalents, while polar ceramides are underrepresented or even missing.

Penetration studies with compounds of different lipophilicity revealed great differences with respect to flux across the membranes between excised human skin and epidermal equivalents. Permeability toward hydrophilic compounds as salicylic acid and caffeine showed increased fluxes by a factor of 20. Mannitol, another hydrophilic compound revealed an increased flux by factors of 20 to 50 depending on the equivalents tested (23). Hydrophobic

substances penetrated skin equivalents 900-fold faster. Reproducibility of penetration between different batches is regarded as an indicator for reproducible barrier function in skin equivalents (21,24). Depending on the compounds tested, one can conclude that reproducibility between batches is dependent on its lipophilicity. Gysler et al. (25) reported a variability of 14% between various batches regarding penetration of prednilosone being better than that of native skin. Garcia (21) demonstrated satisfactory coefficient of variation (CV) at approximately 20% regarding penetration of caffeine, confirming data published by Lotte et al. (24). Reproducibility of penetration of strongly hydrophilic mannitol was poor between batches in all the different models tested, while best for lipophilic lauric acid.

In a recent study by Schäfer-Korting et al. (26), different epidermal equivalents, SkinEthic®, EpiDerm®, and EpiSkin®, have been compared with human epidermis, bovine udder skin, and pigskin in a multicenter approach. Analytes were caffeine and testosterone. It turned out that the human reconstructed epithelia revealed inter-laboratory and intra-laboratory variability. In comparison with the human epidermis, the permeation of the compounds via the reconstructed epithelia are overestimated. Obvious differences were observed between the different equivalents. For testosterone, the model with the poorest barrier was SkinEthic, followed by EpiDerm and EpiSkin, for caffeine barrier was best in EpiDerm.

Thus, the barrier of commercially available epidermal equivalents is still less effective than that of native skin. This is discussed to be an intrinsic property of all epidermal equivalents (27). The different permeation characteristics between the reconstructed tissues indicate that though histological parameters are quite similar and resemble native skin, further research is necessary to reach a common standard achieved by every supplier. The only model resembling native skin in terms of composition and ceramide profile is the re-epithelialized de-epidermized dermis by Ponec (28). With respect to penetration, this model shows best penetration characteristic for caffeine only differing to a factor of 2 in terms of flux from native skin (Ponec: personal communications).

Irritation Testing

The close resemblance of the epidermal equivalents to native human epidermis favors its use for the prediction of skin irritation that is still assessed on animals. Since keratinocytes are the first cells coming into contact with external compounds, they play an important role in the initiation and modulation of skin irritation (29). Markers produced and released by these cells are initial signals for visible clinical signs of irritation as edema and erythema, due to responses of the deeper tissues in human skin.

Early effects of irritation are mostly studied with models consisting only of keratinocytes, pure epidermis models. They are mostly commercially available: EpiDerm, EpiSkin, Apligraf®, and SkinEthic. Further models are in-house developments (30) or used for scientific purposes (31,32).

Recently, Poumay et al. (19) published a protocol that allows any experienced laboratory to produce its own epidermal equivalent. According to the protocol, one obtains a fully stratified epidermis within 14 days that reveals all the characteristic markers of differentiation (keratins 14 and 10, involucrin, and filaggrin).

In 2007, the ECVAM validated a prediction model to predict skin irritation with human reconstructed epidermal models. Analyzed were 58 chemicals, irritants and nonirritants. The assessed endpoints were viability measured by the turnover of MTT and analysis of released interleukin (IL) 1α to increase sensitivity. With the combination of the two endpoints, an overall sensitivity of 91% and a specificity of 79% for EpiSkin were achieved. It is regarded as a full replacement of the rabbit skin irritation test. For EpiDerm, a sensitivity of 57% and a specificity of 85% were obtained; analyzing IL 1α did not result in an improvement of these results. Thus, this system was recommended to be used in tiered testing strategy to assess skin irritation but cannot be regarded as a stand-alone prediction model (33). Catch-up validations of other commercially available systems (e.g., EST-1000®, Advanced Cell Systems®, and others) are undertaken so that several systems from different producers should be available soon to assess skin irritation in vitro.

An OECD guideline for the prediction of skin irritation in vitro does not exist yet.

Predicting potential irritation of ingredients is only one point that interests cosmetic industry. Another point is at least as important as the knowledge about hazardous effects in the predictivity of the irritating effects of formulations.

Measuring the turnover of MTT definitely faces the fact that the epidermal equivalents metabolize it only in the (supra)basal layers. Toxic effects not affecting the lower parts of the epidermal equivalent but the apical layers, as sodium lauryl sulfate (SLS), therefore cannot be predicted and evaluated (34). At least the evaluation of histological sections has to be taken into account. Studies therefore include in addition the determination of inflammatory mediators and enzyme release. Several studies dealt with the detection of inflammatory mediators, as cytokines, chemokines and prostaglandins. IL 1α, constitutively expressed, is one of the most important cytokines since it is released from keratinocytes immediately after membrane damage. Its release resembles data obtained by Lactate dehydrogenase (LDH) release. Further studies looked at IL 6. Though not released by pure epidermal equivalents, it is produced after irritation to model consisting of an additional dermal part (reviewed in 35).

IL 8 has strong chemotactic effects. It is induced by IL 1α and produced by keratinocytes and fibroblasts, therefore transducing effects to deeper parts of the skin. Prostaglandine E3 (PGE3) is the best investigated prostaglandin with respect to application of irritants to skin equivalents. Though some models produce PGE3 in a dose-dependent manner (32), other models fail to show such a relationship (35).

Perkins et al. (36) compared data concerning vitality (MTT), the release of enzymes as LDH and aspartate aminotransferase (AST), and the release of IL 1α after treatment of skin equivalents for definite times toward a human 14 days' repeated patch test, assessing the irritating potency of cosmetic formulation. The results revealed that for the prediction of irritating effects due to cosmetic formulations, endpoints like vitality (MTT) was useful for rank-ordering skin irritancy levels of surfactants. In addition with enzyme release (LDH and AST), these parameters distinguished lower and higher irritancy products. IL 1α was able to distinguish and rank-order the compounds of irritancy between these two extreme points. Another study compared the irritation effects of 22 cosmetic formulations. Endpoints measured in vitro were the determination of the effective time after application when 50% of the tissues lost their viability (ET50), percent of viability left 16 hours after application, the release of IL 1α, and the release of LDH. In vivo irritation was assessed under occlusive conditions by the modified Frosch–Kligman soap chamber patch test, in which the test material is applied repeatedly: the first time for 24 hours, followed by three applications of 6 hours on each of the following three days. Skin reactions are scored on each day until day 5. In addition, skin reddening was measured with a chromameter, and barrier interference was assessed by transepidermal water loss (TEWL) at the beginning and at the end of the study. The best rank correlation in the in vivo and in vitro data was achieved for ET50 followed by MTT at 16 hours and the IL 1α release, while for LDH release correlation was generally low.

Comparing the mean total score of the in vivo evaluation at day 5 with ET50, linear regression analysis gave coefficients of correlation of $r = 0.84$ to $r = 0.94$, depending on the model. Further analysis of the data by contingency tables taking into account a visual score of 2 as a cutoff value between irritancy and nonirritancy and MTT50 values as discriminator revealed equivalent results in all models tested: sensitivity = 92%, specificity = 100%, and concordance of 95%.

Protocols for the prediction of irritating effects of formulations are generally in-house methods. Therefore direct comparisons are hardly possible. Some of the protocols concentrate on the scoring and grading on biochemical parameters (Chatelain, personal communication), others use statistical methods to work out correlation between in vivo and in vitro data.

Generally one can conclude that in vitro assessment of irritation induced by formulations can distinguish between nonirritating and irritating formulations when data are compared with objective endpoint, clinical signs of irritation such as edema, erythema, and fissures. Other more subjective effects like stinging, itching, and pain are hardly to be predicted with the existing in vitro approaches.

Pharmacotoxicology

The reaction to topically applied irritants with the release and production of inflammatory mediators indicates that epidermal equivalents resemble native skin not only in terms of morphology but also in terms of physiology and biochemistry. Different studies deal with the biochemical characterization and metabolic competence of these models to identify them as alternatives for pharmacotoxicological studies. Gysler et al. (37) demonstrated the conversion of topically applied glucocorticoids into their metabolites by the metabolism of the

reconstructed skin models. The double ester prednicarbamate (PC) esterified at position 17 and 21 was hydrolyzed by esterases during its passage through the skin equivalent into the monoester P17EC and later on after passage nonenzymatically to P21EC. No PC itself was detected after passage, since it was totally metabolized. These results were in analogy to those obtained from experiments with native skin.

A fluorinated monoester betamethasone-17-valerate (BM17V) was not affected by esterases because of a missing ester bond at position 21, which passed the skin unconverted. The only metabolites found were BM17V and the nonenzymatically converted BM21V, after permeation of skin equivalents as well as native skin. Thus, the metabolism of PC and BM17V was well reflected by the skin equivalents.

Another study shows the applicability for screening skin-targeted androgen modulators since skin equivalents express type 1 5α reductase (5αR) activity. RT-PCR experiments revealed the expression of a unique 5α R1cDNA fragment, while there were no traces of 5α R2. This reflects the situation in native skin where 5α R1 is highly predominant and regarded as the important enzyme for testosterone turnover.

Topically applied testosterone is metabolized during permeation by 5α reductase mainly to dihydroxytestosterone. When inhibiting the enzyme by finasteride, this metabolic pathway is blocked dose dependently in skin equivalents as the main metabolite 4-androstene-3, 17-dione is produced (38).

In this respect, enzymes of the xenobiotic metabolism are of comparable importance. Activities of phase I enzyme cytochrome P 450 IA1 (CYP IA1) concerning its 7-ethoxyresorufin-O-deethylase (EROD) and 7-ethoxycoumarin-O-deethylase (ECOD) and of phase II enzyme glutathione S-transferase (GST) by 1,4-chlorodinitrobenzene (CDNB) conversion were examined in several skin equivalents (39). Furthermore, NAD(P)H:quinone reductase (NQR) activities were tested.

EROD activities were below detection levels in all tested models, but could be induced by 3-methylcholanthrene. This induction was strongly batch dependent in all the models, since some batches were not inducible at all. With respect to ECOD activity, there was a basal activity in all models. ECOD activity was only inducible in those batches that were inducible for EROD as well. Both activities could be inhibited by clotrimazole.

General GST activity against the standard substrate CDNB was detected in all equivalents. Variation within and between batches of all models did not exceed 20%. Activities were higher than in normal skin except for EpiSkin (40).

NQR is an enzyme that catalyzes the reduction of quinone, compounds present in the environment naturally or anthropogenic. Activities were tested against menadione. All tested models were competent concerning NQR, with SkinEthic showing highest and EpiDerm showing lowest activities. Activities in EpiDerm resembled those of native skin best. Inhibition by dicumarol could be induced in all the tested equivalents.

Studies on the gene expression of enyzmes of the xenobiotic metabolism in full-thickness models showed great resemblence to the in vivo situation. Full-thickness models were separated into dermis and epidermis, as well as native skin. Both compartments were analyzed for their gene expression to show differences in the expression pattern of epidermis and dermis and differences to the in vivo situtation. Phase I enzymes and phase II enzymes are comparably expressed in the different compartments of the native skin. Differences could be seen neither qualitatively nor quantitatively. Genes less strongly expressed could be induced by β-naphthoflavone (41). A review by Gibbs et al. (42) summarizes the actual status of xenobiotic metabolism in skin models compared with native skin.

These studies reveal the metabolic competence of skin equivalents, the physiological regulation of gene expression, and thus their use for pharmacotoxicological studies.

Studies with Melanocytes

For examination of mechanisms of skin tanning and the identification of ingredients influencing this process, the addition of melanocytes to epidermal equivalents is of great advantage. Cocultures of melanocytes and keratinocytes resulted in an enhanced survival of these cells and promoted melanin synthesis (43). Melanocytes and keratinocytes form together a so-called epidermal melanin unit. It typically consists of one melanocyte that is in contact with approximately 35 keratinocytes. Dendrites formed by melanocytes interdigitate into the intercellular spaces. Melanosomes produced within these dendrites are transported into the

keratinocytes. Here they orient themselves toward the nucleus and are organized in the form of an apical cap protecting the nucleus against irradiation. Thus functionality of the melanin unit can be monitored in the in vitro system. A recent study by Yoon et al. (44) shows the applicability of these in vitro systems to screen for melanogenesis affecting compounds. Reconstructed epidermal equivalents contained melanocytes of different origin: of African-Americans, Asians, and Caucasians. Two compounds, melanin-stimulating hormone (MSH) and dihydroxyphenyl alanine (DOPA), known as stimulators of melanogenesis, were examined for their ability to induce melanin content and tyrosinase activity. MSH increased tyrosinase activity in all three types of equivalent. This resulted in an increased content of melanin. In histological sections, Fontana–Masson staining of the melanin revealed an extended pigmentation in the upper layers of the skin.

DOPA increased melanin content, but decreased tyrosinase activity due to competition with the substrate used. Effects were more obvious in models with melanocytes from African-Americans and Asians than from Caucasians.

The key enzyme in the melanogenesis is the tyrosinase regulating the hydroxylation of tyrosine. Therefore known inhibitors affect the activity of this enzyme. Four inhibitors hydroquinone, arbutin, kojic acid, and niacinamid were tested. All compounds inhibited tyrosinase more or less dose dependently in all tissues, with hydroquinone having strongest effects. Melanin content was decreased in all tissues accordingly, whereas Fontana–Masson-stained section revealed a decreased melanin content only in the hydroquinone- and arbutin-treated equivalents.

Though some properties of reconstruced skin need further improvement, many characteristics resemble the in vivo situation. Besides a comparable morphology tissue, equivalents show similar reactions with respect to physiology, whether this is a biochemical answer to irritating compounds, the conversion of pharmacological active compounds by a comparable enzyme system, a resembling xenobiotic metabolism, or a coculture of skin relevant cell types.

Therefore tissue equivalents are used as a reliable tool supporting product development and since recently predicting the skin irritation potential of pure chemicals.

Further efforts are on the way to promote the acceptance of the use of these models also for regulatory acceptance.

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52 Seawater Salts: Effect on Inflammatory Skin Disease

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INTRODUCTION

Use of mineral spa water and seawater has been and continues to be a common treatment modality for inflammatory skin conditions such as psoriasis, atopic dermatitis, and irritant contact dermatitis. Spa water and seawater are noted for their relatively high concentrations of minerals such as strontium and selenium and for their high osmolarity relative to physiological saline (Table 1). Despite widespread use, few studies explore what aspect of seawater accounts for its therapeutic effect and what is its mechanism of action. Recent studies are summarized in Table 2 (1–4).

SEAWATER

Recent in vivo and in vitro studies lend credence to the common practice of applying seawater to inflamed skin. In acute eruptions of atopic dermatitis, seawater exhibited antipruritic effects as evaluated by a significant reduction of visual analogue scores for itching (1). In the setting of irritant contact dermatitis, Pacific ocean water compresses significantly decreased transepidermal water loss (TEWL) and increased skin capacitance compared with the deionized water control when the compresses were applied for 20 minutes at a time for several times over the course of two weeks (5). TEWL measures water barrier disruption, while capacitance measures stratum corneum water content. Thus, the results provide evidence for seawater's ability to inhibit skin barrier disruption and inhibit stratum corneum dryness in irritant contact dermatitis. Seawater has also been shown to be of benefit in psoriasis. In a randomized, double-blinded, controlled study, Dead Sea® salt baths, containing a high mineral composition, were administered daily at 35°C for 20 minutes for three weeks. Relative to the distilled water control, Dead Sea salt baths significantly decreased psoriasis area and severity index (PASI) scores in psoriasis vulgaris patients immediately after treatment, with therapeutic effects still significant one month after the treatment ended. However, there was no statistical difference in PASI scores and patient subjective evaluations between the treatment group that received Dead Sea salt baths and the group that received common salt baths [mostly sodium chloride (NaCl)] of the same osmolality. While this study supports seawater's therapeutic effects, it suggests that osmolality, instead of ion character, may act as the active component in seawater therapy (2).

CATIONS

Sodium

As a substantial component of seawater, sodium has been explored as an explanation for seawater's therapeutic effect. Similar to seawater, compresses with 500-mM NaCl alone have been shown to inhibit the increase in TEWL and increase skin capacitance and, thus, inhibit skin barrier disruption and inhibit stratum corneum dryness in irritant contact dermatitis (5). Sodium compresses for irritant contact dermatitis also decreased blood flow associated with irritation relative to baseline values when applied for 30 minutes twice a day for four consecutive days (6). However, the same study also showed that sodium compresses did not significantly change the skin's clinical appearance as measured by chromametry and clinical

Table 1 Composition of Seawater at 3.5% Salinity

Element	Atomic weight	Parts per million
Sodium (NaCl)	22.9898	10,800
Potassium	39.102	392
Magnesium	24.312	1290
Strontium	87.62	8.1
Selenium	78.96	0.0009
Bromine	79.909	67.3

Note: Parts per million = mg/L = 0.001 g/kg.
Source: Adapted from www.cea-life.com.

scoring. Sodium's role in seawater therapy is further questioned in this study by the finding that there was no significant difference between sodium compresses and cool water compresses, and the idea that neither osmolality nor ions but temperature explains the therapeutic effect of seawater (6). In vitro psoriasis studies also imply that sodium may not account for seawater's clinical value. Psoriasis is characterized by epidermal hyperplasia and heightened mitotic activity. In vitro, sodium salts failed to significantly affect fibroblast proliferation (3).

Potassium

As with sodium, studies yield conflicting results regarding potassium's role in explaining seawater therapy. The 10-mM KCl compresses inhibited skin barrier disruption by inhibiting an increase in TEWL but had no effect on capacitance, and thus, on stratum corneum dryness in irritant contact dermatitis (5). While potassium seems to have some effect on irritant contact dermatitis, its effect on psoriasis is less clear. An in vitro psoriasis experiment showed that potassium salts failed to significantly affect fibroblast proliferation (3), whereas another in vitro study that included two-hour incubations with salt solutions of 0, 50, 100, and 300 mM demonstrated that potassium salts were more effective than those of sodium and magnesium in reversibly inhibiting fibroblast proliferation and that KBr's inhibitory effect was similar to that of Dead Sea water (positive control) (4).

Magnesium

Recent studies suggest that magnesium has a greater influence on inflammatory processes in psoriasis than in irritant contact dermatitis. The 55-mM magnesium salt compresses showed no significant effects on barrier disruption or stratum corneum dryness in irritant contact dermatitis (5). However, magnesium illustrated significant and immediate (within 24 hours of treatment) inhibition of fibroblast proliferation in the in vitro psoriasis studies (3). Additional in vitro and in vivo studies showed that magnesium ions reduced the antigen-presenting capacity of Langerhans cells and are associated with reduced expression of HLA-DR and costimulatory B7 molecules by Langerhans cells (7). This last study hints at a possible role for magnesium in limiting the initial immune response or ongoing inflammation process in psoriasis.

Strontium and Selenium

Long suspected as the reason for the efficacy of Dead Sea water therapy, strontium and selenium have been shown to possess anti-inflammatory properties.

In a double-blind, vehicle-controlled, random-treatment study on irritant contact dermatitis, strontium salts were applied topically as pretreatment or mixed with irritant and were found to decrease the duration and magnitude of inflammation and sensory irritation (stinging, burning, and itching) without local anesthetic effects. Strontium inhibited total cumulative irritation from 56% to 81% according to a patient report, and these findings held true for the broad range of chemically unrelated irritants such as glycolic acid, lactic acid, aluminum chloride, and calcium thioglycolate that were used in the study (8). In another study, strontium exhibited anti-inflammatory effects on the molecular level.

An in vitro, controlled study looked at the effects of strontium and selenium on cutaneous inflammatory cytokines, IL-1α, IL-6, and TNF-α, at concentrations similar to those found in the Dead Sea. A weeklong continuous immersion of both healthy and atopic dermatitis skin in 260-mg/L strontium showed that strontium did not significantly affect cytokine levels in healthy skin. However, the study did show that strontium salts significantly

Table 2 Seawater and Its Effects on Inflammed Skin

Source	No. of patients	Type of dermatitis	Therapy	Duration	Evaluation	Significant results	Conclusion
Yoshizawa et al. (12)	3	Irritant dermatitis	Sea water (Pacific ocean)	20 min, 8× over 2 wk	TEWL, capacitance	↓TEWL, capacitance	Inhibits barrier disruption and stratum corneum dryness
			500 mM NaCl			↓TEWL, ↑capacitance	Inhibits barrier disruption and stratum corneum dryness
			10 mM KCl			↓TEWL	Inhibits barrier disruption only
			55 mM $MgCl_2$			Not significant	
			10 mM $CaCl_2$			Not significant	
Levin and Maibach (6)	9	Irritant dermatitis	H_2O compress	30 min, b.i.d. for 4 day	TEWL, LDF, chromametry, clinical score	↓TEWL, LDF	Both compresses *equally* inhibit barrier disruption and microcirculatory blood flow associated with inflammation but do not affect the clinical score or color
			Physiological saline compress			↓TEWL, LDF	
Celerier et al. (1)	In vitro	Atopic dermatitis	Spa water	1 wk continuous immersion	Inflammatory cytokine levels: IL-1α, IL-6, TNF-α	↓IL-1α, ↓↓↓IL-6, ↓TNF-α	Inhibits all three cytokines, but inhibits IL-6 to a greater degree
			260 μg/L $SrNO_3$			↓IL-1α, ↓↓↓IL-6, ↓↓TNF-α	Sr salts inhibit all three cytokines, but inhibit IL-6 to a greater degree. Sr salts selectively inhibit TNF-α production
			260 μg/L $SrCl_2$			↓IL-1α, ↓↓↓IL-6 ↓↓TNF-α	
			60 μg/L $SeCl_2$			↓IL-1α, ↓↓ IL-6, ↓TNF-α	Se salts inhibit all three cytokines, but inhibit IL-6 to a greater degree. Se salts selectively inhibit IL-1α production
			60 μg/L $SeNaO_3$			↓↓IL-1α, ↓↓↓ IL-6, ↓TNF-α	
			60 μg/L $SeNaO_4$			↓↓IL-1α, ↓↓↓ IL-6, ↓TNF-α	
Hiramatsu et al. (1)	20	Atopic dermatitis	Salt water	4 wk	VAS[a] for itching	5.05 ± 1.53% (pre) to 2.8 ± 2.4% (post)	Decreased itching of acute eruptions

(*Continued*)

Table 2 Seawater and Its Effects on Inflammed Skin (*Continued*)

Source	No. of patients	Type of dermatitis	Therapy	Duration	Evaluation	Significant results	Conclusion
Halevy et al. (2)	30	Psoriasis vulgaris	35°C Dead sea salt bath	20 min q.d. for 3 wk	PASI, patient-subjective evaluation	$34.8 \pm 24\%$ reduction after 3 wk, $43.6 \pm 31.1\%$ reduction after 7 wk	Dead sea salt bath significantly decreased PASI over course of treatment; effect lasted for 1 mo after treatment. However, the Dead Sea salt bath's effect on PASI did not significantly differ from the common salt bath.
Gambichler et al. (2001)	10	Psoriasis	NaCl (24%) immersion followed by phototherapy (280–365 nm)	20 min, 30× over 7.5 wk	Clinical score (desquamation erythema, infiltration of plaques)	68.4% reduction from baseline	Significant decrease in clinical score from, baseline, but effect is, not significantly different from tap water control.
Levi-Schaffer et al. (3)	In vitro	Psoriasis	75 mM NaCl 75 mM KCl 75 mM $MgCl_2$ 75 mM $MgBr_2$ 75 mM KBr	1, 2, or 3 days	Fibroblast proliferation, cAMP levels	$MgCl_2$ inhibits proliferation by $50.7 \pm 2.2\%$; $MgBr_2$ inhibits proliferation by $55.0 \pm 2.3\%$; No treatment significantly affected cAMP levels	Mg salts had a significantly stronger inhibitory effect on fibroblast than other proliferation salt treatments of the same osmolarity. The inhibitory effect was immediate (within 24 hr)
Shani et al. (4)	In vitro	Psoriasis	0, 50, 100, 300 mM NaCl	2-hr incubation	Thymidine incorporation	Maximum reduction 44.4%	Br salts significantly inhibit growth greater than Cl salts. K exerted the greatest growth inhibition with KBr's inhibitory effect similar to that of the diluted Dead Sea brine.
			0, 50, 100, 300 mM NaBr			52.4%	
			0, 50, 100, 300 mM KCl			54.3%	
			0, 50, 100, 300 mM KBr			86.5%	
			0, 50, 100, 300 mM $MgCl_2$			32.5%	
			0, 50, 100, 300 mM $MgBr_2$			47.7%	

[a]Visual analog scale.

inhibited all three cytokines relative to baseline values, but that strontium selectively inhibited TNF-α to a greater degree (9).

A potential role for selenium in reducing inflammatory processes in skin has also been supported by recent studies. In the aforementioned study, healthy and atopic dermatitis skins were also immersed in 60-μg/L selenium solution for one week. In normal, healthy skin, selenium significantly decreased IL-1α cytokine levels but had no effect on IL-6 or TNF-α levels relative to that of the control medium. In atopic dermatitis skin, selenium salts significantly inhibited all three cytokines relative to baseline values but selectively inhibited IL-1α to a greater degree (9). Selenium has also been correlated with the duration and severity of psoriasis and may be related to the protective function of selenoproteins (thioredoxin reductases and glutathione peroxidases) against ultraviolet-induced cell damage and death. Both low plasma selenium and low plasma glutathione peroxidase activity have been seen in psoriasis patients. Patients with a longer history (>3 years) of psoriasis exhibited a significantly lower selenium level compared with patients with a shorter history of psoriasis (<10 months). Selenium also seemed to correlate with the severity of psoriasis in this study. A significant inverse relationship was found between RBC glutathione peroxidase and psoriasis area and severity index scores in individuals with psoriasis of greater than three years (10).

ANIONS

Bromine

Of the few anions in seawater that are studied, bromine affects skin disease processes the most. In an in vitro study of psoriasis with two-hour incubation with Dead Sea brine, NaCl, NaBr, KCl, KBr, MgCl, MgBr at 0, 50, 100, and 300 mM, bromide salts significantly inhibited fibroblast proliferation as compared with chloride salts. When combined with potassium in KBr, bromine's inhibitory effect was similar to that of diluted Dead Sea (positive control) (4). In another in vitro study, magnesium bromide inhibited fibroblast proliferation to a greater extent than magnesium chloride (55.0 + 2.3% vs. 50.7 + 2.2%), lending further evidence that if anions play a part in seawater therapy, bromine, not chlorine, is most likely to be the active anion (3).

With a paucity of studies and small sample sizes in each study, it is difficult to definitively say whether seawater or its individual components offer any clinical benefit in the inflammations of the skin. If seawater is proven to have therapeutic value, further studies will be needed to explore whether it is the synergism between seawater's various components, the osmolality, or individual ions alone that mediate its effect. As inflammatory skin diseases like eczema and psoriasis are accompanied by a defect in permeability barrier function, improving barrier function results in reduced inflammation. Thus, possible mechanisms of action of seawater salts include putative effects on barrier functions. This effect of ions on barrier function has been studied extensively (11,12). Currently, Dead Sea salt is sold in many countries and is used in clinical treatments and private bathtubs, but in much lower concentrations than the Dead Sea itself. Recent interest in the science of alternative medicines may be a stimulus for a more complete biological analysis of these ancient practices.

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53 Allergy and Hypoallergenic Products

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INTRODUCTION

The assessment and detection of the number of contact allergic reactions to cosmetics are not simple. Generally, a consumer who has a problem with cosmetics will consult a doctor only if he or she does not recognize the cause to be a particular cosmetic product, or if the dermatitis persists when the suspected product has been replaced by another, determined by trial and error. Consequently, only a small proportion of the population with cosmetic intolerance problems is ever seen by a dermatologist. Moreover, cosmetic reactions may present in unusual clinical forms, which may evoke an erroneous diagnosis (1–3).

In general, adverse effects are underreported (4), certainly to the cosmetics industry, which obtains its most reliable information in this regard mainly from the relatively few dermatologists who concentrate on cosmetic-intolerance problems and from reports in the literature that are, almost by definition, out of date. Sometimes beauticians and consumers report adverse reactions, but in most cases this kind of information is difficult to objectify unless a dermatologist verifies it.

Application of cosmetic products to the skin may cause irritant, phototoxic, contact, and photocontact allergic reactions as well as contact urticaria. It is generally agreed that most skin-adverse reactions to cosmetic products are irritant in nature and that people with "sensitive skin," as indicated by conditions like atopic dermatitis, rosacea, or seborrheic dermatitis, are particularly liable to develop such reactions. However, contact allergic reactions attract much more attention and thus tend to be overestimated (4). Indeed, the identification of cosmetic allergen is by no means a simple task. It demands special skills and interest on the part of the dermatologist, although labeling of all cosmetic ingredients is facilitating that task. Moreover, there are many factors involved in the sensitization to a specific cosmetic product, all of which have to be taken into account when one seeks an allergen (1,2) (see the following section).

FACTORS CONTRIBUTING TO CONTACT ALLERGIC REACTIONS TO A COSMETIC PRODUCT

Frequency of Use

One may expect the frequently used products to cause more skin reactions than the more exclusive products, simply because more people are exposed to the former. This alone does not imply anything about the quality of these products (the same thing may be said about individual cosmetic ingredients).

Composition

The complexity of a formula can be either positive or negative, as far as its allergenicity is concerned. One of the principles of creating "hypoallergenic" cosmetics and perfumes is the simplicity of formula. The fewer the constituents, the easier it is to identify the offending substance should difficulties arise, and there would be a lesser danger of synergism. The presence of more ingredients leads to an increase in the chance of the skin's sensitization to one of them. However, some investigators recommend placing upper limits on concentrations, rather than advising against the use of any particular ingredient. They may also suggest more complex formulas (5). Preservatives needed for water-based or other easily contaminated products are the common cosmetic allergens. It seems that it is very difficult to combine potent antimicrobial and antifungal properties with low allergenicity. Indeed, it is very difficult to restrict the biological activity of a substance to a single domain.

Concentration of Ingredients

Although the use of low concentrations does not assure complete safety, the incidence of sensitization induction is indeed a function of the concentration of the allergen, at least to some extent. Cases of allergy to the preservative agent methyl (chloro)isothia-zolinone illustrate this problem very well. At first, a 50-ppm concentration of this agent was allowed for use in cosmetic products in the European community, following which this concentration was actually used in some products; there were "epidemics" of contact allergic reactions to it (6). Of late, the frequency of positive reactions has been diminishing considerably, not only because its use is declining and primarily limited to "rinse-off" products (3) but also because its usage concentration has been reduced to about 15 to 7.5 ppm (as the manufacturers recommended). Of course, once a patient has become sensitized, even low concentrations can trigger a reaction.

Purity of Ingredients

It is impossible to refine raw materials to absolute purity. A more or less strict quality control of raw materials and finished products has long been the general practice in modern cosmetic manufacturing. However, one can never rule out the sensitizing potential of impurities in these materials (5).

Common Use of Cosmetic Ingredients in Pharmaceuticals

Patients easily become sensitized to topical pharmaceutical products, which, unlike cosmetics, are most often used on a diseased skin. However, once sensitization has occurred, they may react also to the cosmetics containing the same ingredients (5).

The Role of Cross-Sensitivity

Chemically related substances are likely to induce cross-reactions, and contact eczematous lesions may be maintained in this way. This is especially the case with perfume ingredients, which often cross-react with each other, and applies to all other cosmetic ingredients as well.

Penetration-Enhancing Substances

The chemical environment can substantially affect a person's sensitizing potential of individual chemicals. For example, emulsifiers and solvents enhance skin penetration, and thereby contact sensitization. Penetration-enhancing agents can also be the root of false-negative patch test reactions; the cosmetic product itself may be clearly allergenic (or irritant), although the individual ingredients, abstracted from the environment of the product and tested separately, may not cause a reaction.

Application Site

Some areas of the skin, like the eyelids, are particularly prone to contact dermatitis reactions. A cream applied to the entire face, such as a face care product, along with hair products may cause an allergic reaction only on the eyelids. Moreover, "ectopic dermatitis" [caused by the transfer of an allergen by hand, as often occurs with tosylamide/formaldehyde (i.e., para-toluenesulfonamideformaldehyde) resin, the allergen in nail polish], "airborne" contact dermatitis (e.g., caused by perfumes) (7), as well as "connubial" dermatitis (caused by products shared between partners) (8) often occur only on "sensitive" skin areas such as the eyelids, lips, and neck.

Moreover, the penetration potential of cosmetics is heightened in certain "occluded" areas, such as the body folds (axillary, inguinal) and the anogenital region, because of which the risk of contact sensitization is increased. In the body folds, the allergenic reactions tend to persist for weeks after the initial contact with the allergen. This may be partly attributable to the residual contamination of clothing as well as the increased penetration of the allergen, which is certainly assisted by occlusion and friction (9). Indeed, a reservoir may be formed from which the allergen is subsequently released.

Condition of the Skin

Application on a damaged skin, where the skin barrier is impaired, enhances the penetration of substances and thus increases the risk of an allergic reaction. This is the case with body care products used to alleviate dry, atopic skin and with barrier creams used for protecting the hands, which often suffer from irritancy problems (e.g., dryness, cracking). Sometimes, the

allergic reaction may be limited to certain areas of the skin (areas already affected react more readily to another application of the same allergen) and may even present an unusual clinical picture that does not immediately suggest contact dermatitis. Indeed, contact allergic reactions to preservative agents on the face may present as a lymphocytic infiltrate or even have a lupus erythematous-like picture (3,10).

Contact Time

In the world of cosmetics, a distinction is now being made between leave-on products, which remain on the skin for several hours (e.g., face- and body care products and makeup), and rinse-off products, which are removed almost immediately.

The division between these two kinds of products is not always relevant to the sensitization process, because a thin film can remain on the skin and be sufficient to allow ingredients to penetrate. This occurs, for example, with moist toilet paper (with mainly preservatives as the allergens) and makeup removers.

Frequency of Application and Cumulative Effects

Daily or several-times-a-day usage of cosmetics may cause ingredients to accumulate in the skin and thus increase the risk of adverse reactions. In fact, the concentration of an ingredient may be too low to induce sensitivity in a single product, but may reach critical levels in the skin if several products containing it are used consecutively. This may be the case for people who are loyal to the same brand of products, e.g., day and night creams, foundations, and cleansing products, because a manufacturer will often use the same preservative system for all his products. This should be taken into consideration by companies that use biologically active ingredients such as preservative agents, emulsifiers, antioxidants, and perfumes, because it might well account for many of the adverse reactions to these particular substances. In our experience, intense users of cosmetics are more prone to cosmetic dermatitis than others.

CORRELATIONS WITH THE LOCATION OF THE LESIONS

Like many other contact allergens, cosmetics can reach the skin by several different ways (1,2): by direct application; by airborne exposure to vapors, droplets, or particles that are released into the atmosphere and then settle on the skin (7); by contact with people (partners, friends, coworkers) who transmit allergens to cause "connubial" or "consort" dermatitis (8); by transfer from other sites on the body, often the hands, to more sensitive areas such as the mouth or the eyelids (ectopic dermatitis); and by exposure to the sun with photoallergens.

The most common sources of cosmetic allergens applied directly to the body are listed in Table 1.

THE NATURE OF COSMETIC ALLERGENS

Fragrance Ingredients

Fragrance ingredients are the most frequent culprits in cosmetic allergies (11–15). Katsarar et al., who investigated the results of patch testing over a 12-year period, found an increasing trend in the sensitivity to fragrance compounds, which reflects the effectiveness of the advertising of perfumed products (16). Common features of a fragrance contact dermatitis are localization in the axillae, localization on the face (including the eyelids) and neck, well-circumscribed patches that appear in areas where dabbing-on perfumes are used (wrists, behind the ears), and hand eczema or its aggravation. Airborne or connubial contact dermatitis should be considered as well. Other less-frequent adverse reactions to fragrances are photocontact dermatitis, contact urticaria, irritation, and pigmentation disorders (17).

Sensitization is most often induced by highly perfumed products, such as toilet waters, aftershave lotions, and deodorants, the last of which have been shown to contain well-known allergens such as cinnamal (cinnamic aldehyde) and isoeugenol (18).

As reported in the literature, the fragrance mix remains the best screening agent for contact allergy caused by perfumes, because it would detect some 70% to 80 % of all perfume allergies (19,20). However, it depicts also the need to test with additional perfume allergens.

Table 1 Cosmetic and Cosmetic-Related Dermatitis Caused by Direct Application of the Allergen

Area of dermatitis	Cosmetics that may contain allergens
Face in general	Facial skin care products (creams, lotions, masks), sunscreen products, makeup (foundations, blushes, powders), cleansers (lotions, emulsions), and cosmetic appliances (sponges), perfumed products (aftershave lotion)
Forehead	Hair care products (dyes, shampoos)
Eyebrows	Eyebrow pencil, depilatory tweezers
Upper eyelids	Eye makeup (eye shadow, eye pencils, mascara), eyelash curlers
Lower eyelids	Eye makeup
Nostrils	Perfumed handkerchiefs
Lips, mouth, and perioral area	Lipstick, lip pencils, dental products (toothpaste, mouthwash), depilatories
Neck and retroauricular area	Perfumes, toilet waters, hair care products
Head	Hair care products (hair dyes, permanent-wave solutions, bleaches, shampoo ingredients), cosmetic appliances (metal combs, hairpins)
Ears	Hair care products, perfume
Trunk/upper chest, arms, wrists	Body care products, sunscreens, and self-tanning products, (elbow flexures) cleansers, depilatories
Axillae	Deodorants, antiperspirants, depilatories
Anogenital areas	Deodorants, moist toilet paper, perfumed pads, depilatories
Hands	Hand care products, barrier creams, all cosmetic products that come in contact with the hands
Feet	Foot care products, antiperspirants

Indeed, testing with additional markers, for example, the individual components such as hydroxyisohexyl 3-cyclohexene carboxaldehyde (Lyral®), farnesol, and citral, as well as with the complex natural mixtures (21–25), increases the sensitivity of the testing. Because of the increasing importance of fragrance allergy and to ensure that sensitized consumers are adequately informed, 26 fragrance components are labeled as cosmetic ingredients on the package [Annex 3 (Table 2 of the cosmetic irective (26)]. With fragrance, allergy-associated positive patch test reactions frequently occur and often indicate the presence of common or cross-reacting ingredients in natural products, the occurrence of cross-reactions between simple fragrance chemicals, or concomitant sensitivity. Moreover, oxidation products of fragrance ingredients, such as limonene (27) or resin acids (being the main allergens in colophony), found as contaminants in tree moss (a widely used substitute for oak moss) as well as in oak moss itself (28), play an important role in the allergenic potential of these substances (29).

Preservatives

Among the allergy-causing agents, preservatives are second in frequency to fragrance ingredients; they are important allergens in cleansers, skin care products, and makeup (2,30). However, within this class, important shifts have occurred over the years (30,31).

The methyl(chloro)isothiazolinone mixture was commonly used in the 1980s and was then a frequent cause of contact allergies. This frequency has declined considerably in recent years (3,12). Since then, formaldehyde and its releasers, particularly methyldibromo glutaronitrile—as used in a mixture with phenoxyethanol, better known as Euxyl K400—did gain importance in this regard (12,30–34), although the frequency of positive reactions observed seems to be influenced by the patch test concentration (33,34).

The spectrum of the allergenic preservatives also varies from country to country. For example, in contrast to continental Europe where reactions to the methyl-(chloro)-isothiazolinone mixture and, more recently, methyldibromo glutaronitrile have been the most frequent (12,13,30,31,35), in the United Kingdom, formaldehyde and its releasers have always been much more important, particularly as concerns quaternium-15 (30), although its incidence seems to have slightly decreased of late (36). Parabens are rare causes of cosmetic dermatitis. When a paraben allergy does occur, the sensitization source is most often a topical pharmaceutical product, although its presence in other products can be sensitizing as well (37); this is often the case for other ingredients also. For instance, a young lady, after having previously been sensitized to mefenesin in a rubefacient, presented with an acute contact

Table 2 Twenty-six Substances to be Labeled Regardless of Function and Origin

References in Annex III of the cosmetics directive	Name in the cosmetics directive	INCI name
(67)	Amyl cinnamal	Amyl cinnamal
(68)	Benzyl alcohol	Benzyl alcohol
(69)	Cinnamyl alcohol	Cinnamyl alcohol
(70)	Citral	Citral
(71)	Eugenol	Eugenol
(72)	Hydroxy-citronellal	Hydroxycitronellal
(73)	Isoeugenol	Isoeugenol
(74)	Amyl cinnamyl alcohol	Amylcinnamyl alcohol
(75)	Benzyl salicylate	Benzyl salicylate
(76)	Cinnamal	Cinnamal
(77)	Coumarin	Coumarin
(78)	Geraniol	Geraniol
(79)	Hydroxymethylpentyl-cyclohexenecarboxaldehyde	Hydroxyisohexyl 3-cyclohexene Carboxaldehyde
(80)	Anisyl alcohol	Anise alcohol
(81)	Benzyl cinnamate	Benzyl cinnamate
(82)	Farnesol	Farnesol
(83)	2-(4-tert-butylbenzyl) propionaldehyde	Butylphenyl methylpropional
(84)	Linalool	Linalool
(85)	Benzyl benzoate	Benzyl benzoate
(85)	Hexyl cinnamaldehyde	Hexyl cinnamal
(86)	Citronellol	Citronellol
(88)	d-Limonene	Limonene
(89)	Methyl heptin carbonate	Methyl 2-octynoate
(90)	3-Metyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one	α-isomethyl ionone
(91)	Oak moss extract	EU: *Evernia prunastri* U.S.A.: *Evernia prunastri* (oak moss) extract
(92)	Tree moss extract	EU: *Evernia furfuracea* U.S.A.: *Evernia furfuracea* (tree moss) extract

dermatitis on the face at the first application of a new cosmetic cream containing chlorphenesin, which was used as a preservative agent (data on file). Apparently, it is a potential sensitizing agent (38) and cross-reacts with mefenesin, which is used in pharmaceuticals.

Antioxidants

Antioxidants form only a minor group of cosmetic allergens. Examples are propyl gallate, which may cross-react with other gallates and are also used as food additives, and t-butyl hydroquinone, a well-known allergen in the United Kingdom, but not in Europe (30). Sodium metabisulfite, present in oxidative hair dyes (data on file), may cause allergic contact dermatitis both to the clients and to the hairdressers.

"Active" or Category-Specific Ingredients

With regard to "active" or category-specific ingredients, in contrast to de Groot (3), we found an increase in the number of reactions to oxidative hair dyes (paraphenylenediamine or PPD and related compounds) during the period 1991 to 1996, as compared with the period 1985–1990 (12,13). According to one cosmetic manufacturer (personal communication, L'Oréal, 1997), the use of such hair dyes has more than doubled in recent years. However, the replacement since 1987 of PPD-hydrochloride by a PPD base—a more appropriate screening agent for PPD allergy—may also have influenced the incidence (39). They are important causes of professional dermatitis in hairdressers who also often react to allergens in bleaches (persulfates, also causes of contact urticaria) and permanent-wave solutions (primarily glyceryl monothioglycolate, which may provoke cross-sensitivity to ammonium thioglycolate) (40,41). Tosylamide/formaldehyde (= toluenesulfonamide/formaldehyde) resin is considered an important allergen (4) and is the cause of ectopic dermatitis attributable to nail lacquer,

which may also contain epoxy and (meth) acrylate compounds (3). It often gives rise to confusing clinical pictures and may mimic professional dermatitis (42). Acrylates (methacrylates) are also causes of reactions to artificial nail preparations, more recently to gel formulations, with both manicurists and their clients (43).

Moreover, "natural" ingredients may induce contact allergic reactions as well. Some examples are butcher broom (*Ruscus aculateus*), which is also a potential allergen in topical pharmaceutical products (44), hydrocotyl (asiaticoside) (45), and panthenol (46). Farnesol, a well-known perfume ingredient and cross-reacting agent to balsam of Peru, has become a potential allergen in deodorants, in which it is used for its bacteriostatic properties (47).

Some sunscreen agents such as benzophenone-3, which may also cause contact urticaria, and dibenzoylmethane derivatives have been recognized in the past as being important allergens (3,30,48–50). Indeed, isopropyl dibenzoylmethane was even withdrawn for this reason (3). 4-Methylbenzylidene camphor, cinnamates, and phenylbenzimidazole sulfonic acid are only occasional, sometimes even rare, causes of cosmetic reactions. The use of para-aminobenzoic acid and its derivatives has decreased considerably. Contact allergic reactions to them were generally related to their chemical relationship to para-amino compounds (51), although they were also important photosensitizers (48).

In our experience (12,13,30), the contribution of sunscreens to cosmetic allergy is relatively small, despite the increase in their use as a result of the media attention given to the carcinogenic and accelerated skin-aging effects of sunlight. The low rate of allergic reactions observed may well be because a contact allergy or a photo-allergy to sunscreen products is often not recognized, since a differential diagnosis with a primary sun intolerance is not always obvious. Furthermore, the patch test concentrations generally used might be too low (52), in part, because of the risk of irritancy.

Excipients and Emulsifiers

Many excipients and emulsifiers are common ingredients to topical pharmaceutical and cosmetic products, the former being likely to induce sensitization. Typical examples are wool alcohols, fatty alcohols (e.g., cetyl alcohol), and propylene glycol (13). Emulsifiers in particular have long been regarded as irritants, but their sensitization capacities should not be overlooked. It is imperative, of course, that patch testing be properly performed to avoid irritancy, and that the relevance of the positive reactions be determined. This is certainly the case for cocamidopropyl betaine, an amphoteric tenside mainly present in hair- and skin-cleansing products. Whether the compound itself or cocamidopropyl dimethylamine, an amidoamine, or dimethylaminopropylamine (both intermediates from the synthesis) are the actual sensitizers, is still a matter of discussion (53,54). It is also not clear whether cocamidopropyl-PG-dimonium chloride phosphate (phospholipid FTC (55), an allergen in skin care products, can cross-react with cocamidopropyl betaine. Other emulsifiers and vehicle components that were more recently found to be contact allergens in cosmetics are maleated soybean oil (56), butylene glycol and pentylene glycol (aliphatic alcohols with similar uses to propylene glycol that is considered to have more irritant and allergenic effects) (57,58), ethylhexylglycerin (syn.: octoxyglycerin) (59), methoxy PEG-17 and PEG-22/dodecyl glycol copolymers (alkoxylated alcohols and synthetic polymers) (60), and alkylglucosides (condensation products of fatty alcohols with glucose) (61,62).

Coloring Agents

Coloring agents, other than hair dyes, have rarely been reported as cosmetic allergens. However, with the increased use of cosmetic tattoos (e.g., eye and lip makeup), more treatment-resistant skin lesions might develop in the future (63).

DIAGNOSING COSMETIC ALLERGY

Taking the history of the patient and noting the clinical symptoms and localization of the lesions are critical. Allergen identification for a patient with a possible contact allergy to cosmetics is performed by means of patch testing with the standard series, specific cosmetic test series, the product itself, and with all its ingredients. We can only find the allergens we look for. There are several guidelines for skin tests with cosmetic products that the patients

supply themselves (64). Not only the patch and photo-patch tests, but also semi-open tests, usage tests, or repeated open application tests may be needed to be performed to obtain a correct diagnosis.

HYPOALLERGENIC PRODUCTS

Most of the cosmetic industry is making a great effort to commercialize safest possible products. Some manufacturers market cosmetics containing raw materials that have a "low" sensitization index or a high degree of purity, or from which certain components have been eliminated (5,65) (generally perfume ingredients). Sometimes active preservative agents are also omitted, and in sunscreens immunologically inert physical agents are being used more often than chemical ultraviolet absorbers.

Statements such as "recommended by dermatologists," "allergy-tested," or "hypoallergenic" have been put on the packaging material by manufacturers, to distinguish their products from those of their competitors. Although there are several ways to reduce allergenicity (3), there are no governmentally mandated standards or industry requirements (66).

The latest trend is target marketing to people with a "hypersensitive" or "intolerant" skin, a term often used for the shadowy zone between normal and pathological skin. These would be the people with increased neurosensitivity (e.g., atopics), heightened immune responsiveness (e.g., atopic and contact allergic individuals), or a defective skin barrier, i.e., people with irritable skin such as atopics, those suffering from seborrheic dermatitis (67), or rosacea. This means that part of the cosmetic industry is moving more into the area of pathological skin and that certain products are in fact becoming drugs, often called cosmeceuticals. This has caused a great deal of regulatory concern (68,69), both in the United States and the European Union because it suggests some middle category between cosmetics and drugs that does not yet legally exist. In Japan, however, these products fall in the category of "quasi-drugs."

The meaning of most such claims used nowadays is unclear, both for the dermatologist (65–67) and the consumer, the latter being convinced that the hypersensitive skin is the allergic skin. It is the dermatologist's task to diagnose the skin condition and to provide specific advice about the products that can safely be used. All such problems must be approached individually, at least the contact allergic types, because people sensitive to specific ingredients must avoid products containing them.

Therefore, ingredient labeling can be of tremendous help. Providing the allergic patient with a limited list of cosmetics that can be used is practical and effective (70).

CONCLUSION

The identification of cosmetic allergens is challenging because of the extreme complexity of the problem. This applies not only for the dermatologist who is trying to identify the culprit and advise his patient but also certainly for cosmetic manufacturers who are extremely concerned about assuring the innocuousness of their products. Precise, current, and rapid information about the adverse reactions to cosmetic products is critical in a product design. Apparently, premarketing studies are unable to identify all the pitfalls. Therefore, fruitful communication that is developing between dermatologists and cosmetic manufacturers must be encouraged. Sensitivity to cosmetics can never be totally avoided, but its incidence can be substantially reduced.

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54 Operational Definition of a Causative Contact Allergen—A Study with Six Fragrance Allergens

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INTRODUCTION

Contact allergic dermatitis remains a significant public health problem. Its diagnosis and prevention is complicated by the difficulty of identifying allergens responsible for a patient's condition (i.e., those that have actually caused the allergic contact dermatitis). This paper attempts to provide criteria that can be used as an operational definition of causative allergens. Six fragrance allergens exemplify the application of these criteria. Each is discussed in a separate paper (1–6).

Although predictive tests can identify potential allergens, it is only through clinical diagnosis studies on patients with current contact dermatitis that truly causative allergens can be identified. This is, however, not a simple matter and is complicated by practical difficulties inherent to the technique of patch testing, to the physiological nature of type IV allergy, and by other practical matters such as the available time and willingness of patients to submit to prolonged studies.

Clinical patch testing remains a partially subjective field (7). When a clear reaction is observed, it is not always certain that it has been due to a truly clinically −relevant allergic response. Marks et al. suggested that more than 40% of 3000 patients with suspected allergic contact dermatitis were, in fact, suffering from irritant or some other form of nonallergic contact dermatitis (8). A recent study has shown that there was an association between erythematous reactions to some allergens and irritant reactions to sodium lauryl sulfate (SLS), which is a putative marker for hyperreactive skin, thus allowing many reactions of this type to be classified as irritant rather than allergic in nature (9).

Apart from false-positive reactions from irritancy, there is always a possibility that other false-positive reactions can occur from cross-reactions where patients react to substances, which are not the primary sensitizers and which initially induced the allergic state (10). Similarities in chemical structure or cutaneous metabolism would appear to be major factors in cross-reactivity (11). Over 70 different cross-reacting pairs or families of fragrance ingredients have been catalogued (12,13). Positive correlations of concomitant reactions in different pairs of components of the fragrance mix have also been recorded in poly-reactive patients (14).

False-positive reactions can also arise from phenomena such as the "excited skin syndrome" that occurs after a number of patches result in positive test results, which cannot be reproduced when the patient is retested (15,16). Over 40% of such positive patch reactions are lost on repatching (16–18). Indeed, some studies have involved phased patch testing schedules to avoid false positives due to this syndrome.

Even when there is clear evidence that the reaction is allergic in nature, ascertaining the clinical relevance of the patch test requires knowledge of technical aspects relating to specificity and sensitivity issues (19). In one of the more familiar clinical correlations—nickel allergic contact dermatitis—there is a high ratio of false-positive and false-negative reactions (20). Even when an allergic reaction has been indicated, the chances that an experienced physician will accurately identify the causative allergen from clinical information is about 50% mostly when common allergens are involved, but this is reduced to 10% for less common allergens (21).

There is increasing evidence that diagnostic patch testing may also elicit true allergies, but these allergies are not the cause of the patient's current contact dermatitis. Lachapelle has defined clinical relevance as "the capability of an information retrieval system to select and retrieve data appropriate to a patient's need" (22). In this context, Lachapelle has distinguished

between past (not directly related to the patient's current problems) and current relevance and has devised a system for distinguishing between the two. He has also defined the need to determine the "intrinsic imputability" of a suspected allergen as the "possible (and not necessarily exclusive) cause-effect relationship between each positive test to an allergen and the occurrence of a given chemical event." The approach proposed here uses some aspects of this system. A more recent publication (23) gives strategies for determining true clinical relevance. These require establishing the existence of past exposure and ensuring that the patient's exposure has indeed been responsible for the observed dermatitis. A number of suggestions are also made for improving evidence-based diagnosis of relevance. These include running use tests with implicated products, accounting for possible cross-reactions and a more rigorous and detailed examination of the case in which the clinician must retrieve the pertinent historical data, trace the responsible environmental exposure, and perform the appropriate tests.

While some allergies revealed by patch testing may pertain to past allergic clinical events, others may pertain to allergies acquired by the patient but which have never been clinically manifested (i.e., have never caused contact dermatitis in the patient) (24). There is increasing experimental evidence, mainly in animal tests (25–30) but also in some older studies on humans (31) to show that the threshold dose of elicitation varies in accordance with the conditions of induction. When induction conditions are severe, the elicitation threshold dose is low. When induction occurs under mild conditions, much higher exposures are required to elicit an allergic reaction. This means that it may be possible for patients to have acquired allergies under low exposure conditions (e.g., use of cosmetics or other consumer products resulting in relatively mild exposures to their allergenic components), which will never be elicited during their everyday lives as long as exposure remains low. However, these allergies may be artificially elicited under higher exposure conditions experienced in patch testing. These true positive reactions may not be clinically relevant and indeed may not represent a cause for concern for the patient as they reflect an allergic state, which may never manifest itself clinically.

Diagnostic patch tests are by necessity, purposefully designed to avoid false negatives (i.e., to avoid missing possible causative agents), and to do this, the patch test conditions are intentionally more severe than normal exposure conditions. We have clarified that the patch test dose (single application) is usually higher than use test dose (32,33). This can be seen by comparing relative doses. Taking fragrance allergens as examples, a fragrance ingredient used at 1% in a perfume spray (the product type that produces the highest on-skin level of fragrance), a maximum dermal loading of 26 $\mu g/cm^2$ is obtained (34).[a] Yet the use of diagnostic patch tests with 1% of the same ingredient in 19 mm Hill Top Chambers® will deliver a skin loading of 1770 $\mu g/cm^2$ (35), a 68-fold increase. The use of 2 × 2 cm Webril® patches, 8 mm Finn Chambers®, and a Professional Products® 1.9 × 1.9 cm patch would result in 38-fold, 11-fold, and 21-fold increases, respectively. To this, we should add the (dose enhancing) potentiating effects of occlusion [numerous publications (19,36–39) and the potentiation due to an exceptionally long duration of the 48-hour exposure (39)]. The intentions behind this type of exaggeration are laudable, being aimed at identifying substances to which the patient may be allergic. However, they will not necessarily identify the substance that is primarily responsible for the allergy from which the patient is suffering at that specific time. We return to this particular point in the section "Were These Sufficiently Maximized".

Defining the appropriate concentrations for patch tests balances

(i) nonirritation;
(ii) avoiding active sensitization, and
(iii) an appropriate enhancement of concentration that will identify an allergic individual.

The situation with fragrance allergens remains complex because of the relatively limited data on which to define appropriate patch test concentrations, and until recently, the relative difficulty of obtaining documentation that a given consumer product contains the individual fragrance ingredient and its concentration. However, the International Fragrance Association

[a]This would correspond to a fragrance ingredient present at 20% in a fragrance used at 5% in this type of product.

(40) has recently carried out a number of industry-wide surveys, which have been aimed at determining the highest concentrations currently used in fine fragrance products (i.e., the type of consumer products, which delivers the highest levels of fragrance in terms of concentration and quantity per unit area). On the basis of these levels, we propose in the table (Annex) concentrations that could be used as guides to determine the concentrations in some common patch test systems. These would correspond approximately to the maximum exposure that could be expected from using a consumer product when the dermatologist is confronted with a case of allergic contact dermatitis but has no culprit product to examine.

Depending on whether a response is irritant or allergic has long been a complex challenge. All too frequently, inadequate (nonallergic) controls are available. Excited skin syndrome (15) provides further complexity; most clinicians do little single patch tests to verify this possibility. Brasch (41) and Geier (9) have suggested using their reaction index/positives ratio as a retrospective aid in defining those allergens whose positive responses might, in fact, be irritant instead.

In the ideal, but rarely encountered, clinical situation, the causative role of an allergen will be suggested by its presence in a consumer product that has already been identified as the cause of the patient's allergy. In such cases, the patch test should be conducted at a concentration that is related to the concentration of the suspected allergen in the product.

We suggest below the criteria by which the causative role of an allergen can be attributed to a specific case of clinical contact dermatitis. By far the more important is diagnostic patch testing as this alone can link the substance to the case. The criteria are aimed at determining the degree of confidence we can ascribe through diagnostic patch testing, a specific allergen's causal role in a specific case of allergic contact dermatitis. It is also important, however, to have some measure of the substance's intrinsic allergenic potency. For this reason, we are also presenting a scheme for ascribing a degree of confidence to the results of predictive tests.

These schemes build on a previous approach of Benezra et al. (10), and in subsequent papers; these criteria are put into practice by taking six fragrance allergens recently identified as major fragrance allergens (42). These have been specifically chosen to span the range of likely causality shown by the 24 allergenic substances identified in this new legislation. Two of the substances are considered as frequently encountered allergens (geraniol and amylcinnamic aldehyde), two are among the less frequently encountered (α-iso-methylionone and anisyl alcohol), and two are intermediate in this regard (citronellol and linalool).

DATA OBTAINED FROM DIAGNOSTIC PATCH TESTING OF PATIENTS IN DERMATOLOGICAL CLINICS

The following scoring system is proposed.

Is It A Primary Case Report or A General Review?

Many publications in this area are reviews or statistical studies of patch test results already published elsewhere. While these papers perform an important function, they present a possible source of duplicate reporting whereby the same patch test result is referred twice in the literature. It is therefore important to distinguish between these two types of studies. A higher degree of confidence is attributed to detailed primary studies.

Is Information Provided on the Number and Condition of the Patients?

The number of patients being examined is of importance mainly for epidemiological studies. However when studies do not provide information on the number and nature of the test materials to which individual patients reacted, then it is impossible to estimate the degree of poly-reactivity and possible cross-reactions. It is also important to know if the patients suffer from current eczema or other diseases. A lower degree of confidence is attributed when this information is not provided.

Are the Conditions of Patch Testing Given?

This is of primary importance particularly with regard to the purity of the test material (43). The test material should be clearly identified and degree of purity specified. The presence of potentially more allergenic impurities (e.g., aldehydes in alcohols and autoxidation products)

should also be controlled and indicated. A lower degree of confidence is attributed to results from studies where such verifications are not reported.

Ideally, the report should give a detailed description of the patch test conditions. The type and size of the patch, duration of occlusion, nature of the vehicle, and the concentration of the test material are a minimum of information that is required. The bioavialability of the test material and the dose in quantity per unit area will vary from one type patch test kit to another (35,44). The timing of readings is also of importance.

Unless it is clear from historical evidence, it is necessary to rule out the possibility that irritant reactions are observed. For this reason, it has been recommended to undertake a preliminary test of at least three concentrations on control subjects (45).

A higher degree of confidence is also attributed when it is clear that the patch test conditions have exposed the patient to levels of the allergen that are not disproportionately high with regard to the levels of exposure, which were suspected to have led to the patient's condition. Although there is no clear test to determine whether a positive patch test reaction has revealed the cause of the allergy or some latent subclinical allergy, information on the conditions of patch testing provides valuable information in making a judgment with any degree of confidence in this regard.

Are the Results Reported in Sufficient Detail?

The intensity of positive reactions should be recorded. Numerous authors have expressed concern that a significant proportion of patch test reactions may be irritant in nature. This is particularly the case with weakly positive (1+) scores (46), and it has been proposed that these scores should be handled separately (47). Studies comparing True Test TM and Finn Chambers showed that the fragrance mix gave about 47% irritant and questionable reactions with the former and about 45% with the latter, with a high degree of discordance between the two systems. In other large studies (48,49), about 60% to 70% of the reactions recorded were 1+, and it was speculated that up to 40% were irritant in nature (48). Further, the fact that the skin is not viewed for 24 hours following application of the patch makes it almost impossible to distinguish between quick-developing irritation and delayed contact hypersensitivity.

Scores should be given in a way that allows comparison of reactions experienced by a given patient to different test materials. A higher degree of confidence is attributed to results from studies where this information is provided.

Is It Possible to Ascertain if Patients had Reacted to Other Materials?

Concomitant reactions cloud the issue of causality. Although it is possible that a particular case of allergic contact dermatitis has been caused by several allergens, other explanations for multiple reactions to different suspect allergens will need to be ruled out. For this reason, it is important to know the other substances, which produced positive patch test reactions in each patient.

Can We Rule Out Cross-Reactions?

A lower degree of confidence is attributed to results from studies where uncertainty arises in this regard. When positive reactions occur to different substances in the same patient, and these substances have similar chemical structures, the possibility arises that some of these patch test reactions are in fact false indicators of the true cause of the case of contact allergic dermatitis.

Can We Rule Out "Excited Skin"?

There is also the possibility that false-positive reactions appear because of the excited skin syndrome (15–19). The likelihood of this occurrence can be reduced by carrying out patch tests in a time-phased manner so that the number of patches is minimized. A higher degree of confidence is allocated to studies that have taken such precautions or other measures to ensure that some of the observed reactions are not due to artifacts of this type.

Has the Substance Been Tested in Usage Tests?

Although some uncertainties in these techniques need to be resolved (50), the use of repeat open applied tests (ROATs) and provocative use tests (PUTs) can add important extra evidence of the causative role as they confirm under milder conditions than patch testing the

allergenic role of the substance. If these can be performed on suspected sources of exposure to the patient (e.g., consumer products containing the substance), an even higher degree of confidence is attained (see following section). This is a key to the scheme proposed by Lachapelle (22) for assigning intrinsic imputability.

Has the Substance and the Allergy Been Linked to a Specific Consumer Product or Exposure Situation?

This is perhaps the gold standard for establishing the causal culpability of a suspect. Ideally, this would go further than simply gathering information of the possible causative products [the third criteria of Lachapelle (22)] and include carrying out patch tests on fractions of the(se) causative product(s) until the culpable allergens have been identified by producing reactions to the exclusion of all other components. A number of studies producing convincing results of this type have been published [e.g., Handley and Burrows (51)]. However in such cases, the highest degree of confidence can only be ascribed when it is clear that the patch test conditions have exposed the patient to levels of the allergen, which are not disproportionately high with regard to the levels of exposure, which were suspected to have led to the patient's condition.

The following scoring system has been used.

5: meets all criteria
4: meets all criteria but number of cases is marginal
3: meets criteria but some parameters questionable
2: evidence does not unambiguously indicate causative role of the test substance
1: fails several criteria, results are not considered to be reliable
0: fails all criteria/not primary report in literature of cases cited

DATA OBTAINED FROM PREDICTIVE TESTS IN ANIMALS AND HUMAN VOLUNTEERS

The first part of this analysis examines the likelihood that the designated substance shows an inherent potential to sensitize. This type of information is best obtained from predictive tests, although it should be cautioned that these data only provide information on a substance's intrinsic hazard. The conditions of exposure to the general public may be sufficiently high in some instances to produce reactions in substances, which fail to show any significant sensitization hazard (52). For this reason, the apparent absence of a sensitization potential from predictive tests will not rule out the likelihood that a substance will not cause reactions in sufficiently exposed populations. Given this proviso, we suggest that the degree of confidence should be attributed to predictive tests according to the following criteria.

Was the Test Material Clearly Identified?

The test material should be clearly identified, and the degree of purity should be specified. The presence of potentially more allergenic impurities (e.g., aldehydic impurities in alcohols and autoxidation products) should also be controlled. A lower degree of confidence is attributed to results from studies where such verifications are not reported.

The Type of Test/Type of Test Subjects

The type of test and nature of the test subjects should be clearly specified. If these are human subjects, their dermatological status should have been verified by a dermatologist prior to and, if necessary, during the course of the study. It is also accepted that some types of test are more sensitive than others. Adjuvant tests in guinea pigs have long been regarded as more sensitive than non-adjuvant methods. Some tests have been less well validated than others. A lower degree of confidence is attributed to results from studies that are not as sensitive as others.

Were Details of Test Conditions Provided?

Information on the exact protocols should be provided to ensure that the most sensitive methodology has been applied. The test concentration (and the skin loading as expressed in quantity per unit area) should be neat to the limit of irritation. The choice of the vehicle(s) and type of patches can also have an influence on the sensitivity of the test. A lower degree of confidence is attributed to results from studies where this information is not reported or

deemed less than optimal. Officially approved test protocols should be used for those methods that have been codified in this way [e.g., by Organisation for Economic Co-operation and Development (OECD), 1981 as amended (53)]. There is also accumulating evidence to show that occlusion and ethanol may in fact produce false positives in human studies. This should also be taken into account.

Were These Sufficiently Maximized?

A lower degree of confidence is attributed to results from studies that are deemed to be less than optimally maximized to avoid false-negative results.

Were There Adequate Controls?

These are necessary to ensure that irritancy is not occurring during induction. Tests involving a challenge phase should include challenges to naive subjects to control irritancy. Where ethically possible, the laboratory performing these studies should carry out regular positive control studies using standard borderline allergens. A lower degree of confidence is attributed to results from studies where these controls are not reported to have been used or where they have also produced reactions.

Was the Number of Test Subjects Sufficient?

There are international standards requiring the minimum number of animals to be used in some tests [OECD, 1981 as amended (53)]. Tests on human subjects should generally involve more than these because of the inherent and environmental variability of the test subjects (54), and ideally at least 200 should be used. A lower degree of confidence is attributed to results from studies in which an insufficient number of test subjects were used.

Were the Results Presented in Sufficient Detail?

The intensity of positive reactions should be recorded. Scores should also be followed for individual test subjects to ensure that, for instance, those reacting at one challenge are the same as those who react at subsequent challenges. A lower degree of confidence is attributed to results from studies where this information is not provided.

SCORING

The following scoring system is used:

5: meets all criteria
4: meets all criteria but number of positives is marginal
3: meets criteria but some parameters questionable (e.g., insufficient data provided or test not fully maximized)
2: controls apparently absent or small number of test subjects
1: fails several criteria, results are not considered to be reliable
0: fails all criteria

CONCLUSIONS OF THE STUDIES ON SIX FRAGRANCE ALLERGENS

The accompanying papers on amylcinnamic aldehyde, anisyl alcohol, citronellol, geraniol, linalool, and α-iso-methylionone (1–6) show that when the underlying clinical and experimental data are analyzed according to the criteria outlined above, a clear cause-effect relationship has infrequently or rarely been established and would not necessarily be expected on the basis of the generally weak sensitizing potential of these substances coupled with reasonably low exposure conditions. This is not to say that some of these substances are frequent inducers of type IV allergy in members of the public. It remains to be seen however, how often such allergy, once established, is responsible for any of the cases of allergic contact dermatitis commonly ascribed to these substances.

Schnuch and others have commented extensively on the wisdom and criteria for definition of what is a chemical allergen in man. References (55–57) provide a state of the science.

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ANNEX: PATCH TEST CONCENTRATIONS THAT CORRESPOND TO THOSE EXPERIENCED IN MAXIMUM CONSUMER EXPOSURE

	Maximum exposure from cosmetics		Equivalent concentrations in standard patch test kits[a]			
Test material	Concentration[b]	Quantity/ unit area[c]	Finn Chambers® (8 mm)	Hill Top Chambers® (19 mm)	Professional Products® (1.9 × 1.9 cm)	Webril® (2 × 2 cm)
Amylcinnamique aldehyde	0.89%	23 μg/cm^2	0.08%	0.013%	0.04%	0.023%
Anisyl alcohol	0.57%	15 μg/cm^2	0.05%	0.008%	0.03%	0.015%
Citronellol	0.70%	18 μg/cm^2	0.06%	0.01%	0.03%	0.018%
Geraniol	0.62%	16 μg/cm^2	0.05%	0.009%	0.03%	0.06%
Linalool	0.86%	22 μg/cm^2	0.075%	0.013%	0.04%	0.022%
α-iso-methylionone	0.74%	19 μg/cm^2	0.064%	0.011%	0.035%	0.019%

[a]Does not take account of additional effects of occlusion and 48-hour duration of patch tests. Based on data from Robinson et al. (34)
[b]Data used by RIFM from IFRA surveys. Assumes 20% of fragrance in the cosmetic (fine fragrance product).
[c]From Gerberick et al. (33). Spray-on fragrance product delivers a maximum of 2.6 mg product/cm^2.
. Finn Chambers® (8 mm): 30 mg/cm^2, Hill Top Chambers® (19 mm) 177 mg/cm^2, Professional Products® Patch (1.9 × 1.9 cm): 55.4 mg/cm^2, Webril Patch® (2 × 2 cm): 100 mg/cm^2.
Abbreviation: IFRA, international fragrance association.

55 Anti-Itch Testing: Antipruritics

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INTRODUCTION

Itching, or pruritus, is an unpleasant sensation that provokes a desire to scratch. Chemical, mechanical, thermal, and electrical stimuli can elicit itch (1–5). Mediators of itch, presumably, directly act on nerve fibers or lead to a nerve stimulation cascade whose final common pathway is interpreted in the central nervous system as itching (2–6). Putative receptors for itching are C-fibers with exceptionally low conduction velocities and insensitivity to mechanical stimuli (4–6). Histamine, the prototypical chemical mediator of itch, which is released during mast cell degranulation and mediates its effects in the skin via H_1 receptor (3,5), is the best-known experimental pruritogen (2,3,5,7). Other pruritogens such as compound 48/80 (8,9), substance P (10,11), and serotonin (12) have been studied.

Antipruritics may alleviate or diminish itching sensation. Topical antipruritics such as antihistamines, anesthetics, capsaicin, corticosteroids, and cooling agents are extensively used (8–10). To define antipruritic effects, testing methodologies have been developed (11–13). However, the clinical effects of anti-itch vary, and sometimes it is difficult to compare efficacy between antipruritics. One reason may be inadequate biometrics, as itch is a subjective symptom and to measure its severity is a challenge; its magnitude (intensity) can be only estimated from reports of patients or volunteers. Methodologies have been adopted to evaluate antipruritics that may aid future development of anti-itch products.

This chapter focuses on the evaluation of topical antipruritics; and, further reviews recent investigations involving thermal stimuli-modifying itch (14–16), electronic devices for measurement (8,9,17) as well as alleviation of itch (18), newly found use of known drugs (11,19), questionnaires for assessment of pruritus in atopic and uremic patients (20,21), and possible models for developing new antipruritics (10,11,17).

METHODOLOGIES

Histamine-Induced Itch Human Model

Rhoades et al. (22) examined the inhibition of histamine-induced pruritus by three antihistaminic drugs using a double-blind crossover study on 28 human subjects. These included: diphenhydramine HCl, cyproheptadine, hydroxyzine HCl, and a lactose placebo in identical capsules. All subjects were given intradermal injections of increasing doses of aqueous histamine phosphate in the volar aspect of the forearm to establish their individual threshold levels at which itching occurred. Following the establishment of a baseline, the subjects received two doses of one of the three antihistamines or placebo on four test periods with a one-week interval between test days. Results revealed a fivefold increase above baseline of the histamine dose required to produce pruritus following both cyproheptadine and placebo. This compared to a 10-fold increase following diphenhydramine and a 750-fold increase following hydroxyzine HCl.

Yosipovitch et al. (14,15,23–26) performed human studies to evaluate the antipruritics with this histamine injection, as well as histamine-iontophoresis-induced itch models in man. They also utilized the visual analog scale (VAS) to measure the itch magnitude (intensity). One study compared the effect of antipruritics of a high-potency corticosteroid, clobetasol propionate (CP) ointment versus its placebo in a double-blind manner on 16 healthy volunteers. Additionally, they evaluated the affect of CP and its placebo to thermal sensation and pain (23). They demonstrated that the CP had rapidly decreased histamine-induced itch, but did not alter warmth sensation and thermal pain thresholds. Another study determined

the effect of menthol and its vehicle (alcohol) on thermal sensations, pain, and histamine-induced experimental itch with 18 human subjects (24). Menthol showed a subjective cooling effect lasting up to 70 minutes in 12 of 18 subjects; however, it did not affect the cold and heat threshold, nor did it affect cold and heat pain threshold. Alcohol produced an immediate cold sensation lasting up to 5 minutes in 4 of 18 subjects and lowered the sensitivity of cold sensation threshold ($p < 0.05$). Histamine injection did not change thermal and pain thresholds. Menthol did not alleviate histamine-induced itch magnitude, or its duration. They suggested that menthol fulfills the definition of a counterirritant, but does not affect histamine-induced itch, nor does it affect pain sensation.

Later, they examined the effect of topical aspirin and its model vehicle dichloromethane on histamine-induced itch in 16 human subjects (25). Aspirin significantly reduced itch duration ($p = 0.001$) and decreased itch magnitude ($p < 0.04$). Aspirin and vehicle application did not affect thermal and pain thresholds during histamine-induced itch. Further, they tested the antipruritics effect and thermal sensation of a local anesthetic, 1% pramoxine, and its vehicle control in 15 human subjects (26); pramoxine significantly reduced both the magnitude and duration of histamine-induced itch. The pramoxine also reduced the cold pain threshold but did not affect warm sensation or heat pain threshold.

Recently, they investigated the effect of thermal modulation in histamine-induced itch (14,15). They first investigated the effect of thermal stimuli and distal scratching on skin blood flow and histamine-induced itch in 21 healthy volunteers (14). Thermal stimuli included 41°C, 15°C, and 49°C while scratching was performed using a 7-inch cytology brush. Assessment of itch was done psychophysically using computerized visual analog scale (COVAS, Medoc, Ramat Ishai, Israel), and mapping of skin blood flow was done utilizing a PIM II laser Doppler perfusion imager (LDPI) at baseline, in the different thermal stimuli, after histamine iontophoresis treatment, and after scratching. They found that scratching significantly ($p = 0.01$) reduced skin blood flow and itch; noxious heat significantly increased basal skin ($p \leq 0.001$), but was not significant in reducing blood flow and itch intensity; noxious cold and cooling significantly ($p = 0.007$) reduced itch intensity but not in histamine-induced skin blood flow; and subnoxious warming neither had an affect with both itch intensity nor skin blood flow. They suggest that heat pain and scratching may inhibit itch through a neurogenic mechanism that also affects blood flow.

The other study involved 21 healthy human volunteers and assessed whether (*i*) the sensory perception of itch is attenuated by interactions between thermal and mechanical stimuli, as well as afferent information related to itch; and (*ii*) if interindividual differences in itch perception were related to interindividual differences in pain sensitivity (15). They used a 100-mm COVAS on histamine-iontophoresis applied on the flexor forearms. After 30 seconds, thermal stimuli [noxious cold (2°C), innocuous cool (15°C), innocuous warmth (41°C), noxious heat (49°C)] were delivered repetitively in a random order by a 16×16 mm Peltier device at 3 cm distal to the sight of histamine-iontophoresis. Cytology brush was used to simulate scratching at a constant pressure. Results revealed that noxious heat, noxious cold, and scratching significantly ($p < 0.004$, $p < 0.001$, $p < 0.0001$, respectively) reduced itch via spinal or supraspinal mechanism. A possible explanation favoring supraspinal mechanism is that these three stimuli were sufficient to have called attention from the prefrontal cortex, thus diverting attention away from the itch. The study revealed significant interindividual differences in itch sensitivity to histamine. On the other hand, interindividual differences in itch sensitivity were unrelated to interindividual differences pain sensitivity.

Pfab et al. also used thermal modulation for histamine-induced itch (16). They evaluated the effect of short-term alternating temperature modulation in nine healthy human volunteers and developed a possible methodology for imaging studies using functional magnetic resonance imaging. Histamine induction was done using the skin prick model (27) to the volar aspect of the dominant right forearm of each subject. Skin temperature was modulated, intensity of itch was determined, and the Eppendorf Itch Questionnaire was done by all subjects at the end of the study. Results revealed the mean itch intensity was significantly ($p < 0.001$) higher in the 25°C temperature compared with that in 35°C temperature. Alternating changes in mean itch perception between 25°C and 35°C were notably reproducible. And, the mean descriptive and emotional ratings were also significantly ($p < 0.01$) higher in the 25°C temperature compared with that in 35°C temperature as well. They concluded that a decrease in short-term moderate temperature enhances histamine-induced itch, providing the possibility of further and more

detailed itch investigation by methods usually used for nociception such as functional magnetic resonance imaging.

Weisshaar et al. (28) evaluated the effect of topical capsaicin on the cutaneous reactions and itching to histamine in atopic eczema (AE) patients and healthy human subjects. Capsaicin 0.05% was applied three times daily over a five-day period to the same infrascapular region. The effects of pretreatment upon the pruritogenic and wheal and flare reactions to subsequent histamine iontophoresis were evaluated on the following day. In control subjects, but not in AE patients, capsaicin pretreatment significantly reduced the flare area. Compared with control subjects, AE patients showed a lack of alloknesis (itchy skin) or significantly smaller areas of alloknesis in pretreated and nonpretreated skin. In control subjects, capsaicin pretreatment significantly reduced itch sensations compared with nonpretreated skin, whereas in AE patients no differences were seen. Itch sensations in capsaicin-pretreated skin were significantly lower in control subjects than in AE patients. They concluded that capsaicin effectively suppresses histamine-induced itching in healthy skin but has less effect in AE. The diminished itch sensations and the absence of alloknesis in atopic individuals indicate that histamine is not the key factor in itching in AE.

Thomsen et al. (29) conducted a randomized, double-blind, and placebo-controlled human study to determine the antipruritic ability of topical aspirin in inflamed skin. In 24 nonatopic volunteers, an inflammatory skin reaction was induced in forearm skin at five sites by sodium lauryl sulfate (SLS) contained in Finn Chambers. Aspirin 10%, aspirin 1%, mepyramine 5%, and vehicle were applied to the inflamed and corresponding noninflamed areas 20 minutes before itch induction with intradermal histamine injection. No difference in itch intensities was found after application of aspirin, mepyramine and vehicle, but more itch was induced in aspirin and mepyramine pretreated sites in inflamed skin compared with normal skin ($p < 0.05$). In normal skin, flare areas were smaller after pretreatment with aspirin 10% ($p < 0.05$) and mepyramine ($p < 0.001$), as were wheal areas after mepyramine ($p < 0.01$), compared with vehicle pretreatments. In inflamed skin, flare areas were smaller after pretreatment with aspirin 10% ($p < 0.01$) and mepyramine ($p < 0.001$), as were wheal areas after aspirin 10% ($p < 0.01$), aspirin 1% ($p < 0.05$), and mepyramine ($p < 0.001$). They concluded that despite a significant skin penetration as measured by the influence on wheal and flare reactions, topically applied aspirin did not decrease histamine-induced itch in the model used.

Zhai et al. (30) evaluated the antipruritic effect of hydrocortisone (1% and 2.5%) and its vehicle control on histamine-induced itch and sensory effects in 18 human subjects. In comparison with placebo, 2.5% hydrocortisone significantly ($p = 0.03$) reduced itch duration from 12.6 ± 11.0 to 8.6 ± 8.2 minutes (the reducing rate was 32%) as well as itch magnitude (at minutes 3, 6, 7, and overall). Placebo, 1% and 2.5% hydrocortisone significantly altered ($p < 0.05$) the cold sensation threshold. No treatment altered cold or heat pain thresholds. They suggested that topical application of 2.5% hydrocortisone might be significantly beneficial for the treatment of histamine-induced itch.

They further ascertained the antipruritic effects of topical strontium salts with the histamine-induced-itch model on eight human subjects (31). Strontium nitrate, in comparison with its vehicle control, significantly shortened itch duration from 28.1 ± 5.4 to 18.5 ± 4.2 minutes ($p < 0.01$) and reduced itch magnitude at time points 12 to 20 minutes and overall ($p < 0.05$). They concluded that strontium nitrate may act as a topical antipruritic agent in reducing histamine-mediated itch. Furthermore, they utilized this histamine-induced itch human model to screen and to compare the efficacy of a group of topical antipruritics on 10 individuals who were responsive to histamine-induced itch sensation (32). The pramoxine-containing cream (formulation D) significantly ($p < 0.05$) decreased itch magnitude (within a 20-minute test period), from 2.6 ± 2.1 to 2.2 ± 2.1 cm when compared with its vehicle control; it also significantly ($p < 0.05$) shortened itch duration (15.0 ± 7.4 minutes) in comparison with its vehicle control (20.3 ± 7.0 minutes). Of all the formulations tested, pramoxine-containing cream was the most effective antipruritic in decreasing histamine-induced itch.

Kesting et al. established a reliable model that objectively demonstrates the effectiveness of ear electro-acupuncture by reducing alloknesis areas in experimentally induced itch (18). Thirty-two human healthy volunteers underwent two experiments, both their volar forearms treated with histamine iontophoresis. In the first experiment, 16 were administered with electrical ear acupuncture on the left ear, and the other half, the right ear. Alloknesis were

measured at 5 and 10 minutes posthistamine iontophoresis treatments. In the second experiment, none of them received acupuncture, serving as control. Results revealed after the 5th and 10th minute, the alloknesis areas in the ipsilateral sites, treated with acupuncture, were significantly smaller ($p < 0.05$) than the contralateral untreated sites. And, the contralateral sites of the first experiment compared with the results of the second experiment showed increasing areas of alloknesis in both the 5th and 10th minute, and were not statistically significant in terms of the size of the increasing alloknesis areas.

Scratch Behavior Measurement

Tohda et al. (33) studied the effect of Byakko-ka-ninjin-to (BN), which is composed of gypsum, the root of anemarrhena, ginseng, licorice, and rice on the inhibition of itch using naive/challenged (NC) mouse model of atopic dermatitis (AD). BN (200 mg/kg, p.o.) significantly inhibited the scratching frequency in NC mice, and decreased the skin temperature by 1.97°C.

Electronic Devices for Accurate Measurement of Itch

Orito et al. developed a model for assessing the duration of scratch behavior in mice by evaluating the time course changes in the distance between the animal's hind limbs and the back of the neck. (8) Ten micrograms ($\sim$10 μL) intradermally administered compound 48/80 was used to induce itch to the backs of ICR mice, and their scratch behavior was recorded on digital videotape, as well as the distance between the back and the hind limb (hind limbs were color coded) was measured continuously using an image analysis system (SCLABA system, *Noveltec*, Kobe, Japan). Results for "true" scratching behavior revealed no significant difference among the three thresholds ($p = 0.1$); while the results for duration of scratching recorded during the observation period increased significantly ($p < 0.001$) as the threshold lengthened. This study suggests that the SCLABA system is a good tool for studying factors which may cause itch, and also for evaluation of efficacy of a new antipruritic drug using experimental animals such as NC/Nga mice, a representative model of AD.

Inagi et al. evaluated and characterized scratching behavior using their new apparatus, MicroAct in ICR and BALB/c mice (9). Inductions of scratching behavior were done by: (*i*) intradermally injecting 20 μL of compound 48/80 and 20 μL of physiologic saline in two sets of both ICR and BALB/c mice, the other set served as control; and (*ii*) intradermal administration of 20 μL of appropriately diluted anti-dinitrophenyl (anti-DNP) monoclonal IgE to induce passive cutaneous anaphylaxis (PCA); and (*iii*) to induce contact sensitivity reaction, nine applications of 0.15% of DNCB diluted in acetone were done on the backs of BALB/c mice. Frequency of scratching events ($\geq$3 consecutive scratch behavior or beats), total scratching time, and total number of beats (scratch behavior) detected by MicroAct were the parameters used. Results revealed that MicroAct's tally were comparable with the observer's tally. The frequency of scratching events and total scratching time increased in a dose-dependent manner for both the ICR and BALB/c mice. In the PCA of the ICR mice, the three parameters increased, though not significantly. There was a significant ($p < 0.001$) increase in the three parameters in the induced contact sensitivity in the BALB/c mice.

Benjamin et al. developed a practical method for evaluating scratch behavior by use of a portable digital limb-worn accelerometers suitable for children and adults, in seven atopic children (aged 2–9 years), and seven children (aged 5–7 years) without atopy, utilizing a night video-recording with infrared light as the gold standard (17). Parameters of measuring accelerometer readings were epoch (unit of time assayed) equivalent to two seconds and "burst analysis" (successions of 1 epoch); while for the night video recording were observed as sleeping, scratching, restless movements, and movements under covers–which were clearly defined operationally in the experiment. Results from night video recording revealed a statistically significant ($p < 0.01$) 46-minute less sleep and a greater "scatter" of readings in atopic patients compared with the control group; while results from the accelerometer readings were significantly ($p < 0.01$) clear and consistent, and, though not significant, arm movements resulted higher than lower limbs. Accelerometer scores were highly correlated with the video scores (< 0.01), for scratching, restlessness, and sleeping time.

Others

These include contact allergic dermatitis model (poison ivy), contact irritant dermatitis induced by SLS, etc. (34–36).

Newly Found Use of Known Drugs

Substance P-induced itch was used by Liebel et al. and found that sertaconazole nitrate inhibited contact hypersensitivity and scratching responses in a murine model of pruritus (11). Fifty microliter (50 μL) of 300 μg of substance P dissolved in sterile physiological saline was intradermally injected in male mice to produce itch response, while 50 μL intradermal administration of sterile physiologic saline served as control. Results revealed statistically significant reduction in scratching with sertaconazole nitrate-treated animals ($p < 0.05$), compared with the reduction in scratching in 1% hydrocortisone-treated animals.

Wikström et al. conducted a randomized, double-blind, placebo-controlled study, using κ-opioid agonist nalfurafine in 144 uremic patients with ESRD undergoing hemodialysis (19). Itch intensity was assessed using a five-point scale and revealed a significant ($p < 0.0410$) reduction in itch intensity, as well as the number of excoriations in the body using a three-point scale showed a significant ($p = 0.0060$) reduction. Safety profiles of nalfurafine were evaluated and showed the most common adverse drug reactions were headache, insomnia, vertigo (mediated by the central nervous system) and nausea, and vomiting (mediated by the gastrointestinal system). These adverse drug reactions were transient and were resolved. The results suggest that nalfurafine seem to be both an effective and safe drug in the treatment of patients with ESRD undergoing hemodialysis.

Questionnaires for Itch Assessment

Yosipovitch et al. constructed two questionnaires for itch assessment modifying McGill's pain questionnaire (20,21). The first study utilized a predetermined questionnaire that provided a detailed description of pruritus in AD in 100 atopic Chinese patients (20). The modified questions were aimed to characterize the clinical pattern and sensory and affective dimensions of itch experience in AD. Itch intensity was also measured using VAS. Results revealed: (*i*) prolonged duration of pruritus (descending order) in lower limbs, flexures, upper limbs, and neck; (*ii*) itch intensity peaked twice as much as mosquito-bite itch; (*iii*) itching was most frequent at night, and most patients reported difficulty in falling asleep; (*iv*) daily-life activities that increased severity of the itch were (descending order) sweat, dryness, stress, physical effort, specific fabrics, activity, and hot water. Males significantly ($p = 0.004$) differed with females in terms of activity and physical effort ($p = 0.002$) in increased pruritus; (*v*) major factors found to reduce itch included bathing in cold water and cold ambient environment; (*vi*) associated symptoms were heat sensation, sweating, and pain in the pruritic area; (*vii*) most antipruritic medications have limited long-term effects; (*viii*) itch is bothersome and a major distress to the patient; and (*ix*) the affective score significantly ($p < 0.001$) correlated to itch intensity during its peak. Taken together, the questionnaire was found to be a useful tool in characterizing itch.

The other questionnaire constructed to measure pruritus was based on the short form of the McGill Pain Questionnaire in 145 uremic patients (21). This modified questionnaire included (*i*) patient characteristics; (*ii*) the use of antipruritics; (*iii*) effects of pruritus on sleeping and on mood; (*iv*) effects of dialysis and of daily activities on itch; (*v*) location pruritic sites; (*vi*) sensory and affective scores; and (*vii*) itch intensity measured using VAS. Revalidation of the questionnaire was repeated in 28 subjects after two weeks and revealed no significant ($p > 0.05$) difference in VAS temporal states (onset, pattern, course) and no significant ($p > 0.05$) difference with regard to the sites of the itch between the two questionnaires. Also, the reliability was high ($p < 0.01$).

Studies of Possible Models for Establishing New Antipruritics

As mentioned earlier, Orito et al. developed a model for itch assessment using the SCLABA system and proposed what may be a potential model for development of new antipruritics (8).

Thomsen et al. also proposed two models that could benefit in developing new antipruritics (10,12). The first animal model is to topically apply nonhistaminic antipruritics using serotonin, recognized as a weak local pruritogen in humans (12). Out of the eight

Table 1 Summary Data of Models and Efficacy of Antipruritics

Models	Efficacy	Reference
Intradermal histamine injection-induced itch	5-fold increased above baseline of the histamine dose required, producing pruritus following both cyproheptadine and placebo. A 10-fold increased following diphenhydramine and a 750-fold increase following hydroxyzine HCl.	Rhoades et al. (22)
Intradermal histamine injection-induced itch	Clobetasol propionate ointment rapidly decreased itch but did not alter warmth sensation and thermal pain thresholds.	Yosipovitch et al. (23)
Intradermal histamine injection-induced itch	Menthol failed to show the effect of antipruritics.	Yosipovitch et al. (24)
Intradermal histamine injection-induced itch	Aspirin significantly reduced itch duration and decreased itch magnitude.	Yosipovitch et al. (25)
Intradermal histamine injection-induced itch	Pramoxine significant reduced both the magnitude and duration of itch.	Yosipovitch et al. (26)
Histamine iontophoresis-induced itch	Capsaicin significantly reduced itch sensations.	Weisshaar et al. (28)
Intradermal histamine injection-induced itch	Aspirin did not decrease histamine-induced itch.	Thomsen et al. (29)
Intradermal histamine injection-induced itch	2.5% hydrocortisone significantly reduced histamine-induced itch.	Zhai et al. (31)
Intradermal histamine injection-induced itch	Strontium nitrate showed a good antipruritic effect in reducing histamine-mediated itch.	Zhai et al. (30)
Intradermal histamine injection-induced itch	Pramoxine-containing cream was the most effective antipruritic in decreasing histamine-induced itch.	Zhai et al. (32)
Histamine iontophoresis-induced itch	Noxious heat, noxious cold, and scratching attenuated itch via a spinal or supraspinal mechanism.	Yosipovitch et al. (14)
Histamine iontophoresis-induced itch	Heat pain and scratching reduced itch.	Yosipovitch et al. (15)
Histamine iontophoresis-induced itch	Ear electro-acupuncture reduced alloknesis areas on the forearms.	Kesting et al. (18)
Skin-pricked-histamine-induced itch	A decrease in short-term temperature enhances histamine-induced-itch.	Pfab et al. (16)
Intradermal injection of 8 pruritogens	Histamine and substance P were more pruritogenic in SLS-induced inflamed skin.	Thomsen et al. (10)
Scratch behavior measurement	Byakko-ka-ninjin-to significantly inhibited the scratching frequency in NC mice.	Tohda et al. (33)
Intradermal serotonin induced-itch	Serotonin is a reproducible pruritogen eliciting scratch behavior in rats.	Thomsen et al. (12)
Intradermal compound 48/80-induced itch	MicroAct was comparable with the observer's tally in scratching behavior in mice.	Inagi et al. (9)
Intradermal compound 48/80-induced itch	SCLABA image analysis system was as good as the "true" scratching behavior in mice.	Orito et al. (8)
Intradermal substance P–induced itch	Sertconazole nitrate was comparable with 1% hydrocortisone in reduction of scratching behavior of mice.	Liebel et al. (11)
Lesional skin of atopic patients	Limb-worn accelerometers were comparable with night video-recording in assessing scratch behavior.	Benjamin et al. (17)
Uremia-induced itch	K-opioid agonist nalfurafine was effective and safe treatment in patients with uremia.	Wikström et al. (19)
Predetermined questionnaire (Modified McGill Pain Questionnaire)	Predetermined questionnaire was useful in the assessment of itch in atopic dermatitis patients.	Yosipovitch et al. (21)
Modified McGill Pain Questionnaire	The reliability of Modified McGill Pain Questionnaire was high when compared with the VAS results.	Yosipovitch et al. (20)

substances screened (histamine, compound 48/80, kallikrein, trypsin, papain, substance P, serotonin, and platelet-activating factor) injected intradermally (50 μL per substance) into the rostral back of rats, only serotonin induced excessive scratching, while the rest of the substances were weak or inactive. A dose-response curve was plotted against $\log_{10}$ using different

concentrations of serotonin to evaluate possible systemic effects in: (*i*) 14 rats intradermally and subcutaneously injected with 0.1 and 1 mg/mL (50 μL per dose) to the rostral and caudal back; (*ii*) another four rats were given intradermal serotonin of 10 mg/mL to the caudal back; (*iii*) another 10 rats for each group were given concentrations of 0.01 to 31.6 mg/mL intradermally; and (*iv*) two rats were given concentration of 100 mg/mL each. Video recording was used to objectively count scratch sequences, viewed separately by two investigators, and showed the following: (*i*) number of scratch sequences of injected serotonin related to the rostral back is significantly greater ($p < 0.001$) than that of the caudal back; and (*ii*) number of scratch sequences in the caudal and "other" sites did not produce a significant result. No systemic adverse effects of serotonin occurred at 1 mg/mL, 50 μL. Scratching was probably not due to histamine, since the screening period revealed that histamine did not produce scratching. They concluded that serotonin is a reproducible pruritogen eliciting scratch in rats.

The second study was a randomized, double-blind and placebo-controlled study involving SLS-induced inflamed skin, as well as in normal skin in 32 healthy volunteers, pretreated with 1% SLS in one of their volar forearms, their opposite forearms served as control (10). They evaluated itch intensity, pain, whealing, and redness in 16 subjects given 20 μL of group A battery of substances [substance P, neurokinin A, neurokinin B, histamine (positive control), and physiological saline (negative control)], and the other 16 given were given 20 μL of group B battery of substances [platelet-activating factor, serotonin, trypsin, histamine (positive control), and physiological saline (negative control)], all intradermally injected to both forearms. Results revealed inflamed skin is significantly more pruritogenic than normal skin in substance P ($p = 0.024$) and histamine compared with the control. Neurokinin A, trypsin, PAF, and serotonin only elicited itch in normal skin, while neurokinin B did not elicit itch in both groups. Wheal area was significantly ($p < 0.001$) larger in inflamed skin, though it did not show a significant correlation with itch intensity (Table 1).

CONCLUSION

When measuring itch, several factors must be taken into account: severity, duration, variation between individuals, and subjective differences in determination of itching threshold. To better quantify this subjective response, several approaches have been employed (11–19,27,34–38). The measurement of scratch behavior is problematic, and has been addressed in a variety of ways such as lack of validation and unlikeliness to be reproducible, etc. (12). Since human's verbalization may be more accurate in describing itch sensation, the VAS may provide superior to other methods (11,12,18,19). To evaluate antipruritic drugs, clinical methods may rely on either naturally occurring or experimentally induced pruritus. Methods and judgments based on naturally occurring pruritus better reflects the actually clinical setting (11). However, they have disadvantages including: (*i*) pruritus intensity may fluctuate on its own if the study is conducted over several days since the naturally occurring pruritus may not be stable over time; (*ii*) comparing the pruritic intensity of specific lesions in different patients is often difficult and not always relevant, and (*iii*) adequate controls are difficult to achieve (11).

Histamine-induced itch model was utilized because acute itching is most commonly evoked by chemical stimuli (e.g., histamines) (2). Some individuals do not itch after histamine injection (34,35,38); therefore, to diminish the variation of responses, we suggest that only subjects with histamine-induced itch sensation should be enrolled. This will improve discrimination–an obvious advantage in a screening assay. However, the histamine injection model may induce pain sensation; it may partially interrupt the itch sensation. We note that 1 mL injection appears high and undoubtedly spreads; this large volume has added reproducibly to previous studies (16,17,27,34–38). The VAS score was comparatively low; however, this level (3 cm) was adequate for the discrimination noted. Higher concentrations might be considered in the future.

Alternatively, other itch-inducing models may well be justified in the assessment of antipruritic drugs, complemented by new measuring devices (8–12,14–18,27,39). A recent review of pruritus provides additional insights (40).

Lastly, it is essential that studies of topical antipruritics are well designed and double blind so that resulting data are valid and able to distinguish between effective and noneffective treatments.

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56 Comedogenicity in Rabbit: Some Cosmetic Ingredients/Vehicles

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INTRODUCTION

Several cosmetic ingredients have been shown to be comedogenic using the rabbit ear assay (1,2). On the basis of the animal assay and short-term human clinical studies, the development of comedones has been attributed to prolonged use of cosmetics (1,2). Although there are limitations of the rabbit model in the application of test results to humans (1–4), much information on the comedogenicity of topically applied substances has been based on the rabbit ear assay because it permits rapid screening of many possible offenders. The present chapter is concerned with this theme and reports our finding on the activity of certain raw materials previously investigated.

MATERIALS AND METHODS

To quantify the rabbit comedogenicity data obtained using Kligman's design (1) for acnegenic potential of coded samples in the rabbit ear assay, test and control articles were purchased commercially, which included stearyl alcohol; sodium lauryl sulfate (SLS)—0.1%, 1.0%, 10.0% in petrolatum; butyl stearate—1.0%, 10.0%, 25.0% in petrolatum; isopropyl palmitate 1.0%, 10.0%, 25.0% in petrolatum; myristyl myristate (50% in petrolatum); isopropyl myristate (50% in cold cream); isopropyl palmitate (50% in vanishing cream); isopropyl myristate (50% in vanishing cream); isopropyl myristate (50% in propylene glycol); isopropyl palmitate (50% in propylene glycol); and isopropyl myristate (50% in ethanol). Isopropyl palmitate (δ prime); butyl stearate; paraffin; cetyl alcohol; cocoa butter; decyl oleate; isostearyl neopentonate; isopropyl isostearate; isocetyl stearate; commercial cocoa samples labeled A, B, C, D, E, F, and G; guittard cocoa butter (control); and petrolatum were tested as is.

Samples were stored at room temperature. All test samples were of USP grade.

New Zealand male and female white rabbits, randomly outbred, were delivered at 2.5 kg and acclimated at least four days before entering the study. The rabbit was selected as the test system because of its proclivity to develop comedones (1–6).

Animal identification was via tattoo. The rabbits kept on an 11- to 12-hour light/dark cycle, at room temperature, 71°F to 74°F (22–23°C), and one per cage, were fed commercial laboratory feed (purina chow) and treated potable water available at all times.

The cosmetic ingredient samples were applied to the ears of adult female albino rabbits according to the Rabbit Ear Comedogenic Assay. Each sample was applied daily (Monday through Friday) to the glabrous inner portion of three ears for four consecutive weeks, adding up to a total of 20 applications. Approximately, 0.5 mL or 0.5 gm of the sample was applied with a pipette or syringe, and spread with a glass applicator. Controls consisted of 10% crude coal tar. Coal tar evoked a comedogenic score of 4 on all control animals (data not shown). Ingredient samples were applied to both ears during the tests. At the end of four weeks, each rabbit ear was biopsied and examined for evidence of comedone formation. An elliptical sample, about 2.5 cm long, was blunt dissected down to the cartilage and immersed in water at 60°C for two minutes. The epidermis was peeled off as an intact sheet; its undersurface

examined under a stereomicroscope. Grading was done in a manner similar to the visual grading system (see below).

Scoring System: 0 = none

1 = a few comedones
2 = many comedones
3 = extensive comedones
4 = confluent involvement

RESULTS

Test results (Table 1) indicate that the ester ingredients selected had comedogenic scores that ranged from 1 to 4: isopropyl palmitate, butyl stearate, isopropyl isostearate, and decyl oleate scored highest in its pure form with scores ranging from 3 to 4. Manipulation of testing conditions through dilution of concentration and mixture of ingredients either increased or decreased comedogenic potentials. The addition of vanishing cream to isopropyl palmitate and isopropyl myristate increased their comedogenicities, pushing means scores close to 4, despite a 50% dilution. Isocetyl stearate, myristle myristate, cetyl alcohol, and stearyl alcohol had the least comedogenic potential, with mean scores of only 1 to 2.

SLS was tested in petrolatum at concentrations of 0.1%, 1%, and 10%. Results (Table 2) indicate low comedogenic potentials for these specific combinations. Mean scores were in the range of 1.

Table 1 Comedogenic Scores of Esters and Alcohols

Test article	Mean day 20 (clinical)		Mean (slide biopsy)	
	L	R	L	R
Isopropyl palmitate (δ prime)	3 ± 0	3.6 ± 0.5	3.6 ± 0.5	3.6 ± 0.5
Isopropyl palmitate 1% in petrolatum	1.3 ± 0.5	1.6 ± 0.5	1.3 ± 0.5	1.6 ± 0.5
Isopropyl palmitate 10% in petrolatum	1.3 ± 0.5	1.3 ± 0.5	1.3 ± 0.5	1.6 ± 0.5
Isopropyl palmitate 25% in petrolatum	1 ± 0	1.6 ± 0.5	1 ± 0	1.6 ± 0.5
Isopropyl palmitate in vanishing cream (50%)	4 ± 0	4 ± 0	3.6 ± 0.5	3.6 ± 0.5
Isopropyl palmitate in propylene glycol (50%)	3 ± 0	3 ± 0	3 ± 0	3 ± 0
Isopropyl myristate in cold cream (50%)	3 ± 1	3.3 ± 0.5	3 ± 1	3.3 ± 0.5
Isopropyl myristate in vanishing cream (50%)	4 ± 0	4 ± 0	4 ± 0	4 ± 0
Isopropyl myristate in propylene glycol (50%)	3 ± 1	3 ± 1	3.3 ± 0.5	3.3 ± 0.5
Isopropyl myristate in ethanol (50%)	4 ± 0	4 ± 0	4 ± 0	4 ± 0
Butyl stearate	4 ± 0	4 ± 0	4 ± 0	4 ± 0
Butyl stearate 1% in petrolatum	1 ± 0	1 ± 0	1 ± 0	1.3 ± 0.5
Butyl stearate 10% in petrolatum	1.6 ± 0.5	2 ± 0	2 ± 0	2 ± 0
Butyl stearate 25% in petrolatum	2 ± 0	2 ± 0	2 ± 0	2 ± 0
Isopropyl isostearate	3.6 ± 0.5	4 ± 0	3.6 ± 0.5	4 ± 0
Decyl oleate	3.3 ± 0.5	3.3 ± 0.5	3.3 ± 0.5	3.3 ± 0.5
Isostearyl neopentanoate	2 ± 0	2 ± 0	3 ± 0	3 ± 0
Isocetyl stearate	1 ± 0	1 ± 0	2 ± 0	2 ± 0
Myristle myristate	1.3 ± 0.5	1.3 ± 0.5	1.6 ± 0.5	2 ± 0
Cetyl alcohol	1.3 ± 0.5	1.3 ± 0.5	1 ± 0	1.3 ± 0.5
Stearyl alcohol	1.3 ± 0.5	1.3 ± 0.5	1 ± 0	1 ± 0

Table 2 Comedogenic Scores of Surfactants

Test article	Mean day 20 (clinical)		Mean (slide biopsy)	
	L	R	L	R
SLS 0.1% in petrolatum	1 ± 0	1 ± 0	1 ± 0	1 ± 0
SLS 1% in petrolatum	1 ± 0	1.3 ± 0.5	1 ± 0	1 ± 0
SLS 10% in petrolatum	1.6 ± 0.5	1.6 ± 0.5	1.3 ± 0.5	1.3 ± 0.5

Abbreviation: SLS, sodium lauryl sulfate.

Table 3 Comedogenic Scores of Petrolatum Products and Cocoa Butter

	Mean day 20 (clinical)		Mean (slide biopsy)	
Test article	L	R	L	R
Petrolatum (control)	1 ± 0	1.3 ± 0.5	1 ± 0	1.3 ± 0.5
Paraffin	1 ± 0	1.3 ± 0.5	1 ± 0	1 ± 0
Cocoa butter (#81-C FDA-1B)	3.3 ± 0.5	3.3 ± 0.5	3.3 ± 0.5	3.6 ± 0.5
Cocoa butter (#82-C FDA-2)	3 ± 0	3 ± 0	3.3 ± 0.5	3 ± 0
Cocoa butter A	2.6 ± 0.5	3.3 ± 0.5	2.6 ± 0.5	2.6 ± 0.5
Cocoa butter B	3.3 ± 0.5	3.6 ± 0.5	3.3 ± 0.5	3.3 ± 0.5
Cocoa butter C	3 ± 0	3 ± 0	2.6 ± 0.5	2.6 ± 0.5
Cocoa butter D	3.6 ± 0.5	4 ± 0	4 ± 0	4 ± 0
Cocoa butter E	3.6 ± 0.5	4 ± 0	3.6 ± 0.5	3.6 ± 0.5
Cocoa butter F	3.6 ± 0.5	3.6 ± 0.5	3.6 ± 0.5	3.6 ± 0.5
Cocoa butter G	3.3 ± 0.5	3.6 ± 0.5	3.6 ± 0.5	3.6 ± 0.5
Cocoa butter (control)	3 ± 0	3 ± 0	3.3 ± 0.5	3 ± 0

Tables display comedogenic potential scores ± standard deviation based on a 5-point scale, in which 4 is the highest score. Results listed were obtained at the end of the four-week test period (day 20) using visual and steromicroscopic (slide biopsy) examination. The cocoa butter samples represented different commercial production batches.

Paraffin and petrolatum, used as control vehicles, did not yield significant comedogenic potential scores, whereas cocoa butter displayed high results (Table 3). The majority of comedogenic scores fell between 3 and 4.

DISCUSSION

Among the tested ingredients, decyl oleate, isopropyl palmitate, isopropyl myristate, isopropyl isostearate, isostearyl neopentanoate, isocetyl stearate, myristyl myristate, butyl stearate, and cocoa butter were deemed comedogenic. All of the esters are most commonly found in products such as night cream, wrinkle removal cream, sunscreen, moisturizer, hair care products, lipstick, concealer, antiperspirant, as well as baby care products. Cocoa butter is a prevalent ingredient in cosmetics due to its smooth texture and sweet fragrance. It is found in soaps, lotions, skin care products, and suntan lotion. Since we rely on the use of common household cosmetics to maintain our hygienic regimen, comedogenicity should be considered in the development of cosmetics, skin care products, and topical medications. The Cosmetic, Toiletry, and Fragrance Association (CTFA), the Cosmetic Ingredient Review (CIR), and physicians may consider providing available information about the ingredients of such products to their consumers. These findings are significant and are worth noting; otherwise, consumers may be misguided about the effects of using such products (7).

Data from this study generates a theory for dosage and purity dependence. The initial tests for isopropyl palmitate and butyl stearate were done using the ingredients in their purity at 100% concentration, yielding a mean score of 3.3 and 4, respectively, for week 4. However, when the tests were repeated using isopropyl palmitate and butyl stearate at concentrations of 1%, 10%, and 25% in conjunction with petrolatum, results were significantly different. Scores declined to an average of 1 to 1.7 for isopropyl palmitate and an average of 1 to 2 for butyl stearate at week 4. The results indicate that the comedogenic properties of these ingredients depend on either dosage or purity. Unfortunately, since both purity and concentration were manipulated in one test, it is not possible to determine whether the reduction in the comedogenicity was due to the dilution of concentration, purity, or both. In another case where vanishing cream was added to isopropyl palmitate and isopropyl myristate, comedogenicity escalated to a score of 4, despite a 50% dilution. Is it possible that cosmetic products developed using recommended USP grade have potent acnegenic results? This is a matter that may be worth investigating.

The ultrasensitivity of the rabbit follicle to respond readily to test materials has been extensively documented (1–6,8). Our study sought to quantify comedogenic data collected with the commonly used model: Kligman's rabbit assay. However, the lack of a systematic

database for such information restricts the use of compiled results as a direct correlation to humans. Instead, results from such studies should only be used as a guideline for formulation programs.

To further correlate these results with human reactions, specific studies on humans need to be done. Kligman's four-week patch test conducted on human subjects demonstrates a positive correlation between the animal and human model (4). However, there are two major differences worth noting. First, humans are less sensitive to "acne cosmetica" than rabbits (4). For example, comedogenic substances of scores 1 or 2 that evoke comedones on rabbits may not affect humans (4). Second, the rabbit model takes longer to yield results than the human model. Humans usually experience acute pustulation during the first 24 hours, whereas rabbits typically take three to four weeks to fully express comedones (9). Comedone expression is also present in humans, but it does not occur as frequently as on rabbits. These differences suggest that there may be a different mechanism involved in the production of pustules on humans.

Taken together, this data may be added to dermatotoxicologic ingredient profiles; yet much remains to be done before we can fully comprehend its meaning. Results obtained by Fulton (10) have deemed isopropyl myristate, isopropyl palmitate, isopropyl isostearate, butyl stearate, isostearyl neopentanoate, myristle myristate, decyl oleate, and isocetyl stearate as offenders. However, the CTFA and CIR maintains that the use of all these ingredients is safe at its appropriate concentration (11).

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57 Skin-Whitening Agents

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INTRODUCTION

Skin hyperpigmentation is common and often causes psychosocial distress (1). Therapeutical interventions include whitening agents, chemical peels, lasers, and physical methods (1–3). Although multiple interventions are available, skin-whitening agents, due to their simplicity and convenience, continue to be the mainstay of approach to either lighten skin (individuals who wish to change or modify their skin color) in the cosmetic field or depigment skin (treatment for abnormal hyperpigmentation skin such as melasma, freckles, and actinic lentigines) in the clinical therapy. Commonly used whitening agents include hydroquinone, arbutin, kojic acid, ascorbic acid, and its derivatives. Their efficacy, mechanism, and safety have been extensively reviewed (4). This chapter revises the previous version (4) and updates current progress.

SKIN-WHITENING AGENTS

Hydroquinone

Hydroquinone (1,4-dihydroxybenzene) is used in the photographic, rubber, chemical, and cosmetic industries. In the late 1930s, it was observed that monobenzyl ether of hydroquinone, a chemical used in the manufacture of rubber, caused depigmented skin in some workers (5).

The efficacy of hydroquinone as a skin-lightening agent has been established in both human and animal studies. Clinically, hydroquinone is applied topically in the treatment of melasma, freckles, and senile lentigines as well as postinflammatory hyperpigmentation (PIH). In the United States, hydroquinone is available in concentrations up to 2% as an over-the-counter (OTC) drug and by prescription at higher concentrations (2,5).

Hydroquinone inhibits the conversion of dopa to melanin by inhibiting the tyrosinase enzyme (2,5,6). Other proposed mechanisms are inhibition of DNA and RNA synthesis, degradation of melanosomes, and destruction of melanocytes (2). Electron microscopic studies of black guinea pig skin treated with hydroquinone show the anatomic consequences of this action: (*i*) the melanosome structure is disturbed, resulting in decreased production or increased degradation of these organelles, or both; (*ii*) hydroquinone exposure can ultimately lead to melanocyte degradation; and (*iii*) keratinocytes are spared, showing no apparent injury (5).

Arndt and Fitzpatrick (7), in a non-placebo-controlled study, compared the efficacy of 2% and 5% hydroquinone cream for treatment of pigmentary disorders in 56 patients. Hydroquinone was a moderately effective depigmenting agent in 80% of cases. There was no efficacy difference between the two concentrations; however, 2% hydroquinone was less irritating than 5%.

In another non-placebo-controlled study, Fitzpatrick et al. (8) evaluated the efficacy of a 2% cream of stabilized hydroquinone in 93 patients. Of those patients, 64% showed decreasing hypermelanosis.

Kligman and Willis (9) noted enhanced efficacy with 5% hydroquinone, 0.1% tretinoin, and 0.1% dexamethasone in hydrophilic ointment for the treatment of melasma, ephelides, and PIH on adult male blacks in a non-placebo-controlled study. In contrast, they experienced poor results with each of the aforementioned as monotherapies. However, actinic lentigines were resistant.

Gano and Garcia (10) conducted a 10-week clinical trial in 20 women with melasma. Topical applications of 0.05% tretinoin, 0.1% betamethasone valerate, and 2% hydroquinone were used in a non-placebo-controlled study. There was an objective improvement rate of 65% and a subjective improvement rate of 95%. Side effects were frequent but minimal. Caution is

necessary when using potent fluorinated corticosteroids for prolonged periods on the face since it may result in epidermal atrophy, telangiectasia, rosacea-like erythemas, acne, and perioral dermatitis. Particularly, it may also exert an antimetabolic effect, resulting in decreased epidermal turnover, and, thus, may produce a mild depigmenting effect (11). However, when used in combination with tretinoin and hydroquinone in the treatment of melasma, fluocinolone acetonide 0.01% suppresses biosynthetic and secretory functions of melanocytes, and thus melanin production, leading to early response in melasma, synergy among the three agents, and no significant side effects over an eight-week period (11).

Gellin et al. (12) established a reliable in vivo method to predict the depigmenting action of chemicals on mammalian melanocytes. Black guinea pigs and black mice were used as animal models to screen the depigmenting capacity of several phenols, catechols, and organic antioxidants. Results showed that complete depigmentation on all test sites was achieved with mono-methyl ether of hydroquinone and p-tertiary butyl catechol in the black guinea pig. Less pronounced pigment loss was noted with these chemicals in black mice.

Pathak et al. (13) clinically tested the efficacy of hydroquinone in varying concentrations supplemented with corticosteroids or retinoic acid (tretinoin) in 300 Hispanic women with melasma in a non-placebo-controlled study and concluded that cream or lotion formulations of 2% hydroquinone and 0.05% to 0.1% retinoic acid provided the most favorable results. In addition, avoidance of sun exposure and constant use of broad-spectrum sunscreens are requisite for efficacy. They also suggested that patients should suspend use of oral contraceptives and other agents that promote skin pigmentation.

Sanchez and Vazquez (14) treated 46 patients with melasma, using two versions of a 3% hydroalcoholic solution of hydroquinone. In this non-placebo-controlled study, overall improvement was noted in 88% of the patients and moderate to marked improvement in 36%. Side effects were minimal. Vazquez and Sanchez (15), in a double-blind and vehicle-controlled study, compared a broad-spectrum sunscreen agent with its vehicle in the treatment of melasma in 53 patients who were concomitantly using a depigmentating solution. They reported that 96.2% of those who used the sunscreen agent showed improvement as compared with 80.7% of placebo group. These results suggested that the use of sunscreen might be necessary for efficacy in the treatment of pigmentation disorders.

Clarys and Barel (16) tested the efficacy of an ascorbate-phytohydroquinone complex in 14 patients with actinic lentigo in a non-placebo-controlled study. Objective skin color changes were evaluated with a chromameter. After one month of treatment, a clear depigmentation of the macules was measured.

Haddad et al. (17), in a double-blind, randomized, prospective study, compared the effectiveness of two products with placebo in 30 patients with melasma over three months. In group 1, a 4% hydroquinone cream was applied to one side of the face where placebo applied to opposite sides; group 2, a 5% skin-whitening complex cream was applied to one side of the face where placebo applied to opposite sides. A standard sunscreen was used daily. Group 1 (hydroquinone and placebo) presented an improvement of 76.9% with 25% side effects, and group 2 (skin-whitening complex and placebo) presented an improvement of 66.7% with 0% side effects. They concluded that both depigmentation agents were equally effective in the treatment of melasma; however, the skin-whitening complex seems to be an excellent choice since its adverse effects were nil.

Grimes (18) evaluated a microsponge formulation of 4% hydroquinone with 0.15% retinol on 28 patients of melasma and PIH in a 12-week open-label study. Patients applied the formulation on the full face twice daily (morning and evening). A broad-spectrum sunscreen was applied once in the morning, 15 minutes after application of the test product. Results showed that tested formulation produced significant improvement at all study endpoints (weeks 4, 8, and 12) when compared with baseline. The tolerance of patients to this formulation was good.

Espinal-Perez et al. (19) compared a 5% ascorbic acid cream and a 4% hydroquinone cream on a randomized split-face-designed study with 16 female patients of melasma over 16 weeks. Sunscreen was applied daily throughout the observation period. The hydroquinone side showed 93% good and excellent results, compared with 62.5% on the ascorbic acid side; however, side effects were present in 68.7% (11/16) with hydroquinone versus 6.2% (1/16) with ascorbic acid. They concluded that ascorbic acid may provide a beneficial effect on melasma, with a minimum of adverse effects when compared to hydroquinone.

Ferreira Cestari et al. (20), in a multicenter, open-label, randomized, eight-week clinical trial, compared the efficacy and safety of a triple combination (TC) cream and monotherapy with hydroquinone cream in the treatment of moderate to severe facial melasma of 120 patients. TC cream was significantly more effective than hydroquinone cream (73% vs. 49%). Adverse events (erythema, burning sensation, and desquamation) were similar in both groups.

In some cases, higher concentrations of hydroquinone may be used. The formulations contain concentrations as high as 10% combined with non-fluorinated corticoid creams with or without the additional use of tretinoin or hydroxy acids such as glycolic acid. Extemporaneously compounded preparations are often effective in patients who have failed to respond to lower concentrations of hydroquinone. With controlled use and monitoring, side effects from these preparations have proved minimal (2). However, note that hydroquinone may be quickly oxidized in such formulations.

Hydroquinone occurs in nature as the β-glucopyranoside conjugate (arbutin). Arbutin is a mild agent for treating cutaneous hyperpigmentation, including melasma and ultraviolet (UV)-induced ephelides (21). Arbutin is an active ingredient of the crude drug uvae ursi folium traditionally used in Japan and contained in the leaves of pear trees and certain herbs. Maeda and Fukuda (21) determined arbutin's inhibitory action on the melanin synthetic enzyme and its effects on melanin intermediates and melanin production in cultured human melanocytes. They indicated that the depigmentation effect of arbutin works through an inhibition of the melanosomal tyrosinase activity, rather than by suppression of the expression and synthesis of tyrosinase in human melanocytes. Arbutin was less cytotoxic than hydroquinone to cultured human melanocytes.

Adverse reactions associated with hydroquinone use include both acute and chronic complications. Among acute reactions are irritant dermatitis, nail discoloration, and PIH (5). Although generally assumed to be a common allergen, the documentation of hydroquinone allergic contact dermatitis is weak (5). Hydroquinone use can also induce hypopigmentation and, rarely, depigmentation of treated surrounding normal skin. But, these changes are temporary and resolve on cessation of hydroquinone treatment, in contrast to monobenzone use, which can cause permanent depigmentation (22). Hence, the only indication for monobenzone therapy is in the treatment of severe vitiligo.

A more recent concern regarding the use of hydroquinone is the occurrence of hydroquinone-induced ochronosis, a chronic disfiguring condition resulting, in general, from the prolonged use of high concentrations of hydroquinone (22,23).

Hydroquinone's acute and chronic toxicity toward higher terrestrial organisms appears to be minimal in humans (24,25). In an epidemiologic investigation, 478 persons employed as photographic processors showed no significant excess mortality, sickness/absence, or cancer incidence (24). The reported nephropathy and cell proliferation, as evidence of carcinogenicity, observed in Fischer 344/N rats (26,27), appears to be strain specific and sex specific (27). Hydroquinone was negative in the Ames/Salmonella and Drosophila genotoxicity assays (28). Others suggest that carcinogenic and teratogenic potentials have been inadequately studied (24,29) and that both hydroquinone and benzoquinone produce cytotoxic effects on human and mouse bone marrow cells (30). Hydroquinone in an alcoholic vehicle readily penetrates human forehead skin in vivo following a single 24-hour topical exposure; elimination was complete within five days (31). Wester et al. (32) determined the topical bioavailability, metabolism, and disposition of hydroquinone on humans in vivo and in vitro; dose recovery in urine was 45.3%, of which the majority was excreted in the first 24 hours.

Kojic Acid

Kojic acid, a fungal metabolic product, is increasingly being used as a skin-lightening agent in skin care products marketed in Japan since 1988. It was first isolated from *Aspergillus* in 1907 (33). Kojic acid suppresses free tyrosinase, mainly attributable to chelation of its copper (33–35), and it has been shown to be responsible for therapy and prevention of pigmentation, both in vitro and in vivo (34,36,37).

In Japan, it is used in nonprescription skin care products up to a concentration of 1%. To increase percutaneous absorption and thus therapeutic activity, it is usually used at the highest concentration allowed (33).

Since it is used intensively in foods (such as bean paste, soy, and sake) in some countries, particularly Japan, its oral safety has been studied.

Shibuya et al. (38) investigated the mutagenicity of kojic acid by the Ames test, by the forward mutation test in cultured Chinese hamster cells, and by the dominant lethal test in mice. They concluded that although kojic acid is a weak mutagen in bacteria, it is non-mutagenic in the eukaryotic system either in vivo or in vitro.

Abdel-Hafez and Shoreit (39) tested the mycotoxins, using the dilution plate method; kojic acid may induce some toxins. Fujimoto et al. (40) examined the tumorigenicity of kojic acid in $B6C3F_1$ mice. Three groups of animals were given food containing 0%, 1.5%, and 3.0% kojic acid for six weeks; mice in the groups ingesting kojic acid showed significantly higher frequency of induced thyroid tumors.

But true adverse effects after human oral ingestion have not been demonstrated. Nakagawa et al. (33) noted no signs of relapse of dermatitis or any other adverse effects on sensitized patients upon ingestion of foods containing kojic acid; however, they also noted that topical application may induce allergic contact dermatitis with sensitized patients. They postulated that kojic acid was considered to have a high sensitizing potential, because of the comparatively high frequency of contact sensitivity in patients using one or more kojic acid–containing products.

Majmudar et al. (36) used an in vitro model to evaluate the efficacy, stability, and cytotoxicity of whitening agents. They also conducted a non-placebo-controlled clinical study that indicated that kojic acid in an anhydrous base can induce more skin lightening than in an aqueous base.

Recently, Lim (37) conducted a non-placebo-controlled study to test 2% kojic acid in a gel containing 2% glycolic acid and 2% hydroquinone in 40 Chinese women who had epidermal melasma for 12 weeks. Half of the face was treated with the above formulation. The other half was treated with a formulation that was identical, except that it contained no kojic acid. Results showed similar improvement in melasma on both the sides. More than half (60%) of the melasma cleared in sides receiving kojic acid, whereas less than half (48%) cleared in the side denied kojic acid; in particular, two patients had complete clearance only in the kojic acid–treated side. However, the improvement did not show a statistical difference between the formulations.

Ascorbic Acid and its Derivatives

Ascorbic acid may inhibit melanin production by reducing o-quinones (41), so that melanin cannot be formed by the action of tyrosinase until all vitamin C is oxidized. Because vitamin C is quickly oxidized and decomposes in aqueous solution, it is not generally useful as a depigmenting agent.

Recently, stable derivatives of vitamin C have been synthesized to minimize this problem (41–44). Magnesium-L-ascorbyl-2-phosphate (VC-PMG) is a vitamin C derivative that is stable in water, especially in neutral or alkaline solution containing boric acid or its salt (41). VC-PMG is hydrolyzed by phosphatases of the liver or skin to vitamin C and thus exhibits vitamin C–reducing activity (41).

Kameyama et al. (41) investigated the effects of VC-PMG on melanogenesis in vitro and in vivo. Results from their non-placebo-controlled study suggested that the topical application of VC-PMG was significantly effective in lightening the skin in 19 of 34 patients with chloasma or senile freckles and in 3 of 25 subjects with normally pigmented healthy skin.

Other Agents

Glutathione is a ubiquitous compound found in human bodies. It has recently received attention since it possesses skin-lightening function. The proposed mechanisms of action include (*i*) direct inactivation of the enzyme tyrosinase by binding with the copper-containing active site of the enzyme, (*ii*) mediating the switch mechanism from eumelanin to phaeomelanin production, (*iii*) quenching of free radicals and peroxides that contribute to tyrosinase activation and melanin formation, and (*iv*) modulation of depigmenting abilities of melanocytotoxic agents (45). Villarama and Maibach (45) review the evidence of its involvement in the melanogenic pathway and shed light on its anti-melanogenic effects that have been documented in in vitro and in vivo studies. However, they suggested that to validate the effectiveness of glutathione, randomized, double-blind, placebo-controlled clinical studies in humans are warranted.

Since many predisposing factors—such as pregnancy or exposure to sunlight (in the UVB and UVA ranges)—may cause hyperpigmentation, various systemic drugs and natural products have been used as protective agents. These agents include chloroquine, indomethacin, vitamin C and E, fish oil, and green tea (46).

Funasaka et al. (47) demonstrated that oral vitamin E [α-tocopherol (α-T)] supplementation can improve facial hyperpigmentation: the inhibitory effect of tocopheryl ferulate (α-TF) on melanogenesis was examined biochemically using human melanoma cells in culture. α-TF, solubilized in ethanol or in 0.5% lecithin, inhibited melanization significantly, as did α-T at a concentration of 100 μg/mL, without inhibiting cell growth.

Kobayashi et al. (48) reported that neoagarobiose, a disaccharide, could be useful as a novel whitening agent because it has moisturizing and whitening effects with low cytotoxicity on B16 murine melanoma cells. However, it should be validated on human skin in vivo.

Schmaus et al. (49) recently identified a new potent lightening agent, 4-(1-phenylethyl) 1,3-benzenediol, a dihydroxylated diphenylmethane derivative. Data obtained from in vitro and in vivo studies on human skin showed good lightening effects.

Ando et al. (50) evaluated the effects of unsaturated fatty acids on UV-induced hyperpigmentation of the skin in a placebo (vehicle)-controlled study. Skin hyperpigmentation was induced on the backs of guinea pigs by UVB exposure. Oleic acid, linoleic acid (LA), and α-linolenic acid (0.5% in ethanol), or ethanol alone as a control, were then topically applied daily five times weekly for three successive weeks. Results suggest that the pigment-lightening effects of LA and α-linolenic acid are, at least in part, due to suppression of melanin production by active melanocytes and enhanced desquamation of melanin pigment from the epidermis.

A new combination product composed of 2% 4-hydroxyanisole (mequinol) and 0.01% tretinoin (all-trans-retinoic acid) in an ethanolic solution is being studied for its safety and efficacy as a topical treatment for disorders of skin hyperpigmentation (51). Fleischer et al. (51) evaluated efficacy in a controlled, double-blind trial. Subjects were randomized to treatment with the combination solution, or one of the active components (4-hydroxyanisole or tretinoin), or vehicle twice daily to all solar lentigines and related hyperpigmented lesions on the face, forearms, and backs of hands for up to 24 weeks. The combination solution (2% 4-hydroxyanisole and 0.01% tretinoin) was clinically superior to each of its active components and to the vehicle in the treatment of solar lentigines. Most skin-related adverse events were mild and were similar for both the combination solution and tretinoin treatment groups.

Bissett et al. (52) evaluated the effectiveness of a stable derivative *N*-acetyl glucosamine (NAG) to reduce facial hyperpigmentation in two separate studies. Topical 2% NAG, its vehicle control, 4% niacinamide, and a combination of 2% NAG with 4% niacinamide were compared in an eight-week, double-blind, placebo-controlled, left-right randomized, split-face clinical test. Data showed that 2% NAG was effective in improving the appearance of facial hyperpigmentation. The combination formulation provided the best results.

Hamed et al. (53) reported the efficacy and safety of deoxyarbutin, a new tyrosinase-inhibiting agent both in vitro and in vivo on human skin. They demonstrated that deoxyarbutin has the potential to be as safe and effective as a depigmenting agent and suggested that it may act an as an alternative agent to hydroquinone.

Shigeta et al. (54) evaluated the effect of liposomalization on the whitening activity of LA by using LA in ethanol, hydrogel-containing LA, and hydrogel-containing liposomal LA toward the UV-stimulated hyperpigmented dorsal skin of brownish guinea pigs. In addition, the whitening effect of LA was examined with UV-stimulated hyperpigmented human upper arm skin by using a hydrogel-containing liposomal LA (0.1% LA) and non-liposomal LA (3.0, 10.0% LA). Liposomal LA was significantly more effective than non-liposomal formulations in reducing hyperpigmentation of both pigskin and human skin.

Recently, natural substances extracted from plants have attracted attention as source of potential skin-whitening agent due to their biologically active compounds in medicine, and they may also be isolated in high quantities at low cost. Tengamnuay et al. (55) assessed the heartwood extract of *Artocarpus lakoocha* Roxb for the in vitro tyrosinase inhibitory activity and the in vivo melanin-reducing efficacy in human volunteers. They concluded that *A. lakoocha* has a promising potential for use as an effective and economical skin-whitening agent. Wang et al. (56) examined 25 traditional Chinese herbal medicines on human epidermal melanocytes

to identify the effects of skin-whitening and skin health. They found four herbal preparations to be potent tyrosinase and melanin synthesis inhibitors. However, this finding needs to be validated in vivo on human skin.

Taylor et al. (57) mentioned several combinations of whitening formulations in comparison with monotherapies such as hydroquinone and retinoids to treat the PIH. Findings suggested that 2% mequinol with 0.01% tretinoin solution is a promising alternative for the treatment of PIH.

Chemical peels are also effective and safe in treatment of pigmentation disorders. Grimes (58) investigated the clinical efficacy and safety of a new superficial salicylic acid peel in individuals of skin types V and VI. The patients were pretreated for two weeks with hydroquinone 4% prior to undergoing a series of five salicylic acid chemical peels. The concentrations of salicylic acid were 20% and 30%. The peels were performed at two-week intervals. Results suggested that superficial salicylic acid peels are both safe and efficacious for treatment of melasma and PIH.

Brief data of key skin-whitening agents are summarized in Table 1.

Table 1 Brief Data of Key Skin-Whitening Agents

Whitening agents	Study design	Models	References
Hydroquinone			
2% and 5%	Non-placebo-controlled study	In vivo in humans	(7)
2%	Non-placebo-controlled study	In vivo in humans	(8)
5%, in combination with 0.1% tretinoin and 0.1% dexamethasone	Non-placebo-controlled study	In vivo in humans	(9)
2%, in combination with 0.05% tretinoin and 0.1% betamethasone valerate	Non-placebo-controlled study	In vivo in humans	(10)
Phenols, catechols, and organic antioxidants	Screening	In vivo in black guinea pigs and black mice	(12)
2%, in combination with 0.05–0.1% retinoic acid	Non-placebo-controlled study	In vivo in humans	(13)
3%	Non-placebo-controlled study	In vivo in humans	(14)
In combination of a broad-spectrum sunscreen agent	Double-blind and vehicle-controlled study	In vivo in humans	(15)
Ascorbate-phytohydroquinone	Non-placebo-controlled study	In vivo in humans	(16)
4%, and 5% skin-whitening complex cream	Double-blind, randomized, prospective study	In vivo in humans	(17)
4%, with 0.15% retinol	Open-label study	In vivo in humans	(18)
4%, and 5% ascorbic acid cream	Randomized split-face designed study	In vivo in humans	(19)
4%, in comparsion with a TC cream	Multicenter, open-label, randomized clinical trial	In vivo in humans	(20)
Arbutin	Cell culture	In vitro in human melanocytes	(21)
Kojic acid			
1% and 2%	1) Screening 2) Non-placebo-controlled clinical study	1) In vitro in model 2) In vivo in humans	(36)
2%, in combination with 2% hydroquinone	Non-placebo-controlled study	In vivo in humans	(37)
Ascorbic acid			
10% VC-PMG	1) Cell lines and culture; 2) Non-placebo-controlled clinical study	1) In vitro mammalian tyrosinase and human melanoma cells; 2) In vivo in humans	(41)

Abbreviations: TC, triple combination; VC-PMG, magnesium-L-ascorbyl-2-phosphate.

CONCLUSIONS

The treatment of pigmentation disorders can be a long process. In general, skin-whitening agents are considered modestly effective. However, hydroquinone is still one of the most effective agents for the treatment of hyperpigmentary disorders (59). High concentrations are not recommended, except under a physician's supervision. The application of hydroquinone in combination with certain chemicals (tretinoin, salicylic acid, or corticosteroids) may enhance lightening effects. Recently, chemical peelings with kojic acid, glycolic acid, and trichloroacetic acid, either alone or in combination, have been widely introduced for treatment of hyperpigmentations (57,58,60). However, the real efficacy of whitening agents should be determined in a placebo-controlled study in humans. Non-hydroquinone agents have also been assessed (61), and results have been encouraging. Optimal whitening agents remain a future goal. Recent review of the mechanism and biological, chemical, and clinical aspects of lightening agents provides additional insights (62–64).

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58 Skin Whitening: New Hydroquinone Combination

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DISORDERS OF PIGMENTATION

Pigmentation disorders occur as a result of an increased production of melanin by melanocytes and/or an elevated transfer of melanosomes from melanocytes to basal and suprabasal keratinocytes (1–3). Melanin (eumelanin and pheomelanin) results from the hydroxylation of tyrosine to 3, 4-dihydroxyphenylalanine (DOPA) through the enzymatic action of tyrosinase and the subsequent oxidation of DOPA to dopaquinone (4). Once produced, melanin is transferred to keratinocytes or into the dermis via any of the following processes: (*i*) damage to melanocytes in the basal layer allows for phagocytization by melanophages, releasing melanin into the dermis; (*ii*) melanosomes are directly deposited, through their dendrites, into the dermis; or (3) macrophages migrate into the epidermis where melanosomes are phagocytized, returning them to the dermis.

Melasma

Difficult to treat, melasma, also known as chloasma or "mask of pregnancy," is a relatively common, chronic pigmentary condition typically seen in women of childbearing age. In fact, it is known to appear at any time during a woman's reproductive years and is often associated with pregnancy or oral contraceptive use. Melasma is more common among women of darker skin types. A small percentage of cases, approximately 10%, occur in men, most frequently in those of Middle Eastern, Caribbean, or Asian descent.

Melasma presents as often distinctly demarcated, irregularly shaped light- to dark-brown macules. The blotches usually appear on the upper lip, nose, cheeks, chin, forehead, and, occasionally, the neck. A centrofacial pattern of distribution, involving the cheeks, forehead, upper lip, nose, and chin, is the manifestation most often seen, but there are three identifiable patterns of presentation (2,5). The mandibular pattern and the malar pattern, which affects the nose and cheeks, are less common. Although it most often occurs in skin routinely exposed to the sun, there are reports in the literature of melasma appearing on the nipples and around the external genitalia (6,7).

Etiology

The cause of melasma has not yet been clearly identified, but solar exposure, genetic predisposition, and hormonal influences are considered among the most important factors (6,7). Estrogen and progesterone, nutritional deficiency, and certain antiepilepsy drugs are considered significant causal or aggravating factors in its development (4). Also, Hydantoin and Dilantin have been reported to contribute to melasma in both women and men (7,8).

A history of chronic sun exposure seems to be a necessary precondition for the development of this pigment disorder, and solar exposure is also well known to exacerbate the condition (2,4). Interestingly, in the winter months, when sun exposure is usually less frequent, melasma is typically less noticeable (4). In the absence of other compelling evidence, sun exposure is considered the primary exogenous causal factor in melasma (1,9). Data suggesting a genetic component are sparse, but a few familial cases have been reported (1).

It is not uncommon for women to develop melasma on the upper lip after hot wax application to remove unwanted hair. In fact, this phenomenon is so often reported by patients

[a]Financial conflict statement: Dr. Baumann was an investigator for Stiefel in several clinical trials.

that the author speculates that heat may play a role in melasma development as it does in erythema *ab igne* (a reticulated erythematous hyperpigmented eruption arising after chronic exposure to heat).

It has been observed that women who use oral contraceptives represent the population that most often presents with melasma (6,7). This painless but often stress-inducing condition also frequently affects pregnant women; together, women in these categories comprise the majority of melasma cases. Occasionally, there are menopausal and premenstrual presentations associated with melasma. The low incidence of melasma among postmenopausal women on estrogen replacement therapy suggests that estrogen alone is an unlikely etiological root (4). This is a highly idiopathic condition, varying from patient to patient, within individuals, and even from pregnancy to pregnancy (4). It is also characterized by a high degree of recalcitrance. Melasma may subside in the months following a patient's pregnancy or after discontinuing oral contraceptives, but may still persist, taking up to five years to resolve (7,8). An endocrine etiology has been posited by some (7), but no such mechanism has yet been proved (8). Some ovarian disorders are also correlated with an increased incidence of melasma, but no causal link has been established. The odds of experiencing initial onset of melasma are lower than the likelihood of recurrence once melasma has developed.

OTHER PIGMENTATION DISORDERS

Solar Lentigos

Long-standing data suggest that as many as 90% of elderly patients have one or more solar lentigos (10). Sun exposure, as the name suggests, is responsible for this condition, which is characterized by macular brown lesions usually 1 cm in diameter. Acute or chronic exposure to the sun can induce solar lentigos. The face and backs of hands are the areas most often affected. This condition is resistant to the Kligman formula (11).

Post-Inflammatory Hyperpigmentation

Various skin conditions, such as acne, eczema, and allergic responses, can lead to post-inflammatory hyperpigmentation, also known as post-inflammatory pigment alteration (PIPA), as can more serious cutaneous events, such as burns, surgeries, and trauma. Certain treatments for skin disease or cosmetic conditions can also engender or exacerbate discoloration (e.g., chemical peels and laser resurfacing). PIPA can occur in any skin type, but it most often affects people with darker skin types (12–14). The condition results from an elevation in melanin synthesis in response to a cutaneous attack and can be diffused or localized—its distribution depends on the location of the original insult to the skin.

PIPA presents in areas of previous inflammation as irregular, darkly pigmented splotches (15). Any area of the skin can be involved, but the disorder is especially stressful to patients when it occurs in the face. Not surprisingly then, PIPA is one of the most common conditions prompting patients to visit a dermatologist. PIPA unfortunately tends to recur in susceptible individuals (16).

Treatment

The goals of therapy are to slow the proliferation of melanocytes, inhibit the formation of melanosomes, and promote the degradation of melanosomes (17). Sun-protective behavior is a necessity. As such, patients must use a good high sun protection factor (SPF) sunscreen with UVA protection and make all reasonable efforts to avoid sun exposure. The sunscreen should be worn 24 hours a day. Other practical, behavioral elements of therapy can include UVA screens for car and home windows and protective clothing such as hats.

The Kligman Formula

The "Kligman formula" is a mixture consisting of 0.1% tretinoin, 5.0% hydroquinone, 0.1% dexamethasone, and hydrophilic ointment (11). The daily application of this combination through five to seven weeks resulted in normalized pigmentation of normal adult skin in black males treated for melasma, ephelides, and postinflammatory hyperpigmentation. The removal of one component of the therapeutic regimen resulted in less-efficacious results. The formula,

which has been very popular as a therapeutic option for melasma since its introduction in 1975, is not commercially available now, but can be formulated by a pharmacy. Unfortunately, the stability of these products formulated in a pharmacy is in question. This is because hydroquinone and tretinoin have a tendency to interact and decrease each other's effectiveness. However, the popularity of the Kligman formula led to the status of topical combination therapy as the current mainstay of melasma treatment and resulted in the development of Tri-Luma (discussed later in this chapter) in which tretinoin and hydroquinone have been successfully stabilized.

Other Topical Components

The standard products used to produce hypopigmentation include phenolic and nonphenolic derivatives. Hydroquinone and hydroquinone combination formulations are among the phenolic group; tretinoin and azelaic acid are among the nonphenolic agents (18). Typical topical preparations include hydroquinone 2% to 4%, low-potency steroids, kojic acid, deoxyarbutin, azelaic acid, hydroxy acids, and retinoids. Tretinoin 0.1% has been evaluated as a single agent in the treatment of melasma and favorably reviewed (19,20), but such monotherapy took as long as 10 months, in one study, before the condition improved. A 10-month, randomized, vehicle-controlled clinical study did show that topical 0.1% tretinoin lightened melasma in 28 black patients, with only mild side effects (20). Combination therapy has been considered the mainstay therapy for patients with any skin type, though.

Although most of the remaining discussion here will focus on recent research with a novel combination compound, it is worth noting that other tools in the dermatological armamentarium have achieved favorable results. For example, the addition of glycolic acid to hydroquinone has been shown to promote efficacy by facilitating the penetration of both agents (21). A recently evaluated cream containing 10% buffered glycolic acid, 4% hydroquinone, vitamins C and E, and sunscreen has also been shown to be safe and effective in the treatment of melasma (22). In combination with topical agents, glycolic acid peels and/or Jessner's peels can be used to accelerate the resolution of melasma. Jessner's solution and 70% glycolic acid (combined with tretinoin and hydroquinone between peels) have been shown to work equally well in the treatment of melasma (23).

Kojic acid has also been demonstrated to enhance the efficacy of topical agents. A study in Singapore followed 40 Chinese women treated with 2% kojic acid in a gel containing 10% glycolic acid and 2% hydroquinone on one half of the face and the same application without kojic acid on the other half (24). Patients were observed for 12 weeks, and they showed improvement in melasma on both sides of the face. The side treated with the combination containing kojic acid showed greater improvement, it should be noted. The melasma cleared in 24 of the 40 patients who received kojic acid as compared with 19 of 40 patients treated with the gel without kojic acid.

It is also worth noting that laser therapy has been used with some degree of effectiveness in treating several pigmentary disorders, but has not yet been established as a first-line therapy for melasma (18). However, the Fraxel laser is frequently used in light- and dark-skinned patients after topical melasma regimens have failed or in combination with topical regimens.

A PRESCRIPTION COMBINATION THERAPY: TRI-LUMA

Tri-Luma is a combination of tretinoin 0.05% (retin A), hydroquinone 4.0%, and fluocinolone acetonide 0.01% (a mild steroid). Two 8-week, multicenter, randomized, investigator-blind studies were conducted to compare the efficacy and safety of this triple-combination hydrophilic cream formulation with various dual-combination agents. The same drug concentrations and vehicles were used in all formulations. The dual combinations included tretinoin plus hydroquinone, tretinoin plus fluocinolone acetonide, and hydroquinone plus fluocinolone acetonide.

The theoretical basis for this formulation rests on the prior success of the various first-line components in dual-combination therapies, namely, hydroquinone, tretinoin, and a range of topical corticosteroids. Of particular conceptual importance in the product development are the clinical and experimental data demonstrating the effects of tretinoin and other retinoids in abrogating the epidermal atrophy that can be induced by topical corticosteroids (25,26).

A total of 641 predominantly white female adults (ranging in age from 21 to 75 years) were randomized to the various treatment groups. In both studies, all formulations were applied once daily, at night. Patients enrolled in the study represented Fitzpatrick skin types I through IV and exhibited moderate-to-severe hyperpigmentation.

Results

Significantly more of the patients treated with Tri-Luma (26.1%) demonstrated complete clearing compared with the dual-combination therapy groups (4.6%) at the end of eight weeks (27). Researchers observed complete or near complete clearing of hyperpigmentation in 77% of the aggregate Tri-Luma group compared with 42.2% for fluocinolone acetonide, 27.3% for tretinoin and fluocinolone acetonide, and 46.8% for tretinoin and hydroquinone. Side effects associated with Tri-Luma were transient and mild. The most frequently occurring adverse effects included erythema at the application site, desquamation, burning, xerosis, and pruritus.

Some authors have cautioned against the use of hydroquinone in high concentrations because of its association with inducing ochronosis. Nevertheless, hydroquinone is the most effective topically applied hypopigmenting agent approved by the Food and Drug Administration (FDA) for melasma treatment (18). No ochronosis events were observed among patients on any of the treatment regimens containing hydroquinone 4%.

The use of topical corticosteroids as therapy for melasma has also been discouraged by some authors because of the association with skin atrophy and telangiectasia (28). In fact, protracted use of potent topical corticosteroids is known to engender cutaneous atrophy. When steroids are used in combination with retinoids, however, skin atrophy does not occur. Indeed, the combination of tretinoin application with corticosteroid has been shown to ameliorate the epidermal atrophy induced by the topical corticosteroid while not reducing its activity (26) and is believed to reduce the risk of steroid-induced atrophy (25). The data from this study seem to support this fact because only one patient in the dual-therapy hydroquinone and fluocinolone acetonide group exhibited skin atrophy. Significantly, the one patient that experienced skin atrophy did not receive tretinoin. A 12-week open-label long-term safety study showed a similar safety profile as the previously described eight-week study (27).

The results of the two related studies suggest that the use of this triple-combination agent may be more effective than any of the dual-combination agents in counteracting or inhibiting the pathophysiological mechanism of melasma. Tri-Luma combines three well-established agents in a formulation that appears to be effective and safe in the treatment of melasma. This triple-combination topical therapy also shows favorable tolerability and represents a significant advance in the dermatological armamentarium for melasma.

The concept of such a triple combination is also supported by another recent study. Researchers evaluated the efficacy of a formula containing 0.1% tretinoin, 5% hydroquinone, and 1% hydrocortisone in 25 Korean female patients with melasma recalcitrant to therapy. Patients were evaluated before treatment, then instructed to apply hydrocortisone on their faces for four months and were also assessed four weeks and four months after treatment. Overall, investigators reported statistically significant depigmentation in clinical and histological studies and increased subepidermal collagen synthesis, results that were observed as early as four weeks after hydrocortisone treatment (29).

REGULATORY UPDATE: FDA AND HYDROQUINONE

On August 29, 2006, the FDA withdrew the September 3, 1982 tentative final monograph on over-the-counter (OTC) skin-bleaching products and proposed that no OTC skin-bleaching active ingredients be categorized as generally recognized as safe. Of course, prominent among such products is hydroquinone. A final ruling has not yet been handed down. Should the FDA follow through, thus changing the status of hydroquinone and other skin-bleaching agents, the effect would be to reclassify such products as "new drugs," permitting them to be used by prescription until approval of a new drug application. Until such a ruling, however, hydroquinone products remain available OTC, as this ingredient remains an important first-line therapy for pigmentary disorders. As of the date of publication, a ruling by the FDA does not appear imminent, but no timetable for a decision has been made clear.

ALTERNATIVE WHITENING AGENTS

There are several alternatives to the use of hydroquinone as a hypopigmenting agent, including kojic acid, as described earlier, deoxyarbutin, mulberry extract, Pycnogenol, and vitamin C. Recently, deoxyarbutin [4-([tetrahydro-2H-pyran-2-yl]oxy)phenol] was shown in cultured human skin cells to be less cytotoxic than hydroquinone. It was also demonstrated to exhibit significant tyrosinase-inhibiting activity, and, on xenographs, topical application of deoxyarbutin yielded observable skin lightening over an eight-week period. Further, in a clinical trial, deoxyarbutin promoted the fading of pretanned skin better than hydroquinone or no treatment, with statistically significant differences observed. Investigators concluded that these findings collectively suggest that deoxyarbutin is a potentially effective and safe tyrosinase inhibitor (30).

In a study conducted to investigate the in vitro effects of an 85% methanol extract of dried Morus alba (mulberry) leaves on melanin biosynthesis, mulberroside F (moracin M-6, 3′-di-*O*-β-*D*-glucopyranoside) isolated from the extract was found to inhibit the tyrosinase activity that converts dopa to dopachrome in the biosynthetic process of melanin. Although its activity was weaker than that of kojic acid, researchers concluded that this mulberry extract may be viable as a whitening agent (31).

A 30-day clinical trial of 30 women with melasma in which patients were given one 25-mg tablet of Pycnogenol at each meal, three times daily, was inspired by the observed efficacy of Pycnogenol in protecting against UV radiation. Investigators found that the average surface area of melasma exhibited by the subjects was significantly reduced, demonstrating that Pycnogenol could be effectively and safely used in treatment of this condition (32).

In another study in melasma patients, 16 women were instructed to apply 5% ascorbic acid (vitamin C) cream on one side of the face and 4% hydroquinone cream on the other side nightly for 16 weeks. Patients also applied sunscreen daily throughout the trial. Although the hydroquinone side was associated with better subjective improvement (93% good and excellent results vs. 62.5% for ascorbic acid), colorimetric measures indicated no statistical differences. Further, side effects were more commonly associated with hydroquinone (68.7% vs. 6.2%). Investigators concluded that while a better response was seen with hydroquinone, ascorbic acid demonstrated efficacy in treating melasma while inducing far fewer side effects, justifying its use alone or in combination therapy (33).

CONCLUSION

Many types of skin are susceptible to pigmentation disorders. Such conditions can appear especially prominent in people with dark skin. Traditionally, disorders of pigmentation have been refractory to treatment, frustrating patient and physician alike. Combination therapy, including prolonged use of topical agents, sun avoidance, and, often, in-office chemical peels, has been the mainstay. Laser treatments, with the exception of the Fraxel laser, have been of limited success. A new topical combination therapy, Tri-Luma, has shown great promise, though, in simplifying and improving treatment for these intractable disorders, particularly melasma. In addition, this new combination therapy is effective, tolerable, and easy to use.

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59 Anticellulite Products and Treatments

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INTRODUCTION

Cellulite is a localized condition of subcutaneous fat and connective tissues with the typical visual appearance of the orange peel look of the skin. Cellulite, or more correctly, gynoid lipodystrophy (GLD) affects mostly women and rarely men and is considered as a common aesthetic problem for many women. Cellulite appears generally after puberty and worsens with age. There are preferential places of cellulite: buttocks, thighs, upper part of the arms, knees, and more rarely, the lower parts of the legs and the back of the neck (Fig. 1). It is interesting to note that these preferential cellulite sites are areas in which the typical pattern of adipose deposition is observed (1). Although celllullite may be found in areas with excess adipose tissue, obesity is not necessary correlated with the presence of cellulite (1).

The aims of this chapter are to describe

1. the histological, physiological, and biochemical characteristics of subcutaneous lipodystrophy;
2. the different objective evaluation methods of lipodystrophy; and
3. the different anticellulite treatments available and their efficacy.

There have been only a few review articles on cellulite published since 2000 (1–5).

CLINICAL, VISUAL, AND TACTILE SYMPTOMS OF THE SKIN WITH CELLULITE

There are some typical symptoms, partly subjective (reported by the patients) and partly objective (observed by the investigators), which are very often reported in the case of cellulite (1).

- There is presence of the typical orange peel skin upon normal visual examination and after pinching of the skin.
- Deep palpation of the skin reveals differences in the mobility of fat tissue: presence of micro- and macronodules and fibrosclerosis. Sometimes there is presence of painful subcutaneous nodules through deep palpation.
- There are irregularities in skin surface temperature as observed by thermography. Touching the skin by hand reveals the presence of cold spots in an advanced stage.
- Clinical examination reveals venous stasis and edema.

DESCRIPTION OF THE DIFFERENT STAGES OF LIPODYSTROPHY OF FAT TISSUES

There are different stages in the progression of cellulite. It is difficult to detect cellulite by visual examination and by palpation at the first stages: orange peel skin is not permanently present, only visible after pinching the skin. The clinical symptoms are clearly more visible at later stages of cellulite: permanent orange peel, colder skin areas, diminution in mobility of fat tissue upon palpation, and increased skin sensibility.

Skin surface contact thermographic pictures using thermographic foils give an indication of the degree of cellulite, as the skin surface temperature correlates to some extent with the clinical symptoms of cellulite. On the basis of these thermographic patterns and clinical symptoms, Curri and coworkers proposed a classification of cellulite in four stages (6–11), a classification that has been confirmed by others (12–14). In normal adipose tissues, a fine mesh

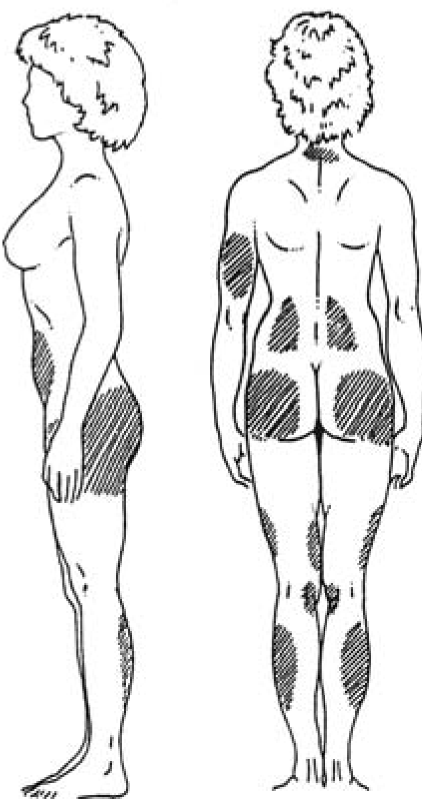

Figure 1 Preferential localizations of subcutaneous lipodystrophy in women.

of blood vessels and lymph vessels supplies this adipose tissue with the necessary nutrients and oxygen and takes care of the removal of the metabolized products. In the early stage of cellulite (stage 1), the capillary blood vessels walls become more permeable, causing leakage of blood plasma from the vessels in between the adipose tissues, which cause an edema in the adipose tissues. In addition probably, problems with the lymph circulation hampers removal of accumulating fluids. The aggregation of adipose cells and the amplification of the fibrillar network of collagen bundles interconnecting the adipose cells hamper blood circulation, leading to some hemostase (stage 2).

Adipose cells aggregates into "micronodules" surrounded by a less-mobile collagen fibers (stage 3). The size of these micronodules is of the order of millimeters. Finally, many of these micronodules aggregate into "macronodules" with larger sizes (2–20 mm) (stage 4). As nerves may be squeezed by these larger nodules, persons with severe cellulite often suffer from a sensitive to painful skin.

Stages 1, 2, and 3 of lipodystrophy are not considered clinically as pathological symptoms but more as aesthetic-cosmetic problems of the skin. Only in stage 4, some clinical symptoms such as an increased skin sensitivity, extensive fibrosclerosis of connective tissue, and very advanced edema are considered as light pathology symptoms. Furthermore, it is believed that the first stages are more or less reversible, whereas the latters stages are almost irreversible and consequently very difficult to treat.

ETIOLOGY OF CELLULITE

Cellulite is probably a multicausal condition, and many hypotheses have been proposed regarding the origin of fat lipodystrophy (1–5).

There is sexual differentiation in the histological distribution of subcutaneous fat lobules in women and in men. The differences between the sexes can be found in the structure of the

septal connective fat tissue. In women, one observes a higher percentage of septa perpendicular to the skin surface and a smaller percentage parallel to the surface as shown in men. Furthermore, in women with cellulite, deep indentations of adipose tissue into the skin were recorded. Using in vivo high frequency ultrasound imaging, Querleux et al. (15), Lucassen et al. (16), and Nuijs and Van Herk (17) confirmed an irregular dermo-hypodermal interface in women with cellulite. Mirrashed et al. (18) and Querleux (19) confirmed by magnetic resonance imaging (MRI) the existence of indentations of adipose tissue into the dermis. Also observed in women with cellulite was an increase in the thickness of the inner fat layer, a higher percentage of septa in the direction perpendicular to the skin surface. Since cellulite is widely present in women, some authors consider cellulite as a secondary sexual characteristic. Although cellulite is not always synonymous with overweight, there is clearly a relation between cellulite and hypertrophy of fat tissues.

Vascular Modifications

These consist in alterations in the microvascular network (mostly venous blood circulation) in the fat tissue leading to a venous stasis (1,2,5). The superficial microcirculation appears to be less efficient, and this results in subcutaneous edema because of the altered permeability of blood vessels and the presence of plasmatic exsudate in the subcutaneous connective tissue. This edema is probably a noninflammatory symptom. However, other authors have suggested an inflammatory basis for its pathophysiology (2). Furthermore, alterations in the reticular fibrillar network surrounding the blood vessels and adipocytes are observed. This fibrosclerosis provokes stiffening and decrease in mobility of fibers. Also, alterations in the interstitial fundamental substance (proteoglycans) are reported. GLD is probably associated with chronic venous problems. Venous insufficiency shows typical symptoms such as possible presence of telangiectasias, heaviness in the legs, cramps in the lower limbs, pain on deep palpation of the skin, and irregularities in skin surface temperature as detected by thermographic examination.

Alterations in the Matricial-Interstitial Unit Surrounding the Fat Cells (1)

The matricial-interstitial unit is formed by fibroblasts (synthesis of macromolecules of the cellular matrix), by the collagen, elastin, and reticular fiber, and by the ground substance (proteoglycans, glycoproteins). Alterations in the structure of the GAG (glycosyl amino glycan) in the perivascular tissues provoke hyperpolymerization and an increase in their hydrophilicity and the interstitial osmotic pressure: edema and hypoxia. Ryan and Curri (9,10) suggested the hypothesis of an increase in water content of subcutaneous adipose tissue in case of cellulite. An increase in the concentration of glycosyl amino glycans, presumably leading to a rise in the amount of water retained in the skin, was suggested. Querleux et al. (15) did not confirm the hypothesis of increased water content in the adipose tissue of women with cellulite except if such water would be located in the connective septa. Modifications in the structure of the proteins of the cellular matrix are observed: alterations of the fibers are followed by sclerosis.

Predisposing Factors

A genetic prediposition factor plays an important role in the development of cellulite (1). More controversial is the observation that white Caucasians tend to have more cellulite than Asians. Also, it appears that Latin women develop more GLD on the hips and thighs than Nordic women (the sale of anticellulite creams and treatments are very much in favor in the Mediterranean countries). A nonbalanced diet with excessive intake of fats and carbohydrates provokes the hypertrophy of fat tissues. A sedentary lifestyle contributes to the aggravation of cellulite, and wearing tight clothes makes venous return more difficult. Smoking provokes alterations in the microcirculation and could favor the formation of cellulite. Other coexisting disorders (hormonal, circulatory, metabolic, gynecologic, nepheotic, and gastrointestinal) may be important and contribute to the development of cellulite.

Modifications and Hypertrophy of Adipose Tissues

Although cellulite is not always synonymous with overweight (some lean persons could present symptoms of cellulite), there is a relation between cellulite and hypertrophy of fat

tissues. First, there is formation of first micronodules and later of macronodules in adipose tissues.

The combined effect of modifications and hypertrophy of adipose issues, alterations in the fibrillar connective tissue, and alterations in the microvascular venous network leads always to the presence of cellulite.

New developments in the aethiopathogenesis of cellulite have recently been described (5). It appears that three main theories on the aethiopathogenesis of cellulite have emerged. These theories indicate the following problems in the edematofibrosclerotic panniculitis (EFP):

1. A different anatomical conformation of subcutaneous tissue in women compared with men
2. A microcirculatory modification
3. An edema resulting from excessive hydropholia in the intercellular matrix

New developments have clearly emphasized the limitations of the three above-described theories. It appears that adipose tissue does not play a significant role in the onset of cellulite: it participates only as a pure inert physical function, producing mechanical tension through its hypertrophy. Today we know that the adipose organ performs complex functions by acting as a system controlling the systemic energy balance, by modulating the food intake and the metabolism of other tissues, and as a glandular system for multiple hormonal secretions. It is known that adipose tissue is able to modulate the blood flowing through it and can secrete numerous substances with the power to regulate the activity of the endothelial cells. In conclusion, these recently identified properties of the adipose tissue are also involved in the pathogenesis of cellulite (5).

OBJECTIVE EVALUATION OF THE SYMPTOMS OF GYNOID LIPODYSTROPHY

There is a variety of physical and pharmacological anticellulite treatments ranging from topical products to oral food supplements or regimens, from manual to mechanical massage, laser, infrared light, continuous or pulsed radio frequencies, etc.

As a consequence of this, there is a need for accurate, sensitive, noninvasive bioengineering methods for the quantitative evaluation of the degree of cellulite (particularly at early stages) and for the objective evaluation of the efficacy of various cosmetic treatments (2–5,14). However, the clinical evaluation of cellulite based either on direct visual examination and palpation of the orange peel skin with a diminution of the mobility of the hypodermis or photograding of photographic pictures taken under well-standardized conditions remain important. The visual evaluation is more closely related to the consumer's considerations and expectations.

The different noninvasive bioengineering measurements are as follows:

- Contact skin surface thermographic measurements using liquid crystals.
- Noncontact skin surface thermography of skin surface using infrared video camera.
- Micro blood circulation using laser Doppler image analysis.
- Ultrasonic skin analysis of skin density. Measurement of thickness of the hypodermis at 10 to 14 MHz and measurement of the surface of the interface between dermis and hypodermis at 20 MHz.
- MRI.
- Skin surface topographical imaging and fringe projection analysis.
- Macroscopic normal and digitalized photographic pictures of the skin surface.

In many studies, there is a confusion between obesity and cellulite (although adipous volume is clearly an aggravating factor for cellulite). Many patients confuse weight gain with the appearance of cellulite, and many commercial anticellulite treatments are in fact slimming treatments. As a consequence of this confusion, the use of antropometric measurements is widely applied to measure the efficacy of the various anti–weight treatments: circumference measurements of hip, both thighs, and individual.

Skin Surface Contact Thermography Using Encapsulated Liquid Crystals in the Evaluation of Cellulite

The principle of the encapsulated cholesteric liquid crystal contact thermography consists of different color plates presenting a pattern of different colors corresponding to about 3°C temperature. Application of the color sheet with uniform pressure on the skin surface and photographic recording of the thermographic pattern using a photographic camera can be made. A qualitative global analysis of the thermographic pictures in relation with the different stages of cellulite can be made (14,20–24). A cellulite-free skin surface thermography shows a uniform color pattern without hypothermic and hyperthermic areas. A cellulite skin surface thermography shows a nonuniform color pattern with the presence of hypothermic (cold spots) and hyperthermic (warm spots) areas. Quantitative analysis of the thermographic pictures can also be carried out by image analysis. Computerized color image analysis gives the mean temperature of the thermogram and respectively the number and the percentage area of the hypo- and hyperthermic areas present on a well-defined skin area. As experimentally observed, an anticellulite treatment will induce an increase in the mean temperature of the skin surface and a decrease in the percentage hypothermic zones (with a concomitant increase in the percentage hyperthermic zones).

This method is rapid, easy to use, and non-expensive for screening subjects for cellulite and for confirmation of the clinical diagnosis.

However, considering the low accuracy and reproducibility of the photographic pictures, quantitative image analysis of the thermograms is very difficult. One observes large interindividual variations in skin surface temperature (large number of subjects is necessary in a study) and long acclimatization time for temperature equilibrium of the skin (influence of external temperature). This method remains a qualitative testing of cellulite at different stages.

Skin Surface Thermography Using Infrared Thermal Imaging System in the Evaluation of Cellulite

Using an infrared video camera, an infrared thermal image of the skin surface is obtained in a noninvasive manner. The thermographic picture can be quantitatively analyzed (14,22,23).

In the validation of this infrared video imaging technique, the problems encountered with liquid crystals are same as those with the contact thermography, such as large interindividual variations in skin surface temperature, long acclimatization time for temperature equilibrium of the skin, and influence of external temperature.

Laser Doppler Imaging System in the Evaluation of Cellulite

Using a laser Doppler perfusion imager, an image of the superficial blood circulation can be obtained. The He-Ne laser light emitting at 633 nm has a penetration power in the skin of only about 300 μm (14,22,23).

This instrument measures the superficial blood flux of the skin (papillary dermis). The blood perfusion of the deeper layers of the skin such as the hypodermis cannot be measured with this technique. However, a high correlation is obtained between the skin surface thermographic pictures and the laser Doppler imaging system when studying skin with cellulite. However, the measurements are delicate (long measuring times during which the volunteer must remain immobile).

Ultrasonic Imaging of the Skin in the Evaluation of Cellulite

High-frequency ultrasound C-mode imaging (10–20 MHz) appears to be a promising method. This noninvasive method has been frequently used both clinically and in research for studying the epidermis, dermis, and hypodermis (24–35). Different authors have used the technique of the measurement of the thickness of the subcutaneous fatty layer using ultrasound imaging at 10 to 14 MHz (27–35); however, the determination of the echographic border line between subcutaneous fat and connective tissues/muscles is very delicate. As a consequence, the determination of the mean thickness of the hypodermis is not very accurate. The interface between the dermis and the subcutaneous fat can be measured using ultrasound imaging at 20 MHz (16,17). The interface between the echogenic epidermis-dermis and the hypoechoic subcutaneous fat is clearly visible, allowing measurements of skin thickness and the surface of this border.

In normal cellulite-free skin, the interface between the dermis and the fat tissue is irregular but rather smooth. In skin with cellulite, this surface is not smooth and very irregular. The surface of this interface is quantified and can be used as a measure of the degree of cellulite. Quantification of the surface of the interface between the dermis and the hypodermis (fat tissue) is possible and can be considered as a measure of the extent of cellulite (16).

Measurement of Skin Surface Topography

Cellulite skin surface presents irregularities (orange peel skin). In principle, the classical skin surface roughness measurements, which are used in cosmetic research, could be applied for studying cellulite. These involve stylus profilometry, image analysis by shadow method and optical focus laser profilometry, topographical skin imaging techniques, and fringe projections analysis (36–42). Stylus profilometry measurements are carried out on soft or hard skin replicas of general small size (2–3 cm^2 area) and have a limited vertical range of roughness capability (maximum 400–500 μm). These techniques are well suited for the determination of the microrelief of the skin surface (50–200 μm), but not for assessing the skin surface with cellulite. Optical focus laser profilometry and fringe projections analysis can be carried directly on the skin surface. The macrorelief of the skin surface can also be evaluated using an optical triangular laser profilometry. This method involves measurements on large size soft replicas with an extended vertical range of skin irregularities (up to 8–10 mm). Quantification of the skin surface macrorelief involves a computerized correction for the curvature of the skin surface with cellulite. Actually the skin surface topography of skin with cellulite could be more easily evaluated using 3-D topographical skin imaging techniques and 3-D map topography from fringe projections.

Digital Photographic Pictures of the Skin Surface

The macrorelief of the skin can be evaluated by taking digital photographic pictures under standardized experimental conditions (with tangential lighting) (1,4). These photographic pictures are then graded visually using numerical scales in double-blind manner by expert independent reviewers for the intensity of cellulite and the efficacy of various anticellulite treatments (43–45). Macroscopic digitalized video pictures (with the use of a CCD camera) of the external part of the thighs were taken after application of a gripping system around the thigh to increase the orange peel look of the skin.

In Vivo Magnetic Resonance Imaging and Spectroscopy

Recently, high-resolution MRI and localized spectroscopy data were published (15,18,19), allowing investigation of subcutaneous adipose tissue in men and in women with and without cellulite. As previously mentioned in this chapter, MRI is very efficient for measuring the thickness, surface, and volume of the adipose tissue. In women with cellulite, an increase in skin thickness and presence of deep indentations of adipose tissue into the skin were noticed. Unfortunately, because of the high cost and limited accessibility of this instrument, this promising technique will not be available for cellulite research for most laboratories and cosmetic companies.

TREATMENTS OF CELLULITE

There are numerous therapies that have been advertised and employed to treat cellulite (1,2,4). Despite the multitude of therapeutic approaches, there is little scientific evidence that any of these treatments really work. A majority of the evidence is anecdotal, subjective, or based upon patient self-evaluation. Only a few anticellulite treatments are validated using noninvasive bioengineering measurements to quantify the degree of cellulite.

The Physiotherapeutic Treatments

Physiotherapeutic treatments such as deep massage and manual and pneumatic lymph drainage stimulate the blood and lymph microcirculation and increase the removal of the extra fluid in the adipose tissues. In addition, these massage techniques will retard the further development of fibrosclerosis and the aggregation of fat cells in nodules. These physiotherapeutic treatments are generally combined with the topical use of anticellulite dermato-cosmetic products (during

massage or pre- or post-massage). Electrolipolysis and mesotherapy are invasive medical treatments of cellulite; these techniques will not be described in this chapter.

Recently, two new physiotherapeutic techniques have been developed in the treatment of cellulite (46,47). One technique combines the use of near-infrared laser light of a continuous radiofrequency wave and mechanical suction (2 rollers with suction), and another instrument combines near-infrared light with a mechanical massage apparatus. These combined physiotherapeutic instruments seem to improve the symptoms of cellulite.

The Topical Dermato-Cosmetic Products

The use of various topical products, generally applied with massage, in the treatment of cellulite and/or as slimming is known for many years (1,2,4). The most used pharmacological topic agents are xanthines, retinoids, and plant extracts. A novel approach to topical anticellulite treatments consists of combining the topical application of the pharmacological product under occlusion as enhancement (bioceramic-coated neoprene short) (48).

Xanthines are common ingredients used in anticellulite products (caffeine, aminophylline, theophylline, or plant extracts rich in xanthines) (1–4,45). Xanthines are used because of their proposed effect on adipocyte lipolysis via the inhibition of phosphoesterase, provoking an increase in AMP. In vitro metabolism studies on fat cells have shown that caffeine could slow down the lipogenesis (uptake of glucose and free fatty acids to synthesize triglycerides) and stimulate the lipolysis (degradation of triglycerides and release of the free fatty acids) in different ways. Furthermore, it appears that caffeine increases the level of a class of uncoupling proteins (UCP) in subcutaneous white adipous tissue adipocytes and may help to reduce the adipose tissue mass.

Retinoids

The use of topical retinol to improve cellulite was proposed by Kligman et al. (49,50), and they demonstrated an improvement in cellulite. Retinol will be metabolized to retinoic acid. These effects may be due to the known effects of retinoids in the dermis modifying the collagen fibers and the network of elastic fibers (1–5). It can be also noted that retinol has an "anti-adipocyte" activity by reducing the differentiation of adipocyte precursor cells in adipocytes (4).

Plant Extracts

The use of plant extracts such as *Centella asiatica*, butcher's broom, horse chesnut, ivy, Ginkgo biloba, Witch hazel, white oak, green tea, lemon, kola, fennel, alguae, barley, strawberry, marjoram, sweet clover, aloe vera, etc. should be noted. The "active" molecules of these plant extracts are probably flavonoids (rutins, rutinosides) or terpenes (ginkgolides).

These slimming/anticellulite plant extracts present properties of stimulation of the peripheral blood circulation and lymph circulation and inhibit further the fibrosclerosis of the fat surrounding collagen matrix. Various algae species such as fucus vesiculosus, laminaria flexicaulis, and ascophyllum nodosum are incorporated in anticellulite cosmetic preparations for their hypothetical beneficial effect on the skin surface. There are very few in vivo scientifically reported studies examining the effects of these plant extracts improving the condition of cellulite (4). The use of anticellulite creams containing various plant extracts seems to be acceptably safe (51); however, the risk for adverse allergic reaction must be taken into account.

ORAL TREATMENTS

Many of the above-mentioned active ingredients are also used in oral anticellulite or slimming treatments of cellulite, and similar to the topical treatments, there are very few scientifically proven clinical studies reported (2–4). These preparations contain mostly various plant extracts and xanthines (caffeine or plant extracts rich in caffeine). These anticellulite food supplements can be used alone or in combination with massage and/or topical creams. The use of plant extracts such as green tea, grape, Ginkgo biloba, and centella asiatica are particularly noticed. It is possible that both oral and topical routes may have a synergic effect and may be the best way to ameliorate the symptoms of cellulite (4).

CONCLUSIONS

Very few anticellulite studies that were performed under well-controlled experimental conditions (double-blind, vehicle-controlled, etc.) and under medical and paramedical supervision are published in the scientific literature (1–5,14).

After a critical overview of these published clinical studies, one can make the following remarks. In the case of simple trials (such as one with treated thigh and other with thigh as control), improvements are always observed. One question remains: Are the improvements the result of the combined action of massage and the active ingredients or solely the result of the massage?

In the case of more elaborated trials (double-blind and placebo-controlled), the results are variable. Some clinical studies clearly show significant improvements in the degree of cellulite found on the treated thigh compared with the placebo thigh. These improvements are significant and clearly visible (confirmed by the subjects themselves), but not very impressive. Other clinical trials indicate that similar significant improvements of cellulite were observed with the inert massage product and the massage product with the active ingredients. These trials substantiate the hypothesis that the cellulite improvements are due to physiotherapeutic treatments such as massage, lymph drainage, or thermal occlusion of the skin and not solely to the so-called active anticellulite dermato-cosmetic ingredients. The majority of cosmetic firms carry out internally clinical trials to evaluate the efficacy of their products. But the results of these clinical studies are generally not published in scientific peer-reviewed journals and are only accessible through the Internet, released press maps, and ordinary publicity in women's magazines. This information, although interesting to consult, is not considered by the author as real scientific publications. Most of the claims presented here are based on anthropometric measurements and in vitro data.

It must be noted that many anticellulite or slimming claims are based on in vitro studies. In vitro metabolism studies on adipocytes have shown that different molecules (caffeine, plant extracts, etc.) can be considered as active ingredients in order to slow down the synthesis of triglycerides and to stimulate the degradation of triglycerides in the adipocyte. However, when using these active molecules in vivo as anticellulite ingredients, one must take into account the limitations in percutaneous penetration of the active molecules through the skin to reach the hypodermis. For example, caffeine penetrates readily into the skin, but scant information is published about the penetration of these plant extracts.

Another problem is related to the concentration of the active products in commercial anticellulite products. It must be assumed that the concentrations of plant extracts are rather low considering the high cost of these extracts and the potential danger of these plant derivatives as allergens. Possibility of problems of photoallergy and photoirritation must be considered.

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60 Baby Care Products

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INTRODUCTION

A great variety of baby skin and hair care products are brought to the market. Those products claim to be specially developed for the delicate baby skin, and therefore specific requirements should be considered when developing products for the care of babies. In order to understand whether babies need different skin and hair care cosmetics than adults, it seems necessary to explain some anatomical and physiological differences of the skin and annexes between both groups. A further distinction can be made between the skin of full-term and premature babies, but in this chapter only the former group is considered. Cleansing and protective cosmetics are available for babies and their safety is in the EU based on exposure-based risk assessment for the individual ingredients as well as for the finished product. Finally, some common baby skin problems and the applicability of cosmetic products under impaired skin conditions are discussed.

ANATOMICAL DIFFERENCES BETWEEN BABY AND ADULT SKIN AND SKIN ANNEXES

A complete overview of the morphological characteristics of baby skin is present in several comprehensive handbooks (1–4). Here some key differences in comparison to adult skin and annexes are given.

In general, a full-term baby possesses all skin structures of adult skin, and anatomically these structures do not undergo dramatic changes after birth. The skin of the newborn could be considered as an "unripe" skin, which progressively adapts during the first weeks and months of life. These adaptations lay at the origin of the physiological differences observed between baby and adult skin.

- The *epidermis* of term infants is well developed and does not show much difference with that of adults (5). Only in prematures, the stratum corneum is lacking a significant barrier function (1). In the stratum germinativum, besides a majority of cylindric keratinocytes, dendritic cells including melanocytes, cells of Langerhans, and Merkel cells are present in a normal number. However, their functionality—namely photoprotection, immunological barrier, and receptor function, respectively—still has to develop progressively. The melanocytes are less pigmented, which explains the pale color of the newborn's skin and which makes sunburn an important risk factor (3).
- At the *basal membrane* of newborn skin, cohesion structures are present in a normal number in comparison with adult skin (1,5).
- In the *dermis*, numerous fibroblasts produce elastic and collagen fibers but fewer in number than in adult life. Most of the development and maturation of the elastic fibers occur after birth, and it is only at the age of three years that the elastic fibers become completely mature. It is known that the dermal matrix differs in composition depending on age. Indeed, during development, the water, glycogen, and hyaluronic acid contents of the matrix decrease, but its dermatan sulfate content increases. This difference in composition probably interferes with the particular turgescence of the newborn's skin (6).

In the embryonic dermal skin, primitive dermal vessels can be seen that differentiate into arterioles, venules, and capillaries. With respect to the vascular system, the subpapillar plexus is not yet completely developed, and the upper layer of the dermis contains a rich but disorganized capillary network, causing the erythematous aspect of the newborn. The cutaneous nerve system is also not yet finalized and may lie at the basis of masked intolerances (3,6).

- The immature *hypodermis* of baby skin consists of small lobules of roundly shaped adipoblasts that are richly vascularized. The fatty acid composition of the triglycerides is more saturated, which results in a higher fusion point of the lipids than measured for adult skin (1,5).
- The *hairs* of a newborn are well developed. Sometimes some lanugo hairs are still observed. After birth, the hairs pass from the anagenic into the telogenic phase. As a consequence, baby's hairs fall out after about eight weeks. Afterward, the hair cycle becomes similar to the one observed for adults, and hairs will be present in different phases. The hairs, however, are very thin and only faintly pigmented, but these phenomena normalize as a function of time (7).
- *Sebaceous glands*, when stimulated by the androgens originating from the mother, are well developed. Their secretions constitute the largest part of the vernix caseosa. That is why at birth, the skin is covered with a white fatty substance. The vernix caseosa is a naturally occurring fetal barrier film produced in late pregnancy (8). Besides the secretion of sebaceous and epidermal lipids, desquamation of maturing fetal corneocytes also takes part in the development of the fetal barrier. The vernix is thought to have multiple overlapping biological functions like moisturization, anti-infective, antioxidant, wound healing, and waterproofing (9,10). Because it lacks desmosomal interconnections between corneocytes, it is also referred to as the "mobile phase" stratum corneum. Removal of vernix lipids can modify the water sorption-desorption profile (11,12).

  The vernix caseosa is taken away during the first washing of the baby. After loss of this protective layer and the onset of a desquamative stratum corneum, the skin is exposed to a much dryer environment than the one present during fetal development (13). Erythema occurs that changes in appearance and gets a more marbered aspect that progressively disappears. This is an adaptation of the microvascular system (6).

  Because of the different biological effects of the vernix caseosa, the question is often raised whether it would not be better to leave this natural film on the baby instead of washing it away. Several publications investigating the effect of immediate bathing of newborns, however, are contradictory (14–17). Vernix distribution is dependent on gestational age, delivery mode, gender, race, and meconium exposure and positively affects skin hydration, skin pH, and erythema. These multiple effects would support its retention on the skin surface after birth (8). Vernix films also retain endogenous chymotrypsin, thus preventing loss of this epidermal enzyme and protecting the epidermal barrier from noxious substances (18). In this respect, the World Health Organization (WHO) developed general guidelines recommending that neonatal bathing should not be undertaken within the first six hours of birth (19).

  In certain cases, large sebaceous glands are observed together with the occurrence of the typical symptoms of so-called *acne neonatorum*. This particularly happens in male newborns and can persist for a few months. It is seen as a temporary effect of the androgens that are present in the mother's blood. Reactivation of the sebaceous glands only occurs later on, around puberty (17).
- The *hydrolipidic layer*, mainly composed of sebum from the sebaceous glands and water originating from eccrine glands and the transepidermal water loss (TEWL), is not fully developed in babies. This protective water-in-oil (w/o) mixture is sometimes even nearly absent, which also has an effect on the skin pH of the newborn (20). Consequently, the observed skin pH imbalance might be responsible for a lower capability to neutralize the alkalinization, especially seen in the diaper area due to urine and defecation.

- The *eccrine sweat glands* are normally formed but their intervention is still immature, affecting thermoregulation. *Apocrine glands* only become functional around puberty (6,21).

PHYSIOLOGICAL DIFFERENCES BETWEEN BABY AND ADULT SKIN

On the basis of TEWL and dermal absorption studies, term infants seem to possess a fully developed stratum corneum with adult barrier properties. Other parameters, such as skin thickness, skin pH, and stratum corneum hydration, show that neonatal skin is adjusting very well to the extra uterine environment. This is rather in contrast to a more steady state situation of adult skin (22).

Dermal Absorption

Dermal absorption in newborn skin is similar to that observed in adult skin. For babies, however, a number of typical risk factors exist (23–25).

1. The *surface area/body weight ratio* is 2.3-fold higher in newborns than in adults decreasing to 1.8-fold and 1.6-fold at 6 and 12 months, respectively (26). Application of the same amount of product on a similar body surface of baby versus adult could result in higher blood and tissue concentrations in the newborn. This ratio is taken up in the intraspecies factor of 10, used in exposure-based risk assessment.
2. *Pharmacokinetic parameters* differ widely between babies and adults and result in reduced clearance and/or a longer half-life of bioavailable substances, thus increasing the potential risk for adverse reactions in babies (Table 1). Premature and full-term neonates tend to show a three- to nine-times longer half-life than adults. However, once the neonatal period is over, often a greater elimination and higher clearance are observed compared with adults bringing back the normal equilibrium (26,27). As referred by Renwick et al. (28), this neonatal period would coincide with the period of lactation (26–30).
3. *In-use conditions of topical products* also play a role. Cosmetic skin care products often are applied onto large body surfaces, e.g., cleansing lotions, sunscreens, etc., increasing not only the risk for local effects but also dermal absorption and potential systemic toxicity. This factor is considered in exposure-based risk assessment.
4. The *diaper area* and nondiapered regions are indistinguishable at birth but show differential behavior over the first 14 days, with the diapered region having a higher pH and increased hydration (31). Special circumstances arise because of the close confining clothes and diapers and the uncontrolled urination and defecation. The close-fitting diaper provides a warm nutritive environment for the proliferation of bacteria (32). Because of the interaction between the urine and the feces, urease becomes activated and converts urea into ammonia, giving rise to alkaline skin pH

Table 1 Potential Differences in Pharmacokinetic Parameters Between Newborns and Adults

Parameters	Newborn	Adult
Plasma binding	+	++
Plasma protein concentration	+	++
Body water	++	+
Fat distribution	+	++
Brain development	+	++
Brain-blood barrier	+	++
Brain volume	++	+
Cyt P_{450} biotransformation	+	++
Conjugation reactions	+	++
Relative liver mass	++	+
Glomerular filtration	+	++
Tubular secretion	+	++

Source: Modified from Refs. 26–30.

levels. As a consequence, fecal enzymes such as lipases and proteases become activated and damage the fragile skin in the diaper area. Despite modern diaper technology, irritant diaper dermatitis can not completely be avoided, favoring dermal absorption of xenobiotics. A number of molecules, which historically have been used in the diaper area, are known to induce systemic toxicity and must be used very carefully and only when indicated, e.g., hexachlorophene, dichlorophene, corticosteroids, boric acid, and ethanol (24). In risk assessment of cosmetics, the margin of safety (MoS) approach is used when defining acceptable human exposure levels. When extrapolating from experimental studies to human, the magnitude of the uncertainty factor must take into account a variety of considerations, such as species differences, sensitive subpopulations, duration and route of exposure, and vehicle or matrix effects. In addition, when the diaper area is irritated, 100% dermal absorption should be used (19,33). Innovative hygiene absorbent and baby care products, however, provide an increasingly good skin compatibility profile, making the frequency and severity of diaper dermatitis declining (34,35).

Transepidermal Water Loss

The barrier function of the skin not only prevents absorption of toxic substances, but also controls TEWL. In particular when skin is damaged, excessive TEWL occurs (36–38). In a healthy, fully developed newborn, TEWL values of 6 to 8 $g/m^2 \cdot h$ water are being measured, depending on the measuring technology (39). TEWL increases proportionally with immaturity, which means that premature children have an increased evaporative heat loss and subsequently a poor temperature control (38,40). Although skin maturation occurs rapidly, fluid and electrolyte shift as well as body temperature have to be controlled frequently (41). Also increased risk of local and systemic toxicity from topically applied substances rises with increasing TEWL or barrier damage (42). In the diaper area TEWL is often defined as skin surface water loss (SSWL) and is used to measure the capability of a diaper to keep the skin dry (43,44).

Defense Against Infection: Skin Thickness, Skin pH, Stratum Corneum Hydration

The water content of the stratum corneum influences the barrier function, dermal absorption, reactivity to irritants, and the skin's mechanical properties. Although healthy infants and adults tend to have similar TEWL values, newborns (until 8–24 months) still present somewhat higher water contents in the horny layer and a greater variation than adults up to one year (38,39,45).

In newborns, skin tends to have a higher pH at birth than a few days later. Among other factors, this higher pH might reflect the influence of the vernix caseosa and the amniotic fluid (both pH values above 7) during the first days of life (38). The pH stabilizes at a slightly acidic range (pH = 5–6), although values of less than 5 also have been reported (31,38,46). Acidic skin protects against pathogenic microorganisms to which the baby is exposed after birth and serves in the defense against infections. Indeed, microbial colonization of the skin starts immediately after birth by so-called saprophytes that are not pathogenic and are credited with protective properties against some harmful microorganisms (5). They require an acidic surrounding for optimal living conditions (39). Whereas the pH value of baby skin is, after a few days, comparable to the pH value of adult skin, the buffering capacity of baby skin is much lower. Therefore, baby skin is more susceptible to pH changes induced by metabolic pathways such as the enzymatic generation of free fatty acids from phospholipids or urocanic acid from histidine, the desquamation process of the stratum corneum with formation of filaggrin and keratohyalin breakdown products, and the formation of pyrrolidone carboxylic acid and N^+/H^+ antiporter (31,47).

BABY CARE PRODUCTS FOR SKIN AND HAIR

From the anatomical and physiological differences between baby skin and adult skin, it appears that frequent contact with xenobiotics, which could damage or disrupt the barrier function of the stratum corneum and change the skin pH, may be at the basis of an increased

dermal absorption, an increased TEWL and the onset of infections (5,39,42). Therefore, exposure-based risk assessment for baby products is key to bringing safe baby cosmetics to the market.

During the development of baby products a number of criteria are taken into consideration:

-High quality of raw materials in terms of purity, stability, and microbiology via appropriate certificates of analysis.

-Skin irritation, which is dose dependent, can be controlled by avoiding well-known irritative ingredients and/or reducing concentration or frequency of application.

-Skin sensitization, triggered by an immunological response, is not restricted to the area of application. Therefore, exposure-based risk assessment is needed to exclude an induction of sensitization in particular for perfume ingredients, even when IFRA-tested or excluding the 26 allergens identified in the 7th Amendment of the EU cosmetic legislation (48).

-As is the rule for adult cosmetics, safety data of baby cosmetics are taken up in a technical information file (TIF) and the risk assessment—approved by a safety assessor —is the driving force behind the safety of baby cosmetics. Usually, special attention is given to the concentration of (*i*) reactive colorants; (*ii*) promotional additives, "natural" and "exotic" ingredients, in particular not well-identified mixtures, plant extracts, and ingredients of animal origin or a questionable, impure source; (*iii*) potential allergens, penetration enhancers, organic solvents (ethanol, isopropanol, highly reactive substances, highly detersive or foaming agents, and antiseptics in particular in daily use products); and (*iv*) concentrations of preservatives.

-It is considered to be good practice (*i*) to add antioxidants to protect unsaturated lipids from oxidative reactions; (*ii*) to adjust the pH of the final product resulting in a skin friendly pH value between 4.5 and 6 after product application; (*iii*) to add chelating or sequestering agents, when appropriate, to prevent heavy metal precipitation and protect the preservative system; and (*iv*) to use skin barrier protective ingredients.

Baby cosmetics can be subdivided in two groups, namely cleansing and protecting cosmetics.

Cleansing Cosmetics

Bath Products

Bathing a baby for five to seven minutes in lukewarm water (35–36°C) usually is sufficient (39,49). Daily bathing is general practice, but not optimal because of the risk of drying out and irritating baby's skin, in particular when aggressive anionics with high degreasing properties are involved (50). Better is to use so-called secondary tensides, including nonionics and amphoterics, or mild anionics such as sulfosuccinates, isothionates, and protein fatty acids condensates. The use of bath oil is preferred over bath foam and bath cream additives particularly when dry, sensitive skin or atopic eczema is present. For optimal effect, the baby is bathed for 5 minutes in plain water, then the bath oil is added and bathing continues for another 5 to 10 minutes (49). Also adding starch to the bath water or using starch-containing bath additives may help to restore an impaired skin barrier (51). In general, the use of bath foam is not suitable for babies because of its high content of primary tensides producing the excessive foam.

Shampoo

Baby shampoo usually has a pH of 6 to 7 and ideally should contain only mild tensides, e.g., mixtures of nonionics and amphoteric substances (7). The shampoo should not be irritating to the eyes. To avoid eye contact the viscosity of the shampoo could be increased. Parents often think that foam is important for the cleansing properties but foam has no cleansing function, and the ingredients required to produce a sufficient amount of stable foam are often quite irritating and not suitable to be used alone in baby shampoos, e.g., alkyl sulfates and alkyl ether sulfates (39,49). Furthermore, it is not necessary to wash baby's hairs every day

since they are neither dirty nor greasy. As the hydrolipidic layer is not yet formed on baby's skin, and sebum production is low, the amount of lipids distributed on the hairs is limited and is easily washed away.

Soap Bars and Syndets

Soaps (salts of fatty acids) liberate, in contact with water, alkali and increase the pH up to values of 10. Also precipitation occurs with calcium and magnesium ions from hard water. On the contrary, syndets (*syn*thetic *det*ergents) do not precipitate with hard water and have an adjustable pH to neutral or slightly acidic. As syndets cover the whole range of synthetic tensides—with exception of the legally protected soap formulations—they can be aggressive (e.g., alkyl sulfates) or mild (isothionates), depending on the choices and mixtures made. Like soaps, they can dry out the skin when not containing lubricant additives. In addition, when soap and washcloth are used in the diaper area, the buffering capacity of the skin is further damaged (35,49). Extensive washing with aggressive tensides disturbs the flora of the newborn skin and can lead to infections (15). In addition, perfumed cleansing products may cause contact allergic reactions due to enhanced skin penetration of the perfume by the presence of anionic soap ingredients (50).

Cleansing Milk

For cleansing of the baby and in particular the diaper zone, liquid cleansers based on oil-in-water (o/w) emulsions are often used, especially when water and washcloth are not well tolerated by the baby skin. Also soft tissues or towelets impregnated with these emulsions are present on the market. They are easy to use and contain anionic and/or nonionic tensides (49). When a baby is prone to contact dermatitis, it is advised to screen the ingredients list because those tissues often contain high concentrations of preservatives, necessary to prevent microbiological contamination of the tissues (52). Mineral-oil impregnated tissues can increase the presence of *Candida* in the diaper area and change the composition of the skin surface lipids.

Baby Wipes

Over the last decade disposable baby wipes have been developed as an alternative to traditional cleansing methods. They usually consist of a nonwoven carrier material soaked with an emulsion-type, watery, or oily lotion. Mineral oil wipes do not efficiently clean hydrophilic components and potentially slip over fecal contaminations. Most emulsion-type lotions are oil-in-water (o/w) and enriched with emollients and surfactants. Because of their high water content, the preservative system is very important to ensure that the product will not be contaminated during its normal lifetime. Products for sensitive skin have also been developed, which offer choice regarding the nonuse of fragrances or additives with which problems have previously occurred. Clinical studies confirmed that high-quality baby wipes are suitable for daily cleansing of the diaper area, of healthy babies as well as of babies with atopic dermatitis (35,53).

Protecting Cosmetics

Face/Body Creams and Body Lotions

Protective creams for the napkin zone are preventive or protect the skin against aggressions from urine, feces, and their interactions. Oil-in-water (o/w) creams do exist, but in case of starting skin damage mostly water-in-oil (w/o) creams or water-free ointments with talc, kaolin, and zinc oxide are advised. Allantoin, bisabolol, aloe vera extract, and silicones are often added to improve water resistance. In winter, barrier creams protect baby's face against freezing cold and wind. The lipid phase often contains petrolatum. These products are particularly effective around the nose and mouth. They usually also contain moisturizers, soothing active ingredients, and nonionic emulsifiers (39).

Powder

Talc powders are not often applied anymore in the napkin area. They absorb moisture, decrease maceration, and prevent irritation of the fragile baby skin (49). Powders,

however, pose a potential inhalation risk and can form on the skin little granules that induce friction. Furthermore, talc is susceptible to contamination with microorganisms and needs sterilization (32,54).

Sunscreens

During the past years, a steady increase of all types of skin cancer has been observed. It is, therefore, very important to inform parents and children about good sun protection. Sun exposure in childhood is seen as a risk factor for skin cancer later in life, as it is known that there is a relationship between skin burning in the prepubertal period and the occurrence of malignant melanoma 10 to 20 years later (55). Therefore, babies and infants should, in first instance, be kept out of the sun and protected by appropriate clothing and hats. Almost 90% of the clothes provide an equivalent protection to sunscreens of SPF 30 or higher, although the protection offered is dependent on weave, color, weight, stretch, and wetness (56,57). Even special protective clothes for children exist today. They have undergone special treatment to filter out UV light (58,59).

It has been reported that sunscreens often are ineffective in preventing sunburns completely, because parents tend to forget to reapply or limit the use to just the upper part of the body. The importance of using multiple sun protection methods to maximize effective sun protection clearly has to be promoted (60). Extreme care should be taken especially during the first weeks and months of life since pigmentation and thermoregulation are not yet fully developed (3). When sunscreens are used, preferably products containing UVA and UVB screens and scattering powders or a sunscreen mix with a high sun protection factor (SPF) should be applied and preferably several times a day (61).

Studies have shown that the application thickness of sunscreen products in adults usually lies between 0.5 and 1.5 mg/cm^2, although the SPF of a product is assessed in vivo at an internationally agreed application thickness of 2 mg/cm^2 (62). Application thickness has a significant effect on the expected protection of the sunscreen. A uniform layer needs to be applied, with special attention to areas like ears, neck, and feet as experience learns that these are commonly skipped (60,63).

The type of UV filter(s) used is important. In the EU, only UV filters taken up in Annex VII of the Cosmetics Directive 76/768/EEC are allowed. For babies and children often micronized and nano forms of ZnO and TiO_2 are used as an alternative to chemical sunscreens (64,65). The popularity of these products results from the fact that they are effective and thought to be safe. As nanotechnology in general is questioned with respect to human health, nanoparticles of ZnO and TiO_2 will also be reexamined for their safe use as UV filters (66).

Recently, the European Commission expressed its concern with regard to the efficacy of sunscreen products and the claims made thereto. Aiming at ensuring sufficient protection against UV light and providing proper information to the general public, Commission Recommendation 2006/647/EC unambiguously states minimum efficacy criteria for sun products: the UVB SPF must at least amount to 6 and the UVA protection factor may not be inferior to one-third of the SPF. In addition, the Commission restricts the UVB SPF values to 8 possibilities, namely 6, 10, 15, 20, 25, 30, 50, and 50+. Higher SPFs are not allowed and mentions such as “100% protection,” “sunblock,” or “all day prevention” are considered misleading and therefore forbidden (67).

The viscosity of sunscreens is important since the products must remain on the skin, even with bathing and sweating. Today most commercially available sunscreens have some water resistance or carry the label of being water resistant, very water resistant, or waterproof, determined in Europe according to Colipa guidelines (68).

Some of the organic UVB filters, like 4-methyl-benzylidene camphor, benzophenone-3, homosalate, octylmethoxy-cinnamate, and octyldimethyl-PABA, have been accused of being endocrine disrupters. The safety profiles of these UV screens were revised by the European Scientific Committee on Consumer Products (69) and considered to be safe for human use. In the meantime, several articles appeared, pointing to potential endocrine disrupting properties of sunscreens (70–76). The issue is not limited to sunscreens alone and appropriate in vivo tests are now being included in the risk assessment process. Efforts are also done to develop in vitro alternatives (77).

APPROPRIATE CARE OF FREQUENT SKIN PROBLEMS

Diaper Rash

Diaper rash is a common condition that refers to irritation on the groin, thighs, buttocks, and perianal area of the newborn. It is caused by the combination of incontinence and diaper use (31). Excessive wetness makes the skin more fragile, and diapers may induce an occlusive effect that makes baby skin more vulnerable. Consequently, hydrated skin is more prone to mechanical damage and chafing of the skin since an increased coefficient of friction is observed, and it may allow irritants to penetrate the stratum corneum more easily. As explained before, not only occlusion but also a higher pH can be an underlying factor, which induces several enzymes-mediated irritations. Alkalinization of the skin increases skin penetration of microorganism and activates fecal enzymes (34,78).

The most appropriate strategy for diaper rash is prophylaxis, and this includes keeping the skin dry. The selection of suitable diapers and frequent diaper changes are important because friction between skin and diaper is often an additional factor. Keeping the baby in an appropriately warmed room with naked buttocks for some limited time period is also quite effective.

In good skin care of the diaper zone, the application of emollients plays an important role, and the application of a thick layer creates an effective protective barrier. ZnO is an often-used component in diaper rash protection products. It adheres well to the injured skin, has adstringent and some mild anti-inflammatory properties, and prevents skin injury or further damage. If the diaper rash shows evidence of *Candida* infection—often seen as satellite lesions extending the rash—antifungal therapy can be indicated (5,39,78,79).

Acne Neonatorum

Mild acne may occur in newborn infants. It consists of closed comedones on the nose, forehead, and cheeks. Pustules, open comedones, and inflammatory pustules may also occur but are less frequent. The cause of neonatal acne is not clearly defined but it is believed to be secondary to the stimulation of the neonatal sebaceous glands by maternal androgens. In boys it is often more pronounced as they have some additional production of testosterone. Neonatal acne requires no treatment as the lesions spontaneously resolve within one to three months (80).

Miliaria

Miliaria is a dermatose frequently observed in neonates. It is a generic term denoting retention of eccrine sweat. Miliaria can be subdivided into three groups: *miliaria crystalline*, *miliaria rubra*, and *miliaria profunda*. The difference between these three types is the level of the skin where the obstruction of the sweat gland occurs. *Miliaria crystalline* refers to an obstruction in the stratum corneum, *rubra* an obstruction within the stratum Malpighi, and *profunda* below the dermoepidermal junction. There is no specific treatment of miliaria. Measures that can be taken consist of regulating the heat and humidity of the environment to reduce sweating. Eventually, the poral obstructions are relieved, but this can take up two or three weeks (81).

CONCLUSION

Full-term newborns have an "unripe" skin, which progressively develops toward adult skin. Anatomically spoken, the differences are limited but some minor changes occurring at birth are responsible for the physiological differences observed between baby and adult skin. The skin of newborns exhibits the same dermal absorption as adult skin, but the circumstances in newborns are very different, thus increasing the risk for dermal absorption, in particular in the napkin area. Also the thermoregulation (TEWL and sweating) of the baby is not yet fully developed, and the skin is easily invaded by infections, the latter often due to subtle pH changes and the immaturity of the defense systems of the skin. This implies that only safe cosmetics with safe ingredients should be used for newborns guaranteed by exposure-based risk assessment. During the development of new baby care products, known potentially eye or skin irritative or sensitizing ingredients are usually limited to a minimum, and profound exposure-based risk assessment has become common practice. One usually aims at simple,

pure, mild, and pathogens-free formulations. Systemic side effects are not to be expected with mild rinse-off products (shampoos, bath additives, toilet bars) but should be carefully looked for when leave-on products for babies are being developed (body milks, hydrating creams, ointments, powders, sunscreens).

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61 Cosmetics for the Elderly

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INTRODUCTION

Skin is the only organ, where signs of aging are evidently visible at the soonest in phenomena such as wrinkle formation, loss of elasticity, uneven pigmentation, loss of moisture, increased roughening, and cutaneous itching. Aging itself is understood as the result of a complex interaction of biological processes that are caused by both genetic (chronological or intrinsic aging) and environmental or behavioral processes (premature or extrinsic aging).

Current expansion of knowledge in modern bio-gerontology widely extends the theories and explanations on mechanisms of aging. They are the basis for scientific approaches in research, aiming to identify new concepts for antiaging treatment of skin. In terms of scientific research activities, the skin's accessibility to noninvasive and slightly invasive biophysical measurements and procedures is definitely advantageous for studying underlying mechanisms of cutaneous aging. In addition, experiments can also successfully be performed on cultured skin cells or on three-dimensional cultured skin models.

Today, consumers of cosmetic products are increasingly expecting a deceleration, cessation, or even a reversal of the underlying physiological processes contributing to the signs of cutaneous aging. These advancing demands require state-of-the-art technological endeavors in cosmetic research and formula development activities, with novel active ingredients, which can perfectly exert their antiaging efficacy in optimized new-formula technologies.

PHYSIOLOGICAL CHANGES IN CUTANEOUS AGING

Unlike internal organs, the skin being the outermost protective barrier is particularly exposed to external influences. As a result of environmental challenges, the aging process in skin is not only influenced by genetic, intrinsic factors, but also accelerated to a far greater extent (80%) by extrinsic factors, especially sun exposure (1). Intrinsic mechanisms of skin aging seem to be only basically involved in formation of fine lines and shallow wrinkles in advanced age. Therefore the research in cosmetic industry is focussed on the identification and qualification of new active principles mainly to fight against extrinsic factors found to be harmful for the skin. One of the most important fields of research is thereby the prevention and repair of sun-induced skin damages, which can occur through several intracellular as well as extracellular mechanisms.

Reactive Oxygen Species

It is widely accepted that UVB irradiation causes DNA damage more or less directly, whereas UVA light induces damages via the generation of reactive oxygen species (ROS) in a more indirect manner (2,3). During sun exposure, endogenous absorbers of UV light and photosensitizers in human skin (riboflavine, porphyrine, tryptophan, urocanic acid, etc.) can be involved in the generation of ROS. These UV-induced ROS are believed to be the main factors for age-related damages in skin, apparent as deep wrinkles and furrows, which are mechanistically summarized by the term "photo-aging."

Dermal Changes

With increasing age and because of UV irradiation, aging skin shows an increasing imbalance between assembly and breakdown of collagen—one of the primary compounds of the dermis—toward breakdown. This results in an overall collagen decline of approximately 1% per year per unit area of skin surface (4). The reasons for this are lower levels of new synthesized collagen, a shift in the ratio of collagen types (5), and an increased activity of collagen-degrading enzymes such as the collagenase MMP-1 (6). Aside from this, UV light also influences other important

dermal components such as elastin and glycosaminoglycans (GAGs). It causes the accumulation of elastotic material, a nonfunctional mass of elastic fibers (7) and loss of GAGs in the dermis. This lack of regeneration of the dermal connective tissue, i.e., structural reorganization of collagen and elastin, decreased GAGs, together with a reduction in the tissue fluid content and water-binding capacity, seems to play an important role in the formation of wrinkles. Even in young skin, the regeneration of collagen (over the period of months) is a relatively slow process. Thus the tensile characteristics of the skin are altered, so that it becomes generally thinner, less elastic, and less resistant to stress. Phenotypically, this extrinsically caused and accelerated premature skin aging is manifested in an advanced state as the formation of coarse, deep furrows and folds, as well as aggravated elastoses. Even though the mechanisms of both extrinsic and intrinsic skin aging cause a fundamental change in the appearance of the skin, the contribution of the extrinsic portion, however, seems to be predominant.

Epidermal Changes

A characteristic feature of aging skin is the declining ability to regenerate, being particularly evident in the longer time span needed for renewal of the epidermal layer. This so-called epidermal turnover takes about 28 days in young adult skin and may increase to 40 to 60 days with age (8). Furthermore, as skin gets older the UV-induced tanning intensity becomes more irregular, but the scientific knowledge about the physiology on development of age spots or melasma is still insufficient. The appearance of age spots can be a result of the decreasing ability of melanocytes to distribute the produced melanin packets (melanosomes) to the surrounding keratinocytes equally, or a localized overproduction of melanin. One can only speculate whether this is primarily caused by an uneven distribution of melanocytes in skin or rather a dysregulation of physiological processes in melanocytes.

Other Aspects

Furthermore the process of premature skin aging leads to an impairment of the denticulation of the epidermal/dermal junction zone and to a reduction in the number of so-called papillae, each of which harbors a blood capillary growing out of the dermis. These structural changes are considered as histological hallmarks of aging skin morphology, which is accompanied by the reduction of the capillary diameter, as well as capillary density in aged skin (9). A well-functioning blood capillary system contributes to an adequate nutrient supply to the upper skin layers, and thus to the structural integrity and complexion of skin.

The immunological defense system is also significantly reduced with increasing age. Thus intensive sun exposure can promote neoplastic cell transformation (e.g., melanoma) and the incidence of skin tumors increase with age. As a consequence protection of skin of all ages against the negative effects of sun irradiation is the most important task in keeping skin healthy and young. Often described and subjectively felt, dry aged skin cannot be only attributed to the distinctive defect of the epidermal water barrier of the horny layer. Rather, it can be attributed to regenerative processes, as well as a worsening in the water storage capacity, caused by a diminished production of cutaneous moisturization factors (e.g., amino acids, hyaluronic acid, pyrrolidon carbon acid, and glycerine) able to bind water in the horny layer. Besides the reduced water-retention capacity, the age-dependent reduction in sebum secretion of the sebaceous glands also plays a role in the formation of dry aged skin. As the sebaceous glands seem to be predominantly hormonally regulated, age-dependent decline and changes in the hormonal system worsen condition and function of aged skin.

An overt example of the endocrine influence on skin aging is the exacerbation of dry skin and increased wrinkle formation that occur with menopausal hormonal changes, and specialized hormone treatments can lead to an improvement of old skin. These new scientific insights have facilitated a scientific merging in the fields of dermatology and endocrinology.

ACTIVE COSMETIC INGREDIENTS AND THEIR POTENTIAL

Aging consumers experiencing dry skin tend to favor rich skin-care formulations that include *moisturizers* with high water-binding properties, e.g., glycerine. Increasingly, modern cosmetics attempt to satisfy these consumer demands for products with preventative or even regenerative performance. Besides preventing early skin aging, products must also smooth

or improve the appearance of wrinkles as well as retard the weakened regenerative potential of the skin (epidermal turnover). Modern skin research in cosmetic industry has already revealed several ways to specifically target the biological needs of aged skin.

In addition, to avoid intensive sun exposure, protection of the skin against UV-dependent oxidative stress can be provided by the use of products with highly efficient *UV filter* technologies. It has to be considered that older skin is even more sensitive to UV exposure. Because of the age-dependent atrophy of the skin (10) UV radiation penetrates more deeply and damages increase and can accumulate. This effect leads to a need for high photoprotection, so product formulations should employ an efficient UVA/B filter combination. Besides UVB protection, which delivers erythemal protection, UVA filter performance plays a decisive role in the prevention of photoaging, because UVA radiation is a key factor in the production of ROS and the subsequent activation of collagen degrading enzymes.

As physiological events in skin are based on physical and chemical processes including redox-cascades, the skin has developed an *antioxidative defense* system as a direct protective barrier against endogenous and exogenous environmental oxidative stress factors (e.g., UV-light). These endogenous antioxidative protectants of enzymatic and nonenzymatic antioxidant systems (11) are concentrated to a higher value in the epidermis compared with that in the dermis. Substances such as flavonoids, vitamins A, C, and E, coenzyme Q10, as well as carotinoids, are components of a healthy diet and can replenish and support the cutaneous system in its protective function. Topical application of substances such as vitamins E and C as well as, in particular, the plant-derived flavonoid derivative, α-glycosylrutin (AGR), show a large protective potential against premature UV light–induced skin aging (12). This positive activity, however, preempts that topically applied antioxidants will adequately interact with the natural endogenous redox system of the skin. Therefore, not all of the known oxidative substances achieve the desired protective effect when applied to the skin. The water-soluble and thus bio-available flavonoid AGR can build up a skin-protective depot in the living layer of the skin, in which the inherent glutathione redox system protects against oxidative damage and UV-induced inflammation is reduced (12–14). Similarly, the water-soluble antioxidant vitamin C can, among other activities, function as a co-factor in the collagen synthesis thereby supporting skin regeneration in deeper layers. Knowing the causative involvement of UV-induced oxidative stress reactions in the cutaneous aging process (15), the best prevention and radical modulation can be reached, and to some extent improved, by providing focused, customized topical treatment strategies.

For treatment of age-damaged skin, particularly for antiscaleness and antiwrinkle efficacy, countless principles are available on the market, which are based on the removal of the outer horny layers of skin (*exfoliation* or peelings). Commonly used agents are so-called α-hydroxy acids (AHAs), most often endogenous metabolites (lactate) or other naturally occurring substances such as fruit acids. Depending on the substance used, the respective depth of treatment in skin can be determined by adjusting the topical concentration and treatment time applied. The activity of these agents is generally based on induction of skin regeneration by exfoliation and subclinical inflammation, which appears to be comparative to a superficial wound-healing process.

Several *antiaging actives* are proven to have beneficial effects on skin aging. Vitamin A and its derivatives have been used as active ingredients in the cosmetic industry for many years. Their activity is essentially based on the interaction of specific nuclear receptors, whose activation regulates, e.g., collagen synthesis, improving the structure of the skin. Regrettably, besides concentration-dependent skin irritant properties, these vitamin A ingredients are also highly sensitive to light-dependent and oxidative processes, greatly reducing their activities. New cyclodextrine-based formulation technologies enable the efficient stabilization of these active ingredients without limiting their activity (16).

All cells, and thus also skin cells, need energy. It is needed to grow, for protection and repair and most important for regeneration and cell division. To maintain this capacity for cellular life the mitochondria, small intracellular organelles operating as small power plants in the cells, are imperative. Besides the mitochondrial energy supply, cells also have a system named "the creatine/phospho-creatine pathway." According to latest insights findings, this occurs in the human skin and is responsible for an extremely fast energy supply (17).

Creatine as well as another energy metabolite, co-enzyme Q10, can both be synthesized in human cells, but from the age of about 30, a reduction in the cellular concentration of these

compounds in the skin can be determined (18). As a fat-soluble oxidative substance, co-enzyme Q10 protects the cell membrane and organelles (19). It especially plays a role in the electron transport system during the energy production (ATP) in cellular respiration of the mitochondria, preventing a chronic energy deficiency in aging cells (20). The topical application of co-enzyme Q10 and creatine, respectively, can activate countless synthetic processes, ultimately resulting in a reduction of wrinkle depth by balancing energy deficits (21). Besides this, the regeneration activity of aged skin can be stimulated by the external application of these active ingredients.

There are different causes, why skin can become irritated. Independent of age, skin is more sensitive to irritation in cold, dry winter, than in summer. Especially people with so-called sensitive skin have to protect their skin against dryness, intensive sun exposure, mechanical stress, and environmental noxiousness. But also, age-related old skin is reported to be more sensitive to irritation due to restricted defense and repair mechanisms. As a consequence, a prophylactic anti-inflammatory treatment appears to be recommendable for skin of elderly.

Special skin-care regimens adapted to the specific needs of sensitive skin are developed and provided by the cosmetic industry. Actives isolated from herbal extracts (e.g., licochalcone A) proved to be effective against mechanical stress such as razor burn, but can also be effective in skin care for dry atopic skin or Rosacea.

PERSPECTIVES

The physiology of skin aging is a complex, multifaceted, and dynamic phenomenon. Even though many molecular causes of the aging process are not understood in complete detail, there is consensus that chronological age alone is not crucial to this process.

In the future, the application of modern molecular and biological methods in skin research such as the DNA chip technology (micro-arrays) and the proteomic technology will allow new insight in the aging process—genes and gene products involved with their genetic control mechanisms. These new technologies, the accessibility of the skin, and the improved possibilities to culture in vivo resembling skin models, will increasingly contribute to a better understanding of the regulation of the intrinsic and extrinsic aging process, and the positive effects of topically applied age-specific agents. In the long term, these technologies can provide new fundamental knowledge about the control mechanisms of the human aging process as a whole.

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62 Antiperspirants

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INTRODUCTION

This chapter presents an overview concerning the current knowledge of antiperspirant actives and their interactions with the human axilla. It is my intention to give the interested reader a short introduction about formulation work, drug delivery systems, and application forms developed for antiperspirant actives. The final section lists references that should be useful for anyone who wants to learn more about a specific topic of antiperspirant technology.

BIOLOGY OF SWEAT GLANDS IN THE HUMAN AXILLA

The axilla region of humans contains apocrine, eccrine, and sebaceous glands. Approximately 25,000 sweat glands/axilla can produce up to 12 g sweat/hr (1). The current understanding concerning the structure and function of sweat glands is that thermoregulation is the only aspect of the body participating in immunological, metabolic, and hormonal aspects of human life (2).

Eccrine Glands

This is the gland responsible for the majority of sweat production. It has a sensory and an excretory function and can be stimulated by emotional and thermal stimuli (3). It produces clear, colorless, and odorless liquid containing 98% to 99% water and 1% to 2% inorganic and organic compounds (4). Inorganic components include NaCl and traces of K^+, Ca^{2+}, Mg^{2+}, Fe^{3+}, and Cu^{2+} ions. Organic components include lactic acid, citric acid, formic acid, propionic acid, butyric acid, urea, and ammonia. Underarm wetness comes mostly from the secretion of eccrine glands. Antiperspirants reduce the amount of sweat only from eccrine glands.

Apocrine Glands

Apocrine glands are apparently a relic from the phylogenetic development of man. These glands start to produce a milky, viscous fluid during puberty on special locations of the body, especially the underarm pit (5). In contrast to eccrine glands, the openings of the glands are not at the skin surface but appear at the hair follicle. Decomposition of apocrine sweat by skin bacteria is responsible for the characteristic malodor of human sweat. Apocrine sweat consists of, besides water, proteins, carbohydrates, and ammonium salts (6). Other investigators have reported that these glands secrete lipids, cholesterol, and steroids (7). Furthermore, it has been shown that androgen-converting enzymes in the apocrine glands are responsible for circulating androgens to dihydrotestosterone (5).

ANTIPERSPIRANTS

Antiperspirants are topically applied products designed to reduce underarm wetness by limiting eccrine sweat production. In the United States, these products are regulated by the Food and Drug administration (FDA) as over-the-counter (OTC) drugs, because they are intended to affect a ''function of the body'' (i.e., in this context, perspiration). Products containing antiperspirant actives have to reduce perspiration to minimum 20% in 50% of the test population under validated test conditions. Test protocols (in vivo clinical trials), to develop a safe and an effective product, have been designed to substantiate the desired claims (8–14).

Comparative quantitative determination of the activity of sweat glands on the forearm after application of aluminum chlorohydrate (ACH) solutions is now possible by combining

the classic starch iodine visualization technique with digital image analysis (15). A noninvasive optical technique that allows the analysis of the function of a number of glands, simultaneously, in vivo was recently reported (16). A new method for parallel testing of up to eight formulations on the backs of volunteers allows a very fast evaluation of product prototypes (1).

Sweat Reduction by Antiperspirants: Current Model/Theory

The reader should be aware that theories concerning the action of sweat-reducing agents depend strongly on the type of actives (aluminum salts, nonionic agents, or ionic agents). The efficacy of antiperspirants based on aluminum and/or aluminum zirconium salts can be understood by the formation of an occlusive plug of metal hydroxide in the eccrine duct (17). Tape-stripping experiments followed by analysis of transmission electron micrographs of an ACH-treated eccrine sweat gland duct show an obstructive amorphous material supporting the theory of a mechanical blockage of sweat glands from diffusion of the soluble ACH solution into the sweat gland and subsequent neutralization to a polymeric aluminum hydroxide gel (18,19). There seems to be no correlation concerning the efficacy of aluminum salts and the location of the plug in the duct, because it is known that, compared with ACH, the more effective Al-Zr compounds do not penetrate as deep as the, also highly effective, $AlCl_3$ solutions (17). The reader is referred to the literature concerning other theories of sweat reduction by aluminum salts (20).

Active Ingredients for Controlling Underarm Wetness—State of the Art

Buffered Aluminum Salts (ACH)

The first antiperspirant, Ever Dry, based on $AlCl_3$, was introduced to the market in 1903 (21). The first cream-containing aluminum sulfate was introduced during the 1930s. The acidic pH value (2.5–3.0) was a drawback of these products, leading to skin irritation in the underarm pit. History tells us that the development of antiperspirant actives with a higher pH value, so-called buffered aluminum chlorides (ACH, pH = 4.0–4.2), was an appropriate step with the additional benefit of reduced destruction of fabric clothes. The formula of this buffering salt is $[Al_2(OH)_5]^+ + (Cl^-)$, or more conveniently $Al_2(OH)_5Cl$.

The historical development from $AlCl_3$ to $Al_2(OH)_5Cl$ can be easily understood by the following consideration:

$$AlClj = {}^{x}h\ Al_2Cl_6\ (\text{substitute } 5\,Cl^-\ \text{ions against OH ions}) => Al_2(OH)_5Cl$$

$Al_2(OH)_5Cl$ is a 5/6 basic aluminum trichloride. The accepted definition of ACH is the ratio of Al to Cl = 2.1 to 1.0. Lower levels lead to ACH $[Al_2(OH)_4Cl_2]$ or to aluminum sesquichlorohydrate $[Al_2(OH)_{4.5}Cl_{1.5}]$—both actives are also generally regarded as safe (GRAS). ACH is supplied as a powder or a 50% solution in water. It can be formulated up to 25%, calculated on an anhydrous basis. The 20% aqueous solution reduces perspiration by 35% to 40% on average (22). Some dyes used in clothing may be acid sensitive and will change color when in contact with an antiperspirant.

The structure of the Lewis acid ACH is very complex, because ACH in water forms the so-called isopolyoxo cations with chloride ions as counterions (23–25). There exist several polymer equilibria of the polycationic aluminum species in water-based systems. Short-chain polycationic species are more effective in reducing sweat.

Aluminum Zirconium Chlorohydrate—Glycine Complexes (AZG or ZAG)

AZG is obtained by reaction of ACH with zirconylchloride. Reaction of the former ingredient in the presence of glycine leads to ZAG complexes. Glycine is used as a buffering agent. These antiperspirant actives form very complex polymeric structures in water. The actives are defined by the ratio of Al + Zr metal-to-chloride ratio and the Al-Zr atomic ratio. The interested reader is referred to the literature concerning available antiperspirant actives (26,27) and nomenclature of the Al-Zr complexes (21,22). These antiperspirant actives were developed especially for anhydrous formulations because they show, compared with ACH, enhanced sweat reduction (28–30). The maximal concentration of ZAG calculated on an anhydrous basis is 20%. They are not allowed to be formulated for use in aerosols.

New Concepts for Controlling Underarm Wetness

Titanium Metal Chelates

The understanding of the complex solution chemistry of aluminum-based antiperspirants gave input to the search for alternative antiperspirant salts. Titanium derivatives, like partially neutralized ammonium titanium lactate (ATL) salts, were shown to be effective in in vitro efficacy tests (31). The titanium metal chelates can be synthesized from the corresponding titanium alkoxides and organic acids allowed by neutralization with ammonia. Under acidic to neutral pH conditions, the ATL active seems to be relatively stable to hydrolysis, and therefore probably is a suitable antiperspirant active in water-based or anhydrous drug delivery systems.

Film-Forming Antiperspirant Polymers

The so-called polybarrier technology is another approach to reduce perspiration by using a polymer that forms an insoluble occlusive film barrier on the underarm skin (32). It was mentioned that the occlusive film is a barrier to the passage of moisture. The main advantages of this technology are reduced skin irritation, applicable after underarm shaving, and higher sweat reduction compared with today's classic antiperspirant salts. The preferred polymer is an olefinic acid amide/olefinic acid or ester copolymer–like octylacrylamide/acrylate copolymer (Versacryl-40). This copolymer can be used alone or in combination with PVP/eicosene copolymer in sticks, roll-ons, or alcohol-based products (33). The reduction of sweat depends on the choice of vehicle and extends in some formulations to 40%.

Lyotropic Liquid Crystals

Certain surfactant/cosurfactant combinations form in water depending on the variables of concentration/temperature instead of micelles' lamellar, hexagonal, inverted hexagonal, inverted micellar, or even cubic phases. The cubic phases can be of micellar or bicontinuous type (34). The water domains in lamellar or cubic phases can swell to a certain degree, while taking up water. The use of this swelling behavior is the basis of a patent where a surfactant/cosurfactant combination is applied to the underarm pit (35). Sweat (water) transfers the applied composition to a lyotropic liquid crystal of cubic structure, thus creating a sweat-absorbing system in the axilla. Oleic acid/glycerol monolaurate is one of the surfactant combinations in the patent. Both components are also well known as deodorizers.

DRUG DELIVERY SYSTEMS AND APPLICATION FORMS FOR ANTIPERSPIRANT ACTIVES

Antiperspirant actives can be formulated in a variety of delivery systems like anhydrous suspensions, water-or hydroalcoholic-based solutions, and emulsions. Typical application forms for antiperspirants are sticks, roll-ons, creams, pump sprays, aerosols, gels, and powders. On a global basis, the three most important product forms are sticks, roll-ons, and aerosols.

Formulation Work

After the decision for the desired application form has been made, the formulator has to decide on the vehicle system for the antiperspirant active. It is the intent of this section to summarize some of the current knowledge concerning the influence of actives with the formula, efficacy of different delivery systems, and the function of the ingredients used in antiperspirants.

Antiperspirant actives, like ACH or ZAG complexes, are soluble in water. Application of a concentrated aqueous solution of an antiperspirant active gives a rather tacky feeling (36). Reduction of tackiness can be best achieved by silicone oils (cyclomethicones) or ester oils like di-(2 ethylhexyl) adipate (27). The acidic pH value (4.0–4.2) has to be taken into account by selecting additional components for the desired drug delivery system. Loss of viscosity and problems of a final formula with color stability are often hints to change the gellant and/or perfume. Aluminum powders in anhydrous systems (aerosols and suspension sticks) often leave visible white residues on skin or clothing. Liquid emollients, like (PPG)-14 butylether or the aforementioned adipate ester, minimize these residues. Another approach is to use the solid emollient isosorbide monolaurate (ICI, Arlamol® ISML) (37). In anhydrous aerosol formulations, the ACH powder settles down and forms a hard-to-redisperse cake at the bottom

of the aerosol can. Suspending aids, like quaternium-18 hectorite or quaternium-18 bentonite, prevent settling of the antiperspirant active and additionally thicken the cyclomethicone oil phase. Usage of fine powders of ACH is another approach to overcome nature's law of gravity.

The reader should be aware that hydrophobic ingredients, like emollients, have an influence on the effectiveness of an antiperspirant active, because a cosmetic oil phase or wax can cover the pores of the eccrine duct. The efficacy of an antiperspirant active, like ACH, is higher in water-containing systems compared with anhydrous formulations. The following rules concerning efficacy might be helpful:

1. Efficacy: aqueous solution > anhydrous suspension.
2. As diffusion of an antiperspirant active in the vehicle and from the vehicle to the skin after application has to be considered, one can further differentiate the expected efficacy trends. Efficacy: aqueous solution > sprayable O/W emulsion > O/W emulsion roll-on > O/W emulsion cream.
3. It is accepted that antiperspirant actives in the outer phase of an emulsion have a higher efficacy than in the dispersed phase. Efficacy: O/W emulsion > W/O emulsion.
4. In water-free systems, the viscosity of the drug delivery system might be of relevance. Suspended ACH in anhydrous vehicles needs to be solubilized after application to the axilla by sweat (water). The effectiveness of suspension sticks depends on the rapidity of active solubilization. The usage of ultrafine powders of ACH is expected to boost efficacy compared with fine powders. Efficacy: low viscous suspension > suspension stick.

The reader is referred to the literature concerning vehicle effects on antiperspirant activity (7,38,39).

Lipophilic ingredients might have an influence on the efficacy of a product, because it is known that the water-soluble propylene glycol can form complexes or hydrogen bonds with aluminum polycationic species thereby altering the efficacy of the salt (40). Also, propylene glycol in high concentrations may result in skin irritations (41). Successful formulation work aims at finding the right viscosity for the product in the desired application form, a lower viscosity during the flow into the underarm pit, and a higher viscosity after application so that the product stays where it was applied. Conventional shear shinning flow curves are characteristic for antiperspirant products. The reader is referred to the literature concerning rheology aspects of cosmetic products (42).

Deodorant/Antiperspirant Sticks

It is at present not easy to give the reader an overview about sticks, because nowadays there exist many technologies to develop this solid delivery system. In Figure 1, an attempt was made to summarize this area. In the following section, only systems of major importance are discussed.

Sticks can be divided into different classes like suspension sticks, gel sticks, and emulsion sticks. Soft sticks have some properties of all the three categories (Fig. 1).

Suspension Sticks

Dry deodorants, or antiperspirant solids, are synonyms for an application form where the active in the form of a powder is suspended in a silicone oil phase. Stearyl alcohol is usually used as the hardening agent. The molten mass crystallizes into a matrix of stearyl alcohol saturated with the silicone oil and suspended particles (43,44). Quaternium-18 hectorite can reduce the settling of the actives. Cyclomethicones give the stick a dry, silky feel; nonvolatile oils, like PPG-14 butylether, minimize white residues on the skin (43). Low-residue sticks can be obtained by using a combination of high-and low-melting waxes and a volatile and nonvolatile silicone-oil combination (45) (Table 1).

Gel Sticks

This class can be subdivided into the following groups: white anhydrous gel sticks, clear anhydrous gel sticks, and clear water-based soap gel sticks. The last class mentioned is discussed in chapter 63.

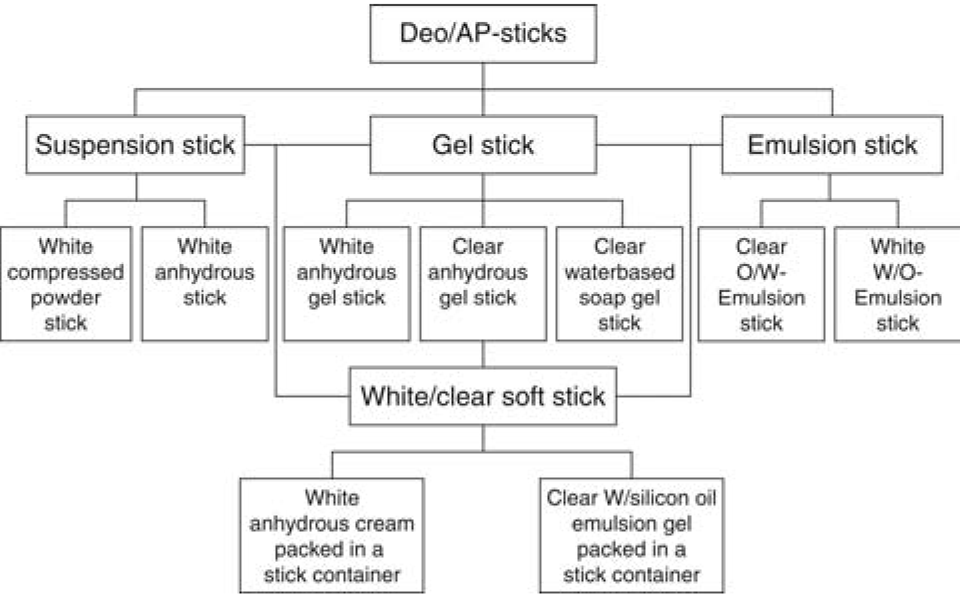

Figure 1 Overview of cosmetic deo/antiperspirant sticks.

Table 1

Suspension stick	Wt%
Stearyl alcohol	20.0
Cyclomethicone	54.0
PPG-14 butylether	2.0
Hydrogenated castor oil	1.0
Talc	2.0
Antiperspirant	20.0
Fragrance	1.0

Abbreviation: PPG, photoplethysmography.

White Anhydrous Gel Sticks

Shear solids, or ultraclear solids, are synonyms for sticks with improved washout performance compared with the classic suspension sticks. They contain *N*-acyl amino acid amides (*N*-lauroyl-L-glutamic acid dibutylamide) and 12-hydroxyacid as gelling agents for an oil phase mixture (e.g., silicone oil/mineral oil). The washout agent is an ethoxylated solubilizer, like Ceteareth-20. These white sticks turn clear after application to the skin (no-residue stick) (46).

Clear Anhydrous Gel Sticks

They are quite popular in the United States, because clarity is associated by the consumer with a lack of white residue on skin, no dangerous ingredients, and high efficacy. A typical gelling agent is dibenzylidene sorbitol [dibenzylaldehyde monosorbitol acetal, (DBMS A)]. This acetal is not stable in an acidic aqueous environment (47). The sticks usually contain a high level of alcohol and/or polyols. At high polyol concentration, the active is regarded to be solubilized instead of suspended in the gel matrix (48). An alternative gelling agent is a polyamide (49) (Table 2).

Emulsion Sticks

They can be grouped into clear O/W emulsions, white W/O emulsions, and clear W/S emulsion gels. The last mentioned is discussed below.

Table 2

White anhydrous gel sticks	Wt%	Clear anhydrous gel sticks	Wt%
N-lauroyl-L-glutamic acid dibutyl amide	5.0	Dibenzylidene sorbitol	2.0
12-Hydroxystearic acid	5.0	Dimethicone copolyol	2.0
Cyclomethicone	40.0	Di-isopropyl sebacate	2.0
Hydrogenated Polyisobutene	15.0	Glycine	1.0
Di-isopropyl myristate	15.0	Dipropyleneglycol	10.0
Antiperspirant powder	20.0	Propyleneglycol	33.0
		Antiperspirant powder	50.0

Source: From Ref. 58.

Table 3

W/O emulsion stick	Wt%
Stearyl alcohol	19.0
Volatile silicone	26.0
Mineral oil	1.0
2-Methyl-2,4 pentandiol	2.0
Polyglycerol-4 isostearate	2.0
ACH solution (50%)	50.0

Abbreviation: ACH, aluminum chlorohydrate.
Source: From Ref. 50.

Clear O/W Emulsions

They contain a high surfactant combination with the active solubilized in the external water phase. The high concentration of surfactants is a disadvantage; no products based on this technology are known to the author (47).

W/O Emulsion Sticks

The water phase containing the active is solubilized by a surfactant, like polyglycerol-4 isostearate. A typical example for an oil/wax phase combination is a mixture of silicone oil/stearyl alcohol (50) (Table 3).

Soft Sticks (Soft Solids, Smooth-Ons)

These sticks can be differentiated into two subgroups, namely, white, anhydrous creams (suspensions), and clear water-in-silicone emulsion gels. Both delivery systems are packed in a container that gives the impression of a stick. The suspension or gel is extruded onto the skin from holes in the top of the stick container to a wide smooth area around the holes.

White, Anhydrous Creams

These creams contain an antiperspirant active, a volatile and nonvolatile silicone oil, and a thickener (*N*-acyl glutamic acid amide).

Clear Water-in-Silicone Emulsion Gels

These formulations can be achieved by adjusting the refractive index of the water and silicone-oil phase. Silicone formulation aids (Dow Corning 3225 C) are mixtures of cyclomethicone and dimethicone copolyol helping to solubilize the active (7,46,48,51). Low surface tension of cyclomethicones facilitates good spreading of a product on the skin and reduces the tackiness of antiperspirant actives.

Antiperspirant Roll-Ons

Roll-on products can be differentiated into several categories (Fig. 2). O/W emulsion-based delivery systems are quite popular in Europe, whereas anhydrous suspension roll-ons or

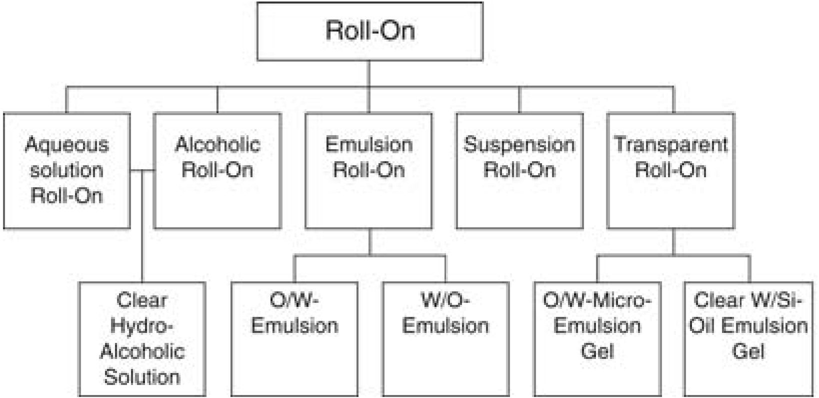

Figure 2 Overview of cosmetic deo/antiperspirant roll-on types.

transparent water-in-silicone emulsions are preferred in the United States. A new trend concerning the size of the roll-on applicator has been identified. Consumers prefer the big-ball format (3.0–3.5 cm), because of the ease of applying the product to the underarm pit (52). The popularity of roll-ons, in general, is because of the nongreasy and nonoily feel in the axilla and the good spreadability of the content on the underarm skin.

Clear Hydroalcoholic Roll-On

This delivery system contains a water/alcohol solution of the antiperspirant active thickened with a water-soluble polymer like hydroxyethylcellulose. The alcohol in the formula gives, compared with the clear aqueous solution-based roll-ons, a fresh sensation in the axilla and facilitates drying of the product. Excellent antiperspirant efficacy is another benefit of hydroalcoholic roll-ons.

O/W Emulsion Roll-On

This delivery system uses ethoxylated surfactants, like PEG-40 stearate, to solubilize an oil phase like mineral oil. The active is dissolved in the outer phase, allowing the formulation of a highly effective product. In alcohol-free formulated systems, microbiological stability has to be checked (Table 4).

W/O Emulsion Roll-On

They are weaker in efficacy because the actives are encapsulated and the external oil phase often gives a sticky feeling.

W/Si Emulsion Roll-On

Silicone oils allow products to formulate on the basis of a "W/O technology," because the skin feel is not comparable with traditional oily components, like ester oils or triglycerides. The concentration of the thickener is reduced compared with sticks based on this type. The technology is discussed under soft sticks (see p. 636).

Table 4

O/W emulsion roll-on	Wt%	Hydroalcoholic roll-on	Wt%
PEG-40 stearate	5.0	Antiperspirant active	20.0
Cetyl alcohol	3.0	PPG-5 ceteareth-20	2.0
Mineral oil	2.0	Water	35.4
Polysorbate-80	1.0	Ethanol	42.1
Glycerin	1.5	Hydroxyethylcellulose	0.5
Magnesium-aluminum silicate	0.8		
Antiperspirant active	20.0		
Water	66.7		

Abbreviations: PPG, photoplethysmography; Mg, magnesium.

O/W Micro-Emulsion Gel

An alternative approach to transparent products uses the phase inversion temperature (PIT) technology. A suitable mixture of surfactants, oils, and water is heated from 60°C to 90°C to give a W/O emulsion above the PIT. During cooling, the mixture shows phase inversion to give white or transparent O/W emulsions; O/W micro-emulsion gels are obtained in the presence of hydrophobically modified water-soluble polymers (53). The technology is explained in more detail in chapter 63.

Suspension Roll-On

The antiperspirant active in powder form is suspended in cylomethicone. The roll-on can be formulated with or without ethanol. Quaternium-18 hectorite is used as a thickener to prevent settling of the active. Consumers in the United States prefer this delivery system, as it does not give a wet feeling after application and because of the easy drying (39). Actives like ZAG complexes give high efficacy to underarm products (Table 5).

Antiperspirant Aerosols

Aerosols, in Europe and Asia, are popular delivery systems for consumers who prefer a hygienic and easy-to-use application form. Typical ingredients for aerosols include isopropyl myristate, isopropyl palmitate, volatile silicone, dimethicone, silica, clays, propylene carbonate, and ethanol. Propellants include propane, butane, and isobutane (Table 6).

As acidic aqueous ACH solutions lead to corrosion of the aerosol can, current aerosol antiperspirant products are formulated as water-free suspensions. The active is suspended as a powder in an oil phase like cyclomethicone or in a mixture of ester oils/cyclomethicone. Agglomeration of solid particles and settling of actives can be minimized by the usage of suspending agents like fumed silica (amorphous silicon dioxide) or clays (bentonite and hectorite). The clays form a weak gel in the presence of an oil phase that can be destroyed by shaking the aerosol can before usage. The gel structure is reformed on standing, thereby holding the active in suspension. Because the organoclays are agglomerated, shear is needed to deagglomerate the platelets, and a polar activator like propylene carbonate or ethanol is used to disperse them and induce the gelation of the oil phase.

The steps involved to prepare an aerosol product can be summarized in the following sequence (7):

1. Preparing bentonite or hectorite clay with the emollient in the presence of the polar activator and shearing the mixture.
2. Adding the antiperspirant active until a uniform agglomeration-free suspension is obtained.
3. Filling the concentrate into the aerosol can and adding the propellant (pressure filling).

Table 5

Suspension roll-on	Wt%
Volatile silicone	65.0
Quaternium-18 hectorite	13.5
Silica	0.5
Antiperspirant powder	20.0
Fragrance	1.0

Table 6

Antiperspirant aerosol	Wt%
Volatile silicone	13.4
Quaternium-18 hectorite	0.8
Ethanol	0.8
Antiperspirant powder	10.0
Propellant (butane/propane)	75.0

Efficacy studies of aerosols, including comparison with other drug delivery systems, have been reported in the literature (30). ZAG complexes are not allowed to be used in aerosols.

Environmental Issues

Aerosols contain volatile organic compounds (VOCs) usually in a weight ratio of propellant to concentrate of 75:25 (54). The environmental impact of VOC, like the reaction with NOX, in the presence of sunlight causes formation of unwanted ozone in the lower atmosphere. U.S. antiperspirant companies especially were forced to reduce VOC emissions by reformulating hydrocarbon propellants and/or exchanging hydrocarbon propellants with the fluorohydrocarbons, 1,1 difluorethane (Propellant 152 a) or 1,1,2,2 tetrafluorethane (Propellant 134 a). The water-soluble dimethoxyethane (DME) is another propellant that is thought to have no impact on the damage of the ozone layer (55).

The current trends in the aerosol market can be summarized as follows:

- higher ratio of concentrate/hydrocarbon propellant
- higher amount of silicone oils
- usage of 1,1 difluorethane (Propellant 152 a)
- formulations with lower vapor pressure
- usage of smaller aerosol cans

Aerosols containing 20% to 50% propellants with a concentrate-propellant ratio from 1.0 to 1.0 to 2.3 to 1.0 have been patented (56).

FUTURE TRENDS

Some new trends in the antiperspirant field concerning new actives and delivery systems have been described in this chapter. Improvements of current formulations and innovative concepts will need the ongoing investigation and better understanding of the interaction of active/vehicle and vehicle/skin. Improving efficacy and skin compatibility is another major trend in the antiperspirant field. New packaging concepts, like the extrudable gels, the big-ball applicator for roll-ons, and reduced-size aerosol cans with ozone-friendly propellants, are probably, in a few years, the state of the art. The influence of perfume components to the skin and the increasing rate of contact allergies attributable to fragrance ingredients have to be closely monitored (57).

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63 Deodorants

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INTRODUCTION

This chapter intends to give an overview on the current knowledge about the origin of underarm odor and the biology of the underarm microflora and its interaction with deodorizing agents. The contents of this chapter have been arranged in particular sequence to facilitate the understanding of rational deodorant product development.

BIOLOGY OF THE UNDERARM MICROFLORA

The resident microflora of the human underarm skin consists of up to $10^6/cm^2$ organisms, e.g., aerobic cocci, lipophilic diphtheroids, and varying species of gram-negative bacteria (1). In the axillae, two types of bacterial flora exist—coryneform bacteria and micrococcaceae such as *Staphylococcus epidermidis*. Coryneform- or *S. epidermidis-dominated* populations are characteristic for human beings. The resident microflora is a quite stable population, not varying a lot between both axillae (2). The organisms are perfectly adapted to their ecological niche with its higher pH value and higher moisture content compared with other skin areas (3). Hair in the axilla, according to the literature, is not a good substrate for bacterial growth; the bacteria prefer to reside on the underarm skin (2). Moisture is required for bacterial proliferation and is secreted especially from the eccrine sweat glands (4). The origin of strong compared with weak underarm odor is associated with a numerical dominance of coryneform bacteria (5). Components of apocrine secretion, e.g., isovaleric acid and androstenone, were proposed to contribute to axillary odor. Hydrolytic exoenzymes of skin bacteria cleave the ester bonds of odorless water-soluble precursors of androstenol to the corresponding volatile steroid (6). Other studies proposed that the key odorants are branched, straight chain, and unsaturated C6-Cn fatty acids (7). (E)-3-methyl-2-hexenoic acid (E-3M2H) is the most abundant fatty acid compared with the rest of C6-Cn fatty acids that contribute to the axillary odor bouquet. Apocrine sweat extracts have been analyzed and concentrations of 0.5 ng/^1 for androstenone and 357 ng/iL for E-3M2H were detected (8). Volatile odor molecules of E-3M2H found in sweat secretions are transported according to the authors in a nonvolatile fashion to the skin surface. Two apocrine secretion odor-binding proteins (ASOB and ASOB2) were identified, carrying 3M2H molecules to the skin surface. Coryneform bacteria liberate the odor molecules from the protein precursor/odorant complex (8).

The reader should be aware that occurrence of these chemical compounds does not mean that all of us can smell them. Individual differences in odor perception for both isomers of 3M2H (9) and for the steroid androstenone are well known (8). Approximately 50% of the adult population is not able to smell androstenones; this anosmia to androstenone—or to 3M2H—is genetically determined.

DEODORANTS

Deodorants are topically applied products designed to reduce underarm odor. They are considered in the United States as cosmetics, while antiperspirants are treated by the FDA as drugs. Deodorants tend to be less irritating than antiperspirants. In Europe, the consumers today prefer deodorants compared to antiperspirants. In the United States the trend is approximately reversed.

Concepts for Controlling Underarm Odor: State of the Art

The current knowledge of the biology of the underarm microflora and the origin of underarm odor is the basis for developing strategies against odor formation. Numerous patents and literature articles disclose the incorporation of chemical compounds for their deodorizing properties. The intention here is to describe and exemplify major strategies, but not all deodorant actives that were developed in the past.

Strategies to reduce underarm odor include the following:

- Antiperspirant active-containing deodorants
- Odor-masking deodorants
- Odor-neutralizing deodorants
- Odor-quenching deodorants
- Enterase inhibitors
- Antimicrobial active-containing deodorants

Antiperspirant Active-Containing Deodorants

Antiperspirant actives such as aluminum chlorohydrate or the Al–Zr complexes (see chap. 62) reduce the secretion of eccrine sweat. Their excellent antimicrobial properties against *S. epidermidis* and coryneform bacteria have been published (10). The acidity of the aluminum salts may be a major factor in bacterial growth inhibition.

Odor-Masking Deodorants

Fragrance compositions (such as perfumes) have been used to mask odors since ancient times. It is conventional to incorporate 0.2% to 1.5% of a perfume in body deodorants (11). They are designed to blend with the underarm odor and thus act as a masking agent. The perception of a perfume may differ significantly between individuals because of different interactions with the skin, washing habits, and specific underarm odor. The fragrance materials are blended to achieve what is known as "top note," "middle note," and "bottom note" components. The first is the refreshing note upon application while the last are the olfactoric components, which stay on after application to the underarm skin.

Perfumes with antimicrobial properties have been described in patents and in the literature (12–14). An additional benefit, especially for emulsion-based products, is that they might also act as a preservative. The increasing rate of contact allergies against fragrance ingredients should be taken into account using this approach to combat underarm odor (15).

Odor-Neutralizing Deodorants

In chapter 62, it was mentioned that odorous C6-Cn fatty acids contribute to underarm odor. Chemical neutralization with sodium bicarbonate (NaHCO3) yields the corresponding odorless soaps (16). This active, however, is not stable for a long time in aqueous compositions. Patents for deodorant applications and usage of NaHCO3, in the presence of antiperspirant actives, have been filed (17,18). Zinc carbonate–containing deodorants are also content of a patent (19).

Odor-Quenching Deodorants

Zinc Ricinoleate

Zinc salts of ricinoleic acid have no bacteriostatic or antiperspirant effect (20). They strongly bind odorous fatty acids, amines, and mercaptanes. Ligand-exchange reactions of ricinoleic acid for odor molecules are probably the reason for the quenching properties of zinc ricinoleate (21). Interactions with perfume components in a deodorant formulation may weaken the desired quenching effect of the odor molecules after topical application to the underarm.

Metal Oxides

The oxides of calcium, magnesium, and zinc form in the presence of fatty acids in the corresponding metal soaps (22). Zinc oxide particles aggregate to form a massive lump. This leads to clogging of aerosol products (23). Hybrid powders were developed in which the metal oxide covers the surface of a spherical nylon powder (23). The advantage of this technology is

the increased surface area of zinc oxide and thus enhanced odor-quenching efficacy and the reduced particle aggregation in aerosols.

Esterase Inhibitors

Zinc Glycinate

The inhibition of exoenzymes from the underarm bacteria should also result in odor reduction. Zinc glycinate has been described as a suitable active (24). Antimicrobial tests showed no inhibitory effect against *S. epidermidis* or against the lipophilic diphtheroid bacteria supporting the suggested mechanism against microbial exoenzymes.

Triethylcitrate

The optimal pH value for development of underarm odor caused by coryneform bacteria is approximately about pH 6 in axillary extracts (25). Shifting the skin surface pH to the acidic side should decrease the activity of skin esterases, which are proposed to be responsible for degradation of underarm secretions. Triethylcitrate was proposed to form citric acid by an enzymatic process on the underarm skin. In 1991, it was shown that this active has no pH-reducing effect after application to the underarm skin (26). Nevertheless, deodorants containing this active are still in the market.

Antimicrobial Active-Containing Deodorants

This approach is currently the most commonly used strategy to prevent underarm odor. Ethanol is probably one of the best-known actives for deodorization (27). Additional efficacy is normally required for a long-term deodorization, and this can be achieved by the additional usage of fragrance, an antiperspirant active, or other antimicrobial actives (famesol, phenoxyethanol, etc.).

Triclosan (2,4,40-Trichloro-20-Hydroxydiphenylether)

This active has a broad-spectrum antimicrobial activity against most gram-positive and gram-negative bacteria, molds, and yeasts. The presence of triclosan in antiperspirant sticks and roll-ons leads to a higher reduction of the bacterial microflora versus the triclosan-free antiperspirant composition (28). Triclosan is also used in skin care products, hand disinfectants, and household products (29).

Glyceryl Fatty Acid Ester

Mono- and oligoglyceryl fatty acid esters such as glyceryl monocaprylate, monocaprinate, monolaurate, and diglyceryl monocaprinate are effective deodorizers (30). Combinations of glyceryl monolaurate with farnesol and phenoxyethanol showed synergistic efficacy effects against coryneform bacteria (31). The advantage of this ingredient combination over the first generation deodorant actives such as triclosan is attributed to their higher biodegradability and their selective bacterial action. These actives are all naturally occurring in plants and animal species. In addition, it could be demonstrated that combinations of mono- and oligoglyceryl fatty acid esters with a variety of natural antimicrobials (e.g., wool wax acids) displayed a synergistic antimicrobial efficacy against underarm bacteria and serve as highly effective deodorant actives (32–35). Products containing such actives have been successfully marketed for a number of years.

Sucrose Fatty Acid Ester

The fatty acid esters of sucrose are well known as emulsifiers in food products (36). Sucrose can be substituted on eight hydroxyl groups with fatty acids. The antimicrobial potential depends strongly on the substitution degree of the sucrose. Sucrose monostearate and sucrose monolaurate have been described as deodorizers in the literature and in patents (37–39).

Glycerolether

2-Ethylhexyl glycerolether (octoxyglycerol) is a clear liquid with good solubility in cosmetic oils, polyols, and alcohol but only moderate solubility in water (0.2%). Synergistic

antimicrobial activity with other ingredients has been described (40). This active has become popular recently in European deodorant formulations.

New Concepts for Controlling Underarm Odor

Ongoing research activities focusing on a better understanding of the interaction between underarm skin/skin microflora and skin microflora/odor formation, in combination with the discovery of highly selective actives, today allow more specific designs for deodorant products. In the next sections, some of the new trends are discussed in detail. New concepts for controlling underarm odor include the following:

- Chitosan
- Bacterial enzyme inhibitors
- Odor-inhibiting precursor mimics
- Product and skin-mediated perfume transformations
- Antiadhesives

Chitosan

Chitin is a naturally occurring polysaccharide (e.g., in insects, lobster, crabs, or fungi) containing N-acetylated D-glucosamine units. Deacetylation of the amino group leads to the slightly water-soluble chitosan. The deodorizing properties of chitosan and the combination of this active with aluminum salts have been the subject of a patent (41).

Bacterial Enzyme Inhibitors

The enzyme amino acid (3-lyase) is, according to a patent filed in 1990, a catalyst for the formation of underarm odor (42). This enzyme is located in odor-releasing bacterial cells and cleaves the apocrine precursors of sweat components, such as amino acids with the structure unit COOH–CH–(NH2)–CH2–S–R, to the corresponding odorous sulfur products. Several classes of enzyme inhibitors such as derivatives of hydroxylamines, 3-substituted amino acids, cycloserine, and pyridoxal were identified.

Odor-Inhibiting Precursor Mimics

Another approach to the inhibition of the above-mentioned enzyme f-lyase is to provide an alternative substrate for the bacteria that cleave the structure unit CH(NH2) CH2–O–C(O)–R instead of the sulfur-containing amino acid sequence (43). This approach leads to the corresponding nonodorous ingredients, such as benzoic acid, or to pleasant odor-generating substances, such as phenylacetic acid.

Product- and Skin-Mediated Perfume Transformations

The physical and chemical interaction of a perfume with the underarm skin is a very complicated matter. Research activities in this area focused on the question, which components of a perfume stay on and above the skin after topical application (44). Headspace analysis is one of the techniques to gain more information concerning skin/perfume interactions. It could be demonstrated that the long lastingness of a fragrance can be achieved by using a prodrug (ester, acetale) of a perfume ingredient (45). The esters or acetales of a fragrance composition hydrolyze on human skin because of the slightly acid pH value. The hydrolysis products (acids, alcohols, and aldehydes) impart a pleasant smell to the underarm skin. These product- and skin-mediated perfume transformations are especially suitable for alkaline formulations such as soap-based deodorant sticks. The advantage of the perfume precursor approach is attributed to a prolonged fragrance impression of a deodorant after topical application to the underarm skin.

Antiadhesives

An alternative concept to reduce the amount of skin bacteria in the underarm skin is the antiadhesion approach. The understanding of the adhesion mechanisms of the resident underarm microflora to the skin surface is the basis for developing strategies against bacterial

adhesion. Numerous skin microorganisms adhere preferentially to specific sites on various body surfaces. For example, *Staphylococcus aureus* and *Pseudomonas aeruginosa* adhere to collected nasal epithelial cells (46). *Corynebacterium xerosis* binds to epidermal cells whereas yeasts species such as *Candida albicans* bind to corneocytes. Structures of the skin specifically involved in adherence to the underarm bacteria are thought to be proteins, oligosaccharide structures, lipids, and hydrophobic surfaces. Imitation of these adhesion motifs by saccharides, oligosaccharides, polysaccharides, and glycoproteins allows one to inhibit the bacterial adherence to the skin. Additionally, it was discovered recently that among others, sucrose esters such as sucrose myristate and sucrose laurate have antiadhesive properties to various microorganisms including the typical microflora of the underarm skin (47).

DRUG-DELIVERY SYSTEMS AND APPLICATION FORMS FOR DEODORANT ACTIVES

Products designed to reduce underarm odor can be formulated in a variety of delivery systems such as suspensions, water or hydroalcoholic solutions, and emulsions. Typical application forms are sticks, roll-ons, creams, pump sprays, aerosols, and gels. Sticks, roll-ons, and aerosols are discussed in detail in the chapter "Antiperspirants. Lowering the amount of an antiperspirant active, such as aluminum chlorohydrate, in an antiperspirant is one option to formulate a deodorant. In this case, the antiperspirant active has only deodorizing properties and nearly no impact on the eccrine sweat glands. Deodorants can be formulated in acidic, neutral, or alkaline environment. Designing a deodorant, the formulator should have in mind the following points:

- Long-term deodorization
- No irritation potential
- Good solubility of the active in the delivery system
- Selection of a stable fragrance
- Viscosity control of the product
- Good skin feeling of the product

Protocols for the in vitro and in vivo evaluation of deodorants have been designed. The reader is referred to the literature (48). A new method for in vivo evaluation of antimicrobial agents was recently developed, where the underarm bacteria were translocated to the forearm allowing the simultaneous evaluation of multiple deodorizers in an individual (49).

Deodorant Sticks

Deodorant sticks are solidified by 6% to 8% of sodium stearate. The deodorizing agent and a fragrance are dissolved in a hydrophilic carrier. Two stick categories can be differentiated, the ethanol-based and the propylene glycol-based sticks (50).

Transparency is usually achieved by usage of a high polyol content. Clarifying agents for sticks such as PPG-14 butylether, Cocamide DEA, Lauramide DEA, Steareth-100 have been patented (51,52). Ethanol-based sticks are preferred if it is the intent of the formulator to create a cooling sensation for the consumer. Shrinkage of the stick has to be taken into account because of evaporation of the alcohol. Propylene glycol-based sticks tend to be more resistant to shrinkage, and solubilization of a fragrance is easier in some instances (53) (Table 1).

Table 1

Deodorant stick	Wt%	Deodorant stick	Wt%
Water	16.0	Water	3.0
Ethanol	75.5	Propylene glycol	10.0
Deodorizer	1.0	Deodorizer	1.0
Sodium stearate	6.5	Sodium stearate	8.0
Fragrance	1.0	PPG-3 myristyl ether	77.0
		Fragrance	1.0

Deodorant Aerosols

Spray products containing a solution of an antimicrobial active in an ethanol and/or propylene glycol carrier, blended with a liquefied propellant, are typical for deodorant aerosols. The difference from an antiperspirant active containing aerosol is that the deodorizer is solubilized in an alcohol- or polyol-based formulation and not suspended. Deodorant sprays provide a dry skin feeling to the underarm skin because they are anhydrously formulated.

Typically, 20% to 60% of the sprayable contents of an aerosol reach the skin, because the liquefied hydrocarbon propellant vaporizes as it is sprayed (54). Propane, butane, and isobutane are the most commonly used propellants. They condense to form a clear, colorless, and odorless liquid with densities of 0.51 to 0.58 g/mL at 20°C (55). These propellants are inflammable in the presence of air or oxygen. Labeling of cosmetic aerosols concerning flammability risks of volatile organic compounds and volatile solvent abuse is discussed in detail in a recently published review (56). Aerosol containers can be fabricated from tin-coated steel, tin-free steel (chromium-coated steel), or aluminum. Numerous types of aerosol can cause corrosion, and testing for it was recently discussed in the literature (57). The environmental issues of aerosols are explained in greater detail in the chapter "Antiperspirants" (Table 2).

The formulator of an aerosol has to optimize the following parameters to get a dry deodorant product:

- Spray rate
- Spray shape
- Particle size, concentrate/propellant ratio
- Fragrance/deodorizer concentration
- Pressure of the aerosol can

Deodorant Pump Sprays

Hydroalcoholic Pump Sprays

An alternative to aerosols is pump sprays. This category is quite popular in Europe, whereas it is of lower interest for the consumers in the United States, because they tend to prefer a dry application form, like the anhydrous sticks. Pump sprays allow a good dosage of the formulation to be delivered to the underarm skin in a hygienic way. They consist of low-viscosity hydroalcoholic solutions of a deodorizer and a perfume. Usually a solubilizer, such as PEG-40 hydrogenated castor oil, is incorporated into the formulation to maintain a clear and homogeneous solution (Table 3).

Table 2

Deodorant aerosol	Wt%
Alcohol	42.0
Laureth-4	0.5
Deodorizer	1.0
Fragrance	0.5
Isobutane	47.6
Propane	8.4

Table 3

Pump spray	Wt%
Water	35.6
Alcohol	60.0
PEG-40 hyd.	2.0
Castor oil	–
Deodorizer	2.0
Fragrance	0.4

Table 4

PIT-emulsion pump spray	Wt%
Glyceryl stearate, ceteareth-20, ceteareth-10, cetearyl alcohol, cetyl palmitate (Emulgade SE)	4.5
Ceteareth-20	1.0
Dioctyl cyclohexane	5.0
Dicaprylylether	5.0
Deodorizer	2.0
Aluminum chlorohydrate	5.0
Water	77.5

Source: From Ref. 60.

PIT-Emulsion Pump Sprays

A disadvantage of hydroalcoholic pump sprays is the alcohol content in the formulation that may contribute to unwanted side reactions especially in the shaved axilla. Beiersdorf AG in Hamburg, Germany, introduced to the European market under the brand name "Nivea®" a new pump spray on the basis of an emulsion in 1995. The sprayable low-viscous deodorant is based on the phase inversion temperature (PIT) technology. Suitable mixtures of ethoxylated surfactants, oils, and water in the presence of antiperspirant and deodorizing actives are heated to 60°C to 90°C. Cooling the resulting W/O emulsion to room temperature yields, via a PIT process, a finely dispersed bluish-white O/W emulsion (58–60). The droplet size distribution of such PIT emulsions ranges from 80 to 250 nm. The above-mentioned pump spray contained a skin-friendly deodorizing combination of glyceryl monocaprinate and wool wax acids in an alcohol-free delivery system (Table 4).

Microemulsion Pump Sprays

Hydroalcoholic pump sprays are usually transparent, whereas sprayable PIT emulsions are white or bluish-white products. Sprayable alcohol-free and additionally transparent pump sprays were recently introduced into the European market (e.g., Basis pH; Beiersdorf AG, Hamburg, Germany). Transparency of an emulsion is achieved when the size of the droplets is below 100 nm. This O/W microemulsion can be obtained with and without the PIT technology but needs careful selection of ingredients and considerable fine-tuning (61). The main advantage compared with classical microemulsions is the low surfactant concentration (<10%). Furthermore, it could be demonstrated that, in the presence of hydrophobically modified water-soluble polymers, the above-mentioned technology allows the formulation of gels, sprayable gels, roll-ons, sticks, and aerosol products (62).

FUTURE TRENDS

The deodorant market has undergone some remarkable changes concerning the principles to reduce underarm odor in the last years. It is expected that the search for effective, skin-friendly actives with a highly selective action against the cutaneous underarm microflora will lead to long-lasting and safe deodorants. Improvements in understanding how microorganisms adhere to human skin should facilitate the development of new strategies to reduce underarm odor. Improvements of aerosols with no/low impact to the environment or aerosol alternatives, such as sprayable emulsions, are probably in a few years in the portfolio of every deodorant-selling company.

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64 Revulsive Products: Way of Action and Evaluation of Their Efficacy

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INTRODUCTION

In Webster's dictionary, we found the following descriptions: "revulsive"—(*i*) to pull away and (*ii*) an act or technique of turning or diverting a disease or blood from a diseased region in one part of the body to another (as by counterirritation); "rubefacient"—substance for external application that causes redness of the skin.

Revulsive products (i.e., rubefacients and urticants) are known for several clinical and nonclinical applications. Clinically, they are used in the treatment of neuropathological [diabetic neuropathy, postherpetic neuralgia (PHN)] and/or muskeloskeletal disorders (e.g., osteoarthritis, rheumatoid arthritis, muscle soreness, and back pain). Nonclinically, they are used in some sports as passive warming-up products and in the cosmetic industry as an ingredient in skin products (1).

The capital active ingredient in these topical formulations is a nicotinate derivative [methylnicotinate (MN), hexylnicotinate (HN), and benzylnicotinate (BN)] or capsaicin. Nicotinates provoke an elevation of arachidonic acid and prostaglandin levels (prostaglandin D2). The produced prostaglandins act on the neuroreticular tissue of the arteriovenous anastomoses of the dermal vascular plexuses by means of an endothelium relaxant factor. The latter provokes a relaxation of the vascular smooth muscles resulting in an augmentation of the cutaneous circulation and a flooding of the superficial veins. This nonimmunological immediate contact reaction is visible as an erythema (2).

Capsaicin is an alkaloid, derived from chili peppers, with analgesic properties. Capsaicin binds to nocisensors in the skin, excitating neurons, which results in itching, pricking, or burning with a cutaneous vasodilation (3).

Stimulation of afferent C fibers with release of substance P is hypothesized, while the desensitization after prolonged treatment is believed to occur due to a depletion of substance P (3).

The topical application of capsaicin has equally been used to study reflex mechanisms of dermal vasodilatation (4,5) and as an experimental pain inducer to study the underlying nocisensoric mechanisms (6). Threshold levels of sensitization to increasing capsaicin concentrations have been used to study sensitive facial skin (7).

Besides these clinical and practical applications, rubeficiants (especially nicotinates) are often used in more fundamental research toward percutaneous penetration processes. The quantification of the physiologically induced vascular response has been found to be a good indicator for the skin bioavailability of these topically applied substances.

The response is often quantified by measuring the perfusion of the skin microcirculation (laser Doppler velocimetry), the skin color (redness), or the skin temperature. The laser Doppler instrument measures the increased perfusion of the arterial plexuses, while the skin color is not only an indication for the increased flux in the arterial part of the skin microcirculation but also for the flooding of the venous capacitance vessels.

This chapter describes the way of action and factors influencing the pharmacodynamic response to nicotinates under different experimental conditions, followed by possible clinical applications. For capsaicin, the emphasis will be mainly on clinical applications since this revulsive product is not often used for fundamental research. Finally, the use of revulsive products in physiotherapy (sport) will be discussed.

NICOTINATES

The onset of the nicotinate contact reaction depends on the derivative used. Application of MN (hydrophilic) results in an immediate response as measured with the laser Doppler instrument, while HN (lipophylic molecule) shows a lag time up to five minutes (8).

The intensity of the reaction is concentration dependent (9,10). Concentrations between 1 and 100 mM are reported. The duration of the response varies from 20 minutes up to 60 minutes for the laser Doppler response and up to 90 minutes for the color response. An increased temperature is noticed up to 90 minutes post application (10).

The pharmacodynamic response varies in function of the anatomical skin site (11). The strongest response was recorded at the forehead and the chest, while the response was weaker at the abdomen, forearm, and thigh. A significant relation was found between the nicotinate response and skin characteristics, such as TEWL, stratum corneum hydration, skin temperature, baseline perfusion of the microcirculation, and sebum gland density.

In the experiments of Marrakchi and Maibach, reactivity was tested on different regions of the face (forehead, nose, cheek, nasolabial and perioral areas, and chin), the neck, and the volar forearm (12). Experiments were carried out on young (29.8 ± 3.9 years) and older (73.6 ± 17.4 years) population. For both the age groups, the areas on the face and neck were more sensitive to HN compared with the forearm. A different sensitivity pattern for the face was detected between the two age groups, while peak values were significantly higher in the older group for the forehead, cheek, and nasolabial area. The authors explained the differences between the age groups by the photoaging effect on the sebum glands with more and enlarged glands in the older subjects. Roskos et al. did not find an effect of age when applying MN in young and older subjects (13).

Issachar et al. found a significant correlation between the percutaneous penetration of MN and sensitive skin. The intensity of the response to the nicotinates differed significantly between normal and sensitive skin, while the duration of the inflammation was comparable (14).

Racial differences in barrier function were demonstrated by quantification of the nicotinate response with a laser Doppler instrument (15,16).

Berardesca et al. compared nicotinate responsiveness in Caucasians and black volunteers before and after a delipidation of the challenged skin area (15).

A lower cutaneous response was noticed for the blacks compared with the whites under both experimental conditions. Kompaore and Tsuruta compared Asians, blacks, and Caucasians with a nicotinate challenge. The lag time between nicotinate application and onset of the vascular response was used as an indication of barrier permeability. Permeability was strongest in Asian skin, weaker in Caucasian skin, and weakest in black skin (16).

The response to nicotinates is significantly reduced after oral treatment with anti-inflammatory drugs (17,18) and with topically applied anti-inflammatory drugs (19–23). The reduced response in the presence of topically applied anti-inflammatory drugs was used as a model to study iontophoresis (24). In these experiments, the reduction in the MN response was used as an indicator for the presence of diclofenac in the stratum corneum.

In their experiments with penetration enhancers Tanojo et al. found a reduced lag time in the HN response after pretreatment of the skin with propylene glycol. The combination of propylene glycol with oleic acid was not more effective than propylene glycol alone (25).

The microvascular sensitivity (tested with increasing nicotinate concentrations) was increased in diabetic patients compared with controls, while the maximal microvascular responses were comparable (2). Similar findings were observed by Caselli et al. in healthy control subjects and diabetic neuropathy patients (1). These authors propose the addition of MN in the moistening products used to reduce the development of diabetic foot (1,2).

A reduced response was noticed in patients with Huntington's disease (26).

CAPSAICIN

Capsaicin (trans-8-methyl-*N*-vanillyl-nonenamide) is an alkaloid derived from the common hot pepper plant of the nightshade (*solanacae*) family. Capsaicin represents the main constituent of the total pungent acid amides present in the capsicum species and is responsible for the red-hot chili taste (27).

Besides being considered as food additive, capsaicin has also gained human exposure as oral supplement or topical analgesic (28).

Capsaicin is a selective agonist for the transient receptor potential (TRP) channel. The TRPV1 receptor is a ligand-gated, nonselective cation channel expressed on a subpopulation of primary small-diameter sensory Aδ fibers and C fibers, responsive to noxious heat and mechanical and chemical stimuli (29,30). The topical application of capsaicin on the human skin excitates the TRPV1-expressing nocisensors, resulting in an itching, pricking, burning sensation with a cutaneous vasodilation, hyperalgesia, and allodynia (30). Stimulation of afferent C fibers with release of neuropeptides, predominantly substance P is hypothesized, while the desensitization after prolonged treatment is believed to occur because of a depletion of substance P (31). Capsaicin inhibits axonal transport of neurotransmitters by depressing the release of the nerve growth factor (NGF) (32).

The resulting hypoalgesia is due to degeneration of epidermal nerve fibers (31–34). The desensitization of hyperactive nociceptive sensory axons is the basis for therapeutic topical application or intra-articular injections of capsaicin (35).

Peripheral neuropathy, provoked by axonal degeneration of sensory autonomic and motor neurons of the peripheral nervous system, is a common complication of diabetes and chronic alcohol abuse. The manifestations of peripheral neuropathy classically progress from the most distal extremities. Positive symptoms are lancinating pain, paresthesia, numbness, allodynia, and burning and itching sensations (36).

The Capsaicin Study Group (1991) conducted a multicenter, double-blind, vehicle-controlled study to establish the efficacy of topical 0.075% capsaicin cream in relieving the pain associated with diabetic neuropathy. Patients ($n = 252$) were randomly assigned to the capsaicin or placebo group. Capsaicin cream or vehicle was applied on the painful areas four times a day for eight weeks. Statistical analysis showed significant difference in favor of capsaicin compared with placebo for the following parameters: pain relief, decreasing pain relief, and pain improvement on the physician's global evaluation scale. The authors asserted that topical capsaicin cream is safe and effective in treating painful diabetic neuropathy (37).

These findings corroborate the results of the placebo-controlled studies by Scheffler et al. (38) and Tandan et al. (39). They demonstrated the superiority of capsaicin cream 0.075% versus placebo in pain control and improvement of daily activity during the treatment of diabetic neuropathy.

A meta-analysis of four randomized, double-blind, placebo-controlled trials using capsaicin in the treatment of diabetic neuropathy found capsaicin to be more effective than placebo (40). This is in contrast with the findings of Low et al. (41). In their study, using a four times daily application of capsaicin cream versus a nicotinate formulation as placebo, they failed to demonstrate significant improvement in chronic distal painful polyneuropathy after 12 weeks of treatment. Besides using different pain evaluation scales, physiological functions such as sudomotor axon reflex, nerve conductance, and sensory examinations were carried out before, during, and after the experiment (41).

PHN is the most common complication of Herpes Zoster. The incidence of Herpes Zoster and its associated complications mainly occur in older patients (42). The pain associated with PHN is often referred to as neurogenic pain and generated as a result of neural dysfunction and therefore unresponsive to conventional analgesics including opiates (43).

The hypothesized mechanism for capsaicin-induced analgesia in PHN is the interference with the biosynthesis of the neuropetide substance P, which has an important role in the central transmission of nociceptive signals (44).

Two published double-blind, placebo-controlled studies evaluated the clinical effectiveness of topical capsaicin cream in the treatment of PHN. The reported results of both the studies were in favor of capsaicin (0.075%) cream versus placebo. McCleane demonstrated that the mixed application of glyceryl trinitrate and capsaicin cream was significantly more effective than placebo in reducing pain. This combination had a positive effect on the tolerability of treatment with capsaicin cream in patients with osteoarthritis (44).

Painfull cutaneous disorders such as psoriasis, nostalgia paresthetica, and atopic dermatitis are characterized by intense itching, scaling, and erythema (45,46). Nonhistaminic itching, in contrast to histaminic itching, is difficult to treat, and therapeutic applications are often uneffective (47). The release of substance P increases the vascular permeability and the number of mast cells in the skin (48). The responsiveness of several forms of urticaria to

capsaicin treatment may be related to the effects of capsaicin on the microvasculature of the skin (47). The topical application of capsaicin seems to be effective in the treatment of a variety of painful clinical conditions affecting the skin (40,47,49). However, the absence of a "burning placebo" as a control vehicle makes it difficult to conduct double-blind studies, and further research is needed to assess the clinical effectiveness of capsaicin.

Detection threshold determined by applying increasing concentrations of capsaicin on facial skin was used as an objective parameter for sensitive skin (7). Using that procedure Jourdain et al. detected subjects with low and high threshold. This threshold level corresponded with the self-declared level of sensitive skin. The authors concluded that this skin neurosensitivity test appears to be a promising tool for cosmetic diagnosis of sensitive skin (7).

However, the use of capsaicin, in some cases, is accompanied with adverse effects. Initial exacerbation of symptoms, transient burning, and redness at application site is reported by patients in various capsaicin studies. Because of the need for frequent application in the treatment procedure, these side effects may be the major reason for the poor compliance (50,51).

PHYSIOTHERAPY AND SPORT

Although revulsive products are widely used in the physiotherapy practice, few studies report on the efficacy of these products (10).

In physiotherapy, revulsive products are mainly used for the relief of joint and muscle pain. In sports, these products are often used as passive warming-up for activities in cold environments. As active ingredients, the formulations contain analgesic substances, such as salicylates, camphor, menthol, oil of turpentine, and MN or HN as vasodilatory substances (10). Their effect is mainly because of vasodilatory components provoking a thermal effect in the superficial layers of the skin. Analgesic components (e.g., salicylates) may be added in the formulations, but the target tissue of these analgesic substances is not the skin but the underlying muscle, tendon, or joint tissue. Evaluation of the efficacy of regional therapy is beyond the scope of this chapter.

Our literature search did not find reports on the efficacy of revulsive products as used in physiotherapy. We evaluated the effects of three commercially available topical products commonly used in physiotherapy practice.

The thermal effect of these products was evaluated by means of noninvasive measurements of biophysical skin properties. Product 1 was a W/O (Water/Oil) emulsion containing MN at 1.5% and methylglycolisate at 5.0% as active ingredients. Product 2 was an O/W (Oil/Water) emulsion containing MN (1.0%) and α-bisabolol as active ingredients. α-Bisabolol is the active component of camomile and has an anti-irritant effect. Product 3 was a spray containing methylsalicilate (2.0%), menthol (3.0%), and oil of turpentine (5.0%).

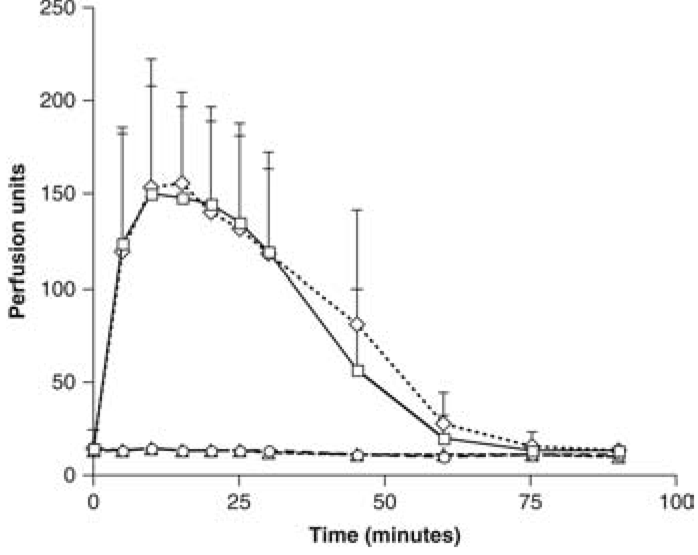

Figure 1 Perfusion of the skin microcirculation in function of time as measured after topical application of product 1 (—□—) product 2 (......◇......); product 3 (- - -o- - -). An untreated control is included (- - -△- - -). Mean ± s.d. ($n = 15$).

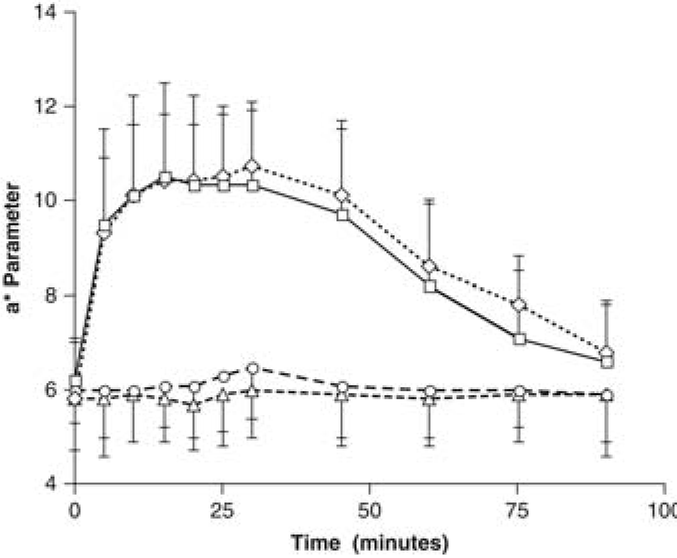

Figure 2 Skin color a* parameter in function of time as measured after topical application of product 1 (—□—) product 2 (......◇......); product 3 (- - -o- - -). An untreated control is included (- - -△- - -). Mean ± s.d. (*n* = 15).

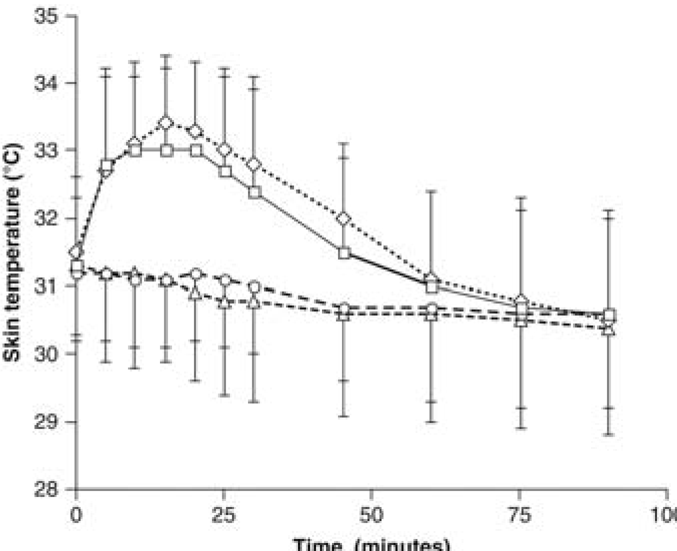

Figure 3 Skin temperature in function of time as measured after topical application of product 1 (—□—) product 2 (......◇......); product 3 (- - -o- - -). An untreated control is included (- - -△- - -). Mean ± s.d. (*n* = 15).

It was found that products containing nicotinates provoked a significant increase in the perfusion of skin microcirculation, a significant increase of the superficial skin temperature, and a significant reddening of the skin color (erythema) (see Fig. 1–3). The maximum increase obtained in skin temperature (±2°C) is believed to have little effect on the underlying tissues. However, the strong sensation in the skin can "pull away" the pain sensation from other tissues.

Comparison of the three measurement techniques gives information concerning the underlying mechanism of erythema production: the arterial vasodilatation causes an increased blood flow (reaching its maximum 10 minutes post application), resulting in an increased amount of blood in the superficial capacitance vessels. This results in reddening of the skin (maximum redness 15 minutes post application), and heat is lost by convection (reaching a maximum 15 minutes post application).

We assume that the effect of single application of revulsive products is mainly due to the thermal effect, which is easily quantifiable. The strong sensations in the dermal and epidermal skin layers will relieve the feeling of pain in other tissues or structures. The thermal effect is equally limited, since a maximal increase in skin temperature of ± 2°C was obtained

by nicotinate application. The heat transfer toward muscles and other structures is limited because of the hypodermal layers, containing mainly adipose tissue, which work as very good isolators (52,53). Moreover, deeper tissues have a temperature closer to the core temperature of 37°C. In our experiments, skin temperature reached about 33°C, which is lower than the temperature of deeper structures! This finding points equally to the inefficiency of these revulsive products, as passive warming-up is often used for sport activities in colder environments. On the contrary, the use of such products will provoke a greater transfer of heat toward the cold environment with a possible negative effect on the thermoregulation.

CONCLUSION

Revulsive products produce a reddening of the skin. This erythema is due to an increased perfusion of the microcirculation after a vasodilation of the arterial plexus at the different skin levels.

Nicotinates act via an endothelium relaxant factor, while capsaicin uses a neurogenic cascade with involvement of substance P.

The more clinical applications of nicotinates aim to increase the perfusion of the superficial microvasculature to obtain increased skin temperature and a kind of pain relief by "pulling away" the pain sensation located in the deeper tissues (gate control). In capsaicin treatments, desensitization is aimed by depletion of substance P at the nociceptive sensors. This can only be obtained by long-term multiple treatment regimes (up to 4 times a day for 12 weeks).

For nicotinates, no side effects are reported, while the adherence to capsaicin treatment is rather weak due to the inconvenience of the side effects at the site of application (burning, itching, etc.).

Despite nicotinates being widely used in physiotherapy (sport), there is only limited evidence for the efficiency of these treatments. Reports on capsaicin treatment indicate a moderate positive effect under different clinical situations. However, most of the designs lack an adequate placebo treatment. In the experiments of Low et al. using a nicotinate solution as "burning placebo," no difference was found between the capsaicin versus the burning placebo in the relief of pain (41). Hence, the use of nicotinates, in different clinical treatments, needs to be further elaborated. The absence of side effects may improve compliance.

Nicotinates are widely used in more fundamental research toward percutaneous penetration, since the quantification of the vascular response can be used as an indicator for the skin bioavailability. Other skin properties, such as the problem of sensitive skin can also be studied using a nicotinate challenge. In more recent reports, a capsaicin challenge has been developed to study the sensitive skin.

The instrumentation available nowadays allows a more precise evaluation and quantification of physiological responses. Using these techniques under experimental and clinical conditions may increase the knowledge and evidence in the use of revulsive products.

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65 Cooling Ingredients and Their Mechanism of Action

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INTRODUCTION

The use of "purified" cooling agents in pharmaceutical and cosmetic preparations only dates back to the late 1880s with the commercial production of menthol from Japanese peppermint (*Mentha arvensis*) oil in Japan (1). The cultivation of peppermint in Japan before the Christian era appears to predate any other country, and menthol is reputed to have been used medicinally for almost as long (2). In the Western world, it was about 1770 that the Dutch botanist, H. David Gaubius, first isolated menthol from the oil of *Mentha piperita* in Utrecht (2,3). Prior to the commercial availability of menthol, the essential oils of peppermint varieties (primarily *M.* and *M. arvensis*) were the sole source for use as cooling minty ingredients. It is significant that at the end of the 18th century only about 900 to 1400 kg of peppermint oils (both *piperita* and *arvensis*) were consumed worldwide (1). By the late 1890s, production had increased to about 175,000 kg (2). In 2007, total peppermint oil production was estimated at more than 26,000,000 kg, with about 21,500,000 kg being the oil of *M. arvensis* (commonly referred to as cornmint oil), which is used mostly for the production of natural *leavo*-menthol (4).

This chapter reviews the use of menthol and new classes of cooling agents that have been discovered since the 1970s. In addition, we briefly touch upon the efficacy of cooling agents as insect repellents. Finally, recent findings on the physiological mechanisms of cold receptors are presented.

COOLING INGREDIENTS

Menthol Background

Before World War II, production of *leavo*-menthol [hereafter referred to as (–)-menthol] was controlled exclusively by Japan and China. In 1939, Japan exported 268,920 kg of menthol, while China's exports in 1940 were 190,909 kg (1). With the advent of war, shipments to the allied countries ceased and major shortages ensued. While synthetic (–)-menthol could be produced from high citronellal feed stocks (e.g., citronella oil and citronella-type eucalyptus oils), this also was no longer an option. However, Japanese and Chinese immigrants in Brazil rapidly began planting *M. arvensis* for menthol production. In 1941, Brazil produced 5000 kg of menthol, rising to 1,200,000 kg by 1945 (1). By the 1960s, Brazil's production peaked at about 3,000,000 kg, while about the same time China began supplying menthol again.

During the 1960s, an oversupply of menthol caused the price to fall to as low as $7.70 to $8.80 per kilogram, and processors reduced production levels. This ultimately led to worldwide shortages and a price spike as high as $50 plus per kilogram in 1974 (with similar price spikes now occurring about every 10 years) (3). As menthol is a commodity, it is sometimes subject to financial speculation, which exacerbates price swings.

In 1958, India began expanding plantings of *M. arvensis*, but, until the late 1980s, the quality was highly variable and often had low menthol content. In the 1980s, new strains were introduced that gave improved oil yields and had menthol contents of 75% to 85%. By 1996, India was producing 6000 metric ton of *M. arvensis* oil and had long surpassed China as the major producer of menthol (3). In 2007, it was estimated that India would produce in excess of 20,000 metric ton of this mint oil. While the bulk of current production is used for local menthol crystallization, significant amounts of oil and crude menthol fractions are exported to Brazil, Taiwan, and Japan for further purification. The residual oil left after crystallizing

menthol still contains 35% to 45% menthol as well as menthones and other typical mint components. Much of this oil (commonly referred to as dementholized cornmint oil) is rectified by distillation and sold for use where normal peppermint oil (ex *M. piperita*) is used (toothpaste, mouthwash, etc.). In addition, some of this dementholized oil is fractionated to isolate the menthones (which can be converted by reduction into (–)-menthol) and other "natural" flavor chemicals.

During the 1970s and 1980s, a number of new routes to synthetic (–)-menthol were developed, only two of which led to long-term commercial success. These processes have been reviewed by both Leffingwell (5) and Hopp and Lawrence (6). Today, the procedure developed by Haarmann and Reimer (now Symrise) on the basis of hydrogenation of thymol to racemic *dl*-menthol followed by selective crystallization of (–)-menthol (via the benzoate ester) is the major process (7).

The Takasago process uses myrcene as the raw material, which is converted to *N,N*-diethylgeranylamine and then asymmetrically isomerized via the chiral rhodium (S)-BINAP (or SEGPHOS) complex to the optically active enamine of citronellal. Hydrolysis yields (+)-citronellal, which is cyclized to (–)-isopulegol by classical methods. On hydrogenation, the isopulegol gives (–)-menthol in high optical purity (8,9). An alternative starting material (instead of myrcene) is isoprene, which can be dimerized to *N,N*-diethylnerylamine. This material can be converted to (–)-menthol in a manner analogous to the myrcene route using rhodium (R)-BINAP as the chiral catalyst (10). Reflecting on the Takasago process, Ryoji Noyori stated in his 2001 Nobel lecture, "*This resulted from a fruitful academic/industrial collaboration. . . .*" (11).

Clark estimates that 2007 worldwide consumption of menthol from all sources (i.e., peppermint oils, natural menthol, and synthetic menthol) is 32,000 metric ton, of which 19,170 metric ton is purified menthol (4).

Table 1 provides our estimate of production in producing countries (or in the case of Symrise and Takasago, company production of synthetic (–)-menthol).

Table 2 provides the 2007 estimated worldwide usage of menthol by consumer product category—on the basis of Clark's data by region (4).

Table 1 Worldwide Sources of Menthol (2007)

Source	Metric ton
India (natural)	9,700
China (natural)	2,120
Symrise (synthetic)	3,600
Takasago (synthetic)	1,500
Other synthetic[a]	1,200
Brazil (natural)[b]	450
Taiwan (natural)[b]	300
Japan (natural)[b]	300
Total[c]	19,170

[a]Other synthetic includes menthol produced from menthone as well as racemic menthol.
[b]Primarily from *Mentha arvensis* oil or crude menthol ex India (or China).
[c]Total menthol volume based on Clark's estimate (4).

Table 2 2007 Estimated Worldwide Consumption of Menthol % by Product Category

Product category	Menthol %
Oral hygiene	28.00
Pharmaceuticals	26.60
Tobacco	25.30
Confectionaries	11.00
Shaving products	7.00
Miscellaneous	2.10

Source: From Ref. 4.

Menthol Chemistry

Menthol is a $C_{10}H_{20}O$ terpenoid alcohol (MW 156.27) with three chiral centers leading to eight possible stereoisomers (4 enantiomeric pairs). The characterization of the stereoisomeric menthols was painstakingly resolved prior to the availability of modern methods by Read (12,13). The structures of the eight enantiomers, with their optical rotations (in ethanol), are shown in Figure 1.

Only the (–)-menthol enantiomer possesses the clean desirable minty odor and intense cooling properties. For example, the (+)-menthol enantiomer is less cooling and possesses a musty off-note odor that is undesirable in most applications. This musty note is also present in racemic menthol (15). The organoleptics and cooling strengths of all of the enantiomers have been reviewed (5,6). Figure 2 provides the cooling thresholds in ppm.

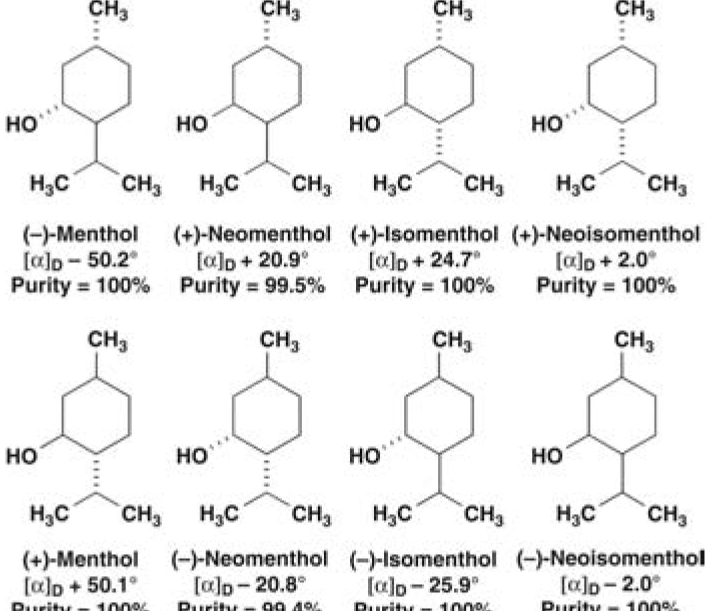

Figure 1 Stereoisomers of menthol. *Source*: From Ref. 14.

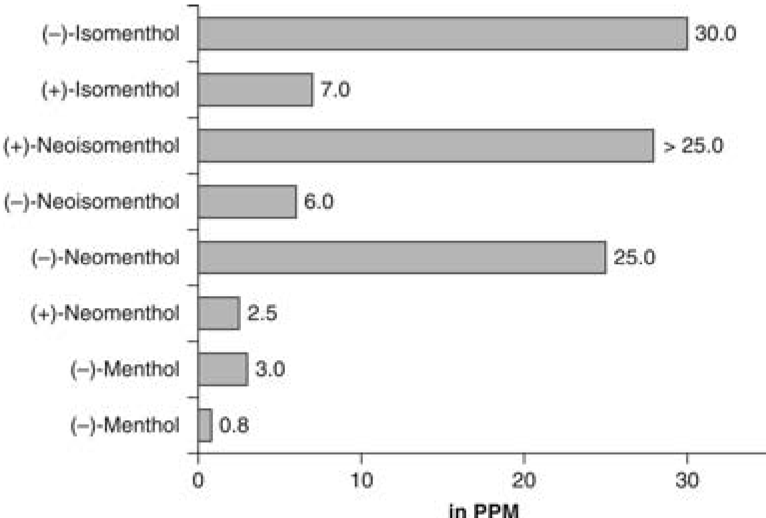

Figure 2 Cooling thresholds (in ppm) (by taste dilution). *Source*: From Ref. 14.

Table 3 Major Impurities in Synthetic and Natural Menthols

Major impurities	Synthetic %	Brazil 1 %	Brazil 2 %	China 1 %	China 2 %	India %
Menthone	0.0069	0.0258	0.0258	0.0135	0.0350	0.0295
Isomenthone	0.0000	0.0069	0.0172	0.0052	0.0123	0.0155
Menthyl acetate	0.0000	0.0100	0.0148	0.0014	0.0128	0.0048
Isopulegol	0.0022	0.1868	0.1651	0.1374	0.1914	0.1789
Neomenthol	0.0032	0.0689	0.1339	0.0951	0.0882	0.1079
Neoisomenthol	0.0000	0.0075	0.0459	0.0352	0.0177	0.0368
Isomenthol	0.0299	0.0099	0.0442	0.0296	0.0248	0.0322
Piperitone	0.0000	0.0053	0.0046	0.0018	0.0031	0.0024
Totals	0.0422	0.3211	0.4515	0.3192	0.3853	0.4080

Source: From Ref. 16.

Natural menthol ex *M. arvensis* oil is normally about 99.0% to 99.6% pure, with the remaining impurities being other constituents found in the cornmint oil. While, in most cases, the mint oil impurities contribute a pleasant peppermint aroma, certain impurities, such as mint sulfide, can also impart less desirable and harsh notes. Thus, odor discrepancies often arise when comparing samples from different companies or countries. To overcome such differences, the skilled technician can add a small percentage (e.g., 0.2–0.4%) of terpeneless peppermint oil ex *M. piperita* (or redistilled dementholized cornmint oil), which adds the desirable sweet peppermint top note. Table 3 compares the major impurities present in synthetic menthol and natural menthol samples from major producing areas (16).

Although not generally commercially available, menthol produced from *M. piperita* oil has a sweeter peppermint top note than that produced from cornmint oil (JC Leffingwell, unpublished observations).

Synthetic (–)-menthol is normally about plus 99.8% pure and has less of the minty top note present in natural menthol. Again, this can be adjusted to increase the mint character, if desired, by the addition of a small amount of terpeneless peppermint oils.

Menthol-Related Cooling Agents

Interest in menthol-related cooling agents began in the late 1950s to 1960s when several tobacco companies began to develop various esters as potential menthol release agents (17–19), some of which now appear on the flavor extract manufacturers association's GRAS list. Among those of interest today is monomenthyl succinate (MMS) (FEMA# 3810) (18), which was later patented by Mane as a cooling agent for general use (20). In addition, menthol ethylene glycol carbonate (Frescolat® MGC), with FEMA# 3805, and menthol propylene glycol carbonate (Frescolat MPC), with FEMA# 3806, were first patented as tobacco flavorants (19), again to be later patented by Haarmann and Reimer for general cooling usages (21).

A number of other menthol-related cooling agents are commercially available: menthone glycerol ketal (Frescolat MGA) (22)—both the racemic (FEMA# 3808) and *leavo* forms (FEMA# 3807); the *leavo* form appears to be the main item of commerce. This material provides a clean cooling refreshing effect and as a partial replacement of peppermint oil has been shown to provide longer-lasting sweetness and a higher cooling sensation in chewing gum (23). (–)-Menthyl lactate (Frescolat ML) is faintly minty in odor and virtually tasteless with a pleasant, long-lasting cooling effect (24). Recently, Erman has shown that the (–)-ML of commerce has the 'S' configuration for the hydroxy moiety, indicating the fact that it is produced by the esterification of (–)-menthol with (S)-(+)-lactic acid (25). 3-(l-Menthoxy) propane-1,2-diol, known as MPD, Coolact® agent 10, TK-10, and coolant agent 10, is another important commercial cooling agent, which, in contrast to menthol, is essentially odorless (26). The cooling threshold (in mouth) is 1 ppm (about 20–100% that of menthol), and the time of cold-feeling maintenance is 20 to 25 minutes for a 100-ppm solution (about twice that of menthol). While the cooling strength of Coolact agent 10 is accepted as being about 20% to 25% that of menthol, it is also noted that "in a Vaseline ointment, 3-(l-menthoxy)propane-1,2-diol shows a cool feeling 2.0 to 2.5 times stronger than that of (–)-menthol" (27). The cool-feeling intensity of the (2S) isomer is 2 to 3 times that of the (2R) isomer and 1.5 to 2 times

superior to that of the racemic modification (28). Similarly, the related menthoxyalkanols, 3-(l-menthoxy)-2-methylpropane-1,2-diol (FEMA# 3849), 3-(l-menthoxy)ethanol (Coolact 5), FEMA# 4154, 3-(l-menthoxy)propan-1-ol, and 3-(l-menthoxy)butan-1-ol have cooling properties (29). Interestingly, cooling compounds such as 3-(l-menthoxy)propane-1,2-diol and 3-(l-menthoxy)-2-methylpropane-1,2-diol when admixed with warming sensates (e.g., vanillyl butyl ether, ginger extract, or capsicum tincture) provide increased warmth and longer-lasting warmth in cosmetic and flavor systems (27,30,31). Conversely, it has also been observed that admixtures of such cooling compounds with the warming sensate vanillin-MPD (the acetal of 3-(l-menthoxy) propane-1,2-diol and vanillin), FEMA# 3904, can increase the duration of cooling sensations (32) (Fig. 3).

(–)-Isopulegol (Coolact P), FEMA# 2962, having a chemical purity of better than 99.7% and an optical purity of not less than 99.7% ee, is odorless and gives a feeling of freshness, crispness, and coolness. The cooling strength is about 20% to 30% that of (–)-menthol (33). The *p*-menthane-3,8-diols (Coolact 38D, PMD38), FEMA# 4053, consist of a mixture of (+)-*cis* and

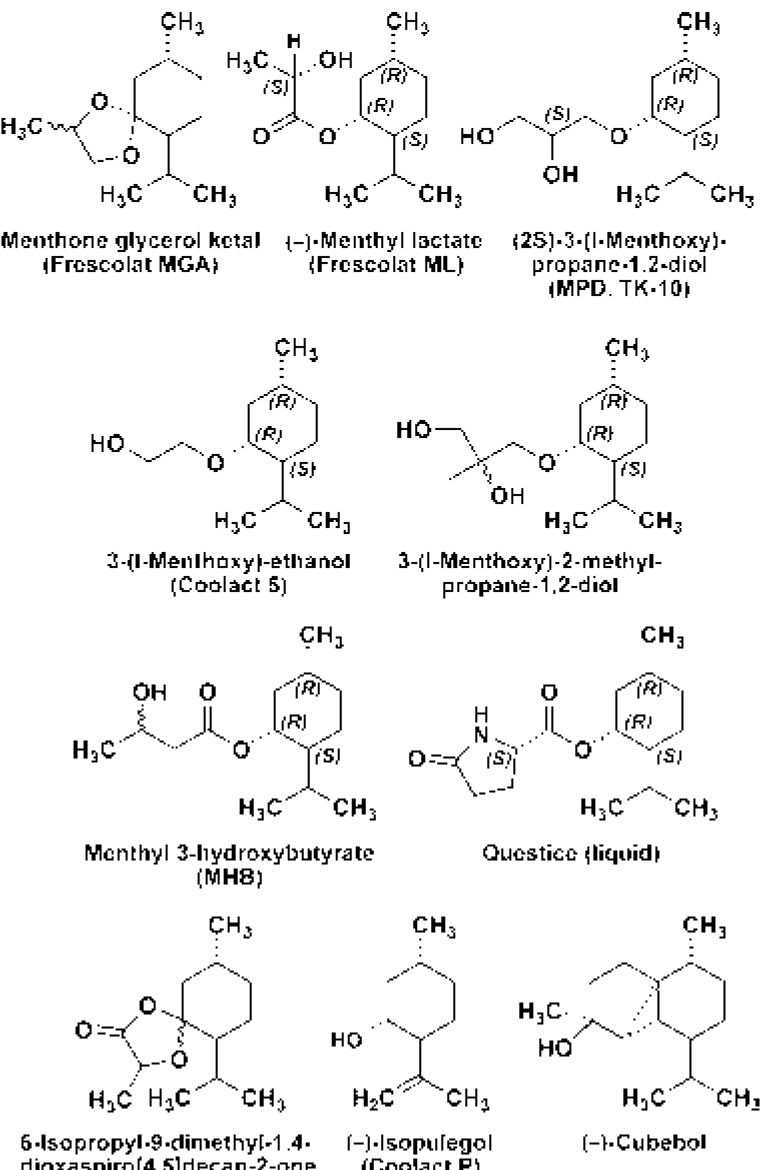

Figure 3 Menthoxy-related coolants.

(–)-*trans* PMD38 in a ratio of ~62:38 and possesses a cooling strength of about 11% that of (–)-menthol (27,34). PMD38 is a nature identical material that occurs in a number of citronellal-rich oils (e.g., *Litsea cubeba*, *Eucalyptus citriodora*) and is also effective as an insect repellant (34–36). (–)-Monomenthyl glutarate (Physcool 2, MMG), FEMA# 4006, is a nature identical cooling agent that has been found in *Litchi sinensis* accompanied by (–)-dimenthyl glutarate (37). It has been described as "probably the longest-lasting oral cooling agent that is commercially available" (38). Recently, an improved synthesis has been reported for both MMG and MMS that minimizes the amount of diester impurities (39). Similarly, (–)-MMS has been confirmed to be nature identical by its isolation from *Lycium barbarum* and *M. piperita* (37). A recent description of MMS indicates that it is virtually tasteless and has well-balanced cooling onset and length of cooling (38). Questice® (menthyl pyrrolidin-2-one 5-carboxylate) was first patented as a composition of matter that acts as a long-lasting cooling and fresh ingredient in toothpaste. The cooling properties are due to the enzymatic hydrolytic release of menthol. A liquid form was produced by reacting (–)-menthol with L-pyrrolidin-2-one carboxylic acid, while a crystalline form was produced when racemic DL-pyrrolidin-2-one carboxylic acid is employed (40). Surprisingly, it did not appear on the GRAS list until 2005 with FEMA# 2155 (41). However, it has long been employed in various cosmetics, lotions, etc. Recently, Erman has shown that the liquid form of Questice is a diastereoisomeric mixture of (–)-menthyl 5-oxopyrrolidine-2-carboxylates with a ratio of the 5S:5R configuration of ~91:8, while the solid form has a ratio of ~46:53 (25). (–)-Menthyl 3-hydroxybutyrate (MHB), FEMA# 4308, is another recent addition to the GRAS list (42). This is reported by workers at Takasago as having a long-acting excellent cooling effect and is odorless and tasteless. Potential uses include foods, drinks, cosmetics, pharmaceuticals, and cigarettes (43). Other workers indicate that the cooling effect is slightly stronger than ML (about 48% the cooling strength of menthol) (44). Firmenich workers have recently found that a diastereoisomeric mixture of the 6-isopropyl-3,9-dimethyl-1,4-dioxaspiro[4.5]decan-2-ones, prepared by reacting lactic acid with *cis* and *trans*-menthones, provides a minty, fresh, *piperita*-type flavor that is remarkable by its strength and cleanness. In combination with other cooling agents (e.g., menthyl succinate or menthol), a synergist increase in cooling strength was found. In particular, the (3S,5R,6S,9R) and (3S,5S,6S,9R) isomers are preferred (45). A patent describes the use of certain esters such as (–)-menthyl methoxyacetate and (–)-menthyl 3,6-dioxaheptanoate as cooling agents (46). In addition to the cooling properties, (–)-menthyl methoxyacetate has a head note and fruity taste resembling that of menthyl acetate, whereas (–)-menthyl 3,6-dioxaheptanoate has a bitter taste. Cubebol, a natural isolate of cubeb oil, in which it normally occurs at levels of 10% to 30% (47), is a sesquiterpenoid alcohol that has a certain stereochemical resemblance to menthol and, while not menthol derived, is included here for completeness. Cubebol has only a very weak smell and taste and provides a refreshing effect that develops in the mouth after a delay of approximately 1 to 2 minutes and lasts for approximately 30 minutes. It has applications in flavors, oral care, pharmaceutical products, etc. (48).

Carboxamide Cooling Agents

During the early 1970s, Wilkinson Sword Ltd. conducted an extensive research program in which they designed and evaluated about 1200 compounds for their cooling activity (49,50). The interest in such compounds related to cooling agents without the minty and volatile side effects of menthol, such as eye irritation, in aftershave lotions, etc. Over 25 U.S. patents were issued on these materials (51). Of these original Wilkinson Sword compounds, three were initially commercialized: WS-3 (*N*-ethyl-*p*-menthane-3-carboxamide) (52), WS-23 (2-isopropyl-*N*,2,3-trimethylbutyramide) (53), and WS-14 [*N*-([ethoxycarbonyl]methyl)-*p*-menthane-3-carboxamide] (52). WS-3 was given GRAS status (FEMA# 3455) in 1975 (54) and WS-23 (FEMA# 3804) in 1996 (55). Interestingly, WS-14 was used as a cooling agent for the Northwind cigarette introduced into test market in 1981. This test market was short lived, but it is not clear if this was because of market failure or concern that the additive testing conducted was insufficient to pass Food and Drug Administration (FDA) scrutiny (56). WS-14 is commercially available as ICE 4000 cooling sensate (57) and finds some applications as a topical cooling agent (Fig. 4).

In 2007, WS-5 [ethyl 3-(*p*-menthane-3-carboxamido)acetate], which is currently the coldest of all commercial cooling agents, was granted GRAS status as FEMA# 4309 (42). It has been found that only highly purified WS-5 is suitable for flavoring purposes (58), as less pure

WS-3 WS-23

WS-14 WS-5

Figure 4 Successful Wilkinson sword coolants.

material exhibits a powerful bitter taste. WS-3 and WS-23 are currently the two largest volume carboxamide coolants. They are widely used in flavors, especially for chewing gum, breath fresheners, confectionaries, and oral care. They also find use in cosmetics (e.g., aftershave lotions). As both WS-3 and WS-23 are solids, there has been considerable interest in developing blends of such cooling agents that provide strong cooling but are easy to handle liquids. For example, it has been found that mixtures of ML, WS-3, and propylene glycol form stable liquid systems (59). It has also been shown that WS-3, WS-5, WS-14, and WS-23, alone or in certain combinations, when mixed with ML (or other coolants such as menthoxypropane-1,2-diol) will form stable liquid systems (60), and such mixtures often give a synergistic increase in cooling sensation. Similarly, eutectic mixtures of WS-3 and WS-23 provide liquid cooling systems (61,62), which can be used either as cooling agents or as flavor and saltiness enhancers.

Another compound that can be classified either as a carboxamide or a menthyl ester is *N*,*N*-dimethyl menthyl succinamide (FEMA# 4230 for the racemate). An International Flavors & Fragrances (IFF) patent (63) describes this as having a cooling onset time of 25 seconds with cooling duration of 11.25 minutes. The taste/sensory profile is "cooling and refreshing on tongue, palate and front gums; fruity flavor with estery top-notes and sour undertones" (at 25 ppm in water). In a chewing gum at 0.2%, it increased sweetness and exhibited a pleasant and substantive cooling effect on the tongue and roof of the mouth.

Other examples of newly discovered carboxamides coolants are a series of analogs of WS-23 [such as *N*-(2-ethoxyethyl)-2-isopropyl-2,3-dimethylbutanamide] patented by Qaroma (64) and aryl carboxamide analogs (with the reversed amide configuration) by Givaudan (65), many with cooling intensities equal to or greater than WS-23. For example, *N*-(1-isopropyl-1,2-dimethylpropyl)-1,3-benzodioxole-5-carboxamide has about 2.2 times more cooling intensity as compared with 2 ppm of menthol (Fig. 5).

N-(1-isopropyl-1,2-dimethylpropyl)-1,3-benzodioxole-5-carboxamide

N-(2-ethoxyethyl)-2-isopropyl-2,3-dimethylbutanamide

Figure 5 Recent WS-23 analogs.

Figure 6 New *p*-menthane carboxamide coolants.

Of particular interest are various aryl *p*-menthane-3-carboxamides, such as *N*-benzo[1,3] dioxol-5-yl-3-*p*-menthanecarboxamide and *N*-benzooxazol-4-yl-3-*p*-menthanecarboxamide, which are reported to have 100 times more cooling intensity than menthol (when compared with menthol at 2 ppm) (66).

In 2004, T. Hasegawa Co. Ltd. patented a new series of strong cooling compounds on the basis of alkyloxy amides of the *p*-menthane series, which exhibit no bitterness; compound HASE-1 is an example (Fig. 6) (67).

Further, Wei (68) has shown that several materials related to WS-5 possess strong cooling with remarkable cooling longevity. For example, the methyl and ethyl ester analogs of WS-5 (referred to as D-Ala-O-Me and D-Ala-O-Et, respectively) are produced from D-alanine (rather than glycine). Similarly, when D-homoserine lactone is employed, the resultant compound is *N*-(R)-2-oxotetrahydrofuran-3-y1-(1R,2S,5R)-*p*-menthane-3-carboxamide (referred to as "D-HSL"), which also is a potent long-lasting coolant. By combining suitable sympathomimetic amine drugs that act as α-adrenergic receptor agonists to form the corresponding *p*-menthane carboxamides, Wei found certain compounds (such as L-phenylephrine *p*-menthane carboxamide, referred to as CPS-195) that were effective as long-lasting coolants and possessed additional therapeutic properties (69). The cooling duration of a number of these, applied to the skin as a 1% wt/vol in a petrolatum-based ointment, versus leading coolants is shown in Figure 7.

In the last 10 years, there has been extensive patent activity relative to physiological cooling agents. Between 1998 and 2007, more than 280 patents were issued (25,63), and from January 2005 to December 2007 more than 300 patent applications have been filed. It is beyond the scope of this article to review all of these. However, it should be noted that a recent activity trend has been the patenting of various combinations of cooling agents, both to achieve improved cooling properties and/or for liquefaction of solid coolants (59,61,70,71). For example, it has been found that blends of menthyl glutarate with low levels of

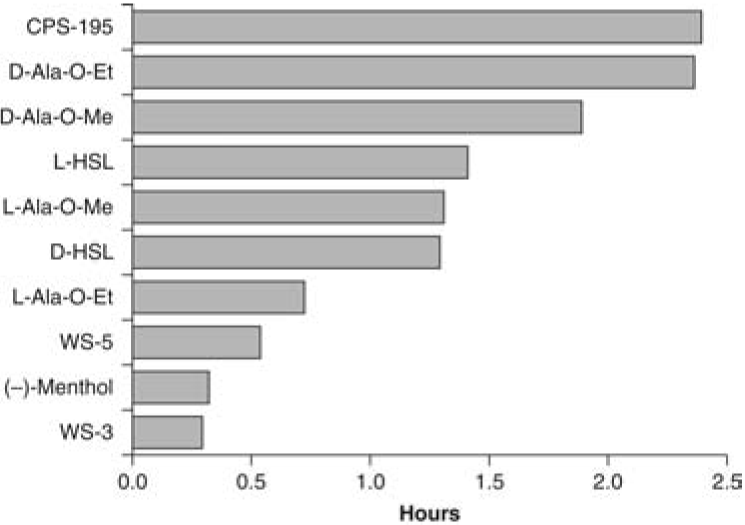

Figure 7 Topical cooling duration 1% in ointment. *Source*: From Refs. 68, 69.

(–)-isopulegol and/or PMD38 exhibit a remarkable synergistic increase in cooling in oral-care products (71).

The relative "accepted" cooling strengths of important coolants are shown in Figure 8 (72,73).

It should be noted that "accepted" cooling strengths, primarily associated with topical skin cooling, do not always agree when compared to oral sensory panel results. This is clearly shown by results obtained by Wm. Wrigley Jr. Company sensory panels comparing 5% sucrose solutions of various coolants versus 100-ppm (–)-menthol, as shown in Figure 9 (71).

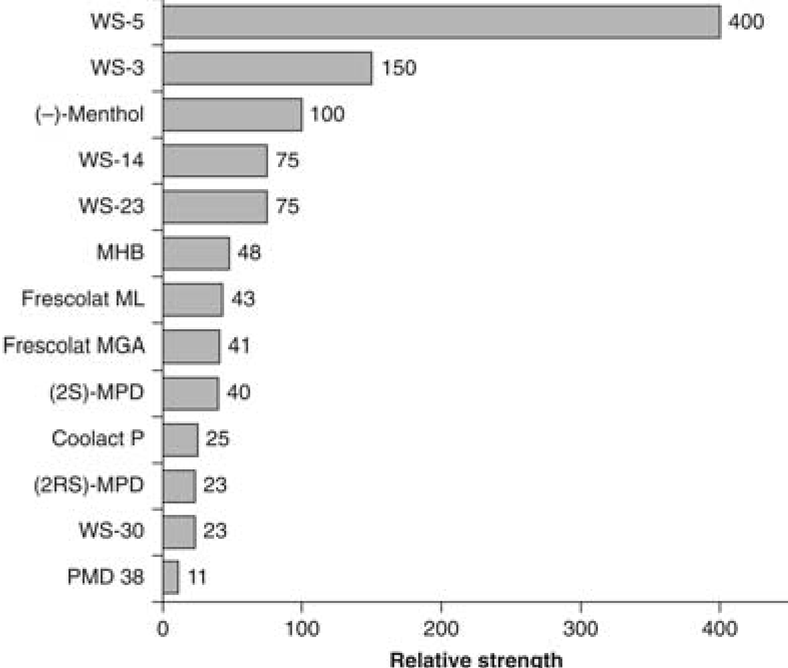

Figure 8 Approximate "accepted" cooling strengths versus menthol (as 100). *Source*: From Refs. 72, 73.

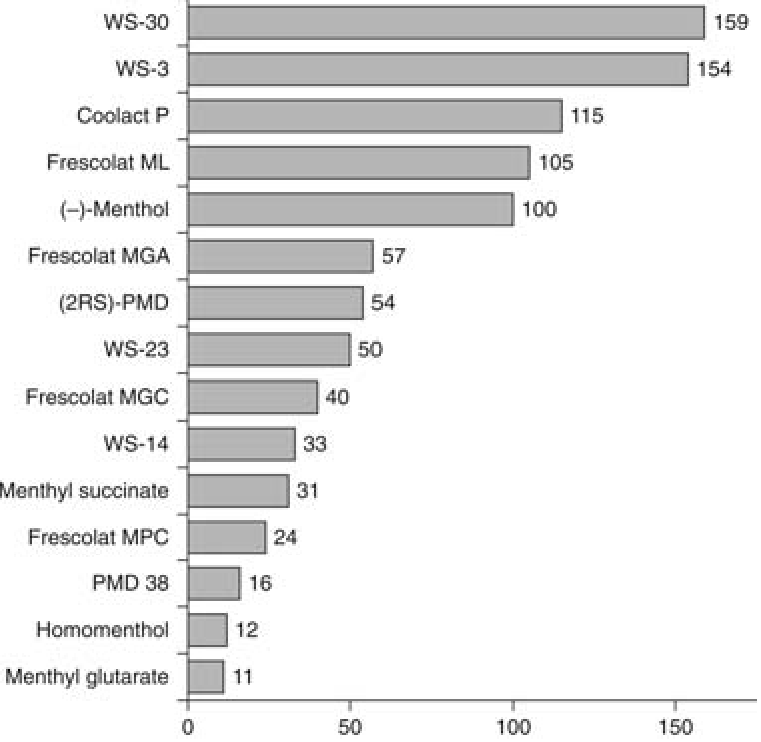

Figure 9 Relative oral cooling in 5% sucrose solutions versus 100 ppm menthol. *Source*: From Ref. 71.

COOLING COMPOUNDS AS INSECT REPELLENTS

As previously mentioned, the PMD38 have shown effectiveness as an insect repellent. Barnard has compared its efficacy against the leading insect repellants DEET, IR3535 [ethyl 3-(*N*-butyl-*N*-acetyl)-aminopropionate], and KBR3023 [sec-butyl 2-(2-hydroxyethyl)piperidine-1-carboxylate] (74,75).

Questice (menthyl pyrrolidone carboxylate) has also been patented as an insect repellent (76) and, Kalbe and Nentwig describe the use of ML or menthol glycerol acetal for repelling mites and other insects (77). Notably, Gautschi and Blondeau of Givaudan have discovered that WS-3 (*N*-ethyl-*p*-menthane-3-carboxamide) and related N-substituted *p*-menthane carboxamides have insect repelling activity against cockroaches equal to or exceeding that of DEET (diethyl-*m*-toluamide) (79). Another Givaudan patent application describes the use of a series of (–)-menthyl carbamates as insect repellents, but is silent relative to their cooling activity (80).

COLD RECEPTORS AND MECHANISM OF ACTION

The underlying process in thermoreception, whether hot or cold, is dependent on ion transport across cellular membranes. Cellular membranes consist of an oily phospholipid bilayer, which would be impermeable to ions such as K^+ or Ca^{2+}, except for receptor protein ion channels.

The flow of ions through these gated ion channels can cause rapid changes in ion concentrations, which in turn produce electrical signals that are the basis for many biological processes (80). In the case of thermoreceptors, these are activated when a thermal (or chemical) stimulus excites primary afferent sensory neurons of the dorsal or trigeminal ganglia (81).

In the last 12 years, there has been tremendous progress in determining the various receptor structural sequences. Thermoreceptors belong to the class of transient receptor potential (TRP) channels of which seven subfamilies exist (TRPC, TRPV, TRPM, TRPA, TRPP, TRPML, and TRPN). Six members of three TRP subfamilies are involved in mammalian

Table 4 Thermoreceptor Agonists

Chemical agonist (botanical source)	ThermoTRP
Capsaicin (hot chilli peppers, e.g., Tabasco®)	TRPV1
Piperine (black pepper corns)	TRPV1
Allicin (fresh garlic)	TRPV1, TRPA1
Camphor (*Cinnamomum camphora*)	TRPV3, TRPV1
Δ-9-Tetrahydrocannabinol (*Cannabis sativa*)	TRPV2, TRPA1
2-Aminoethoxydiphenyl borate (synthetic)	TRPV1, TRPV2, TRPV3
4-α-phorbol 12,13-didecanoate (synthetic)	TRPV4
(-)-Menthol (peppermint)	TRMP8, TRPV3
1,8-Cineole, eucalyptol (eucalyptus)	TRPM8
WS-3 (synthetic)	TRPM8
Icilin (synthetic)	TRPM8, TRPA1
Cinnamaldehyde (cinnamon, cassia)	TRPA1, TRPV3
Allyl isothiocyanate (mustard, horseradish)	TRPA1
Benzyl isothiocyanate (mustard, horseradish)	TRPA1
Phenethyl isothiocyanate (mustard, horseradish)	TRPA1

Abbreviation: TRP, transient receptor potential.
Source: From Refs. 80, 84.

temperature-sensitive thermoreception. The closely related TRPV analogs are activated by heat, TRPV1 (≥43°C), TRPV2 (≥52°C), TRPV3 (22–40°C), and TRPV4 (>~27°C), while TRPM8 (<~28°C) and TRPA1 (<~18°C) are activated by cold (80). Certain types of chemical agonists activate these same thermoTRP channels. TRPV1 was the first thermoreceptor characterized and is referred to as a vanilloid receptor, as it is activated by capsaicin as well as heat. The cold and menthol receptor, TRPM8, was characterized by McKemy, Neuhausser, and Julius (82) and by Peier et al. (83) in 2002. Paradoxically, the cold receptor TRPA1, which is activated by noxious cold to produce a pain-like sensation, produces a human sensorial effect often described as "hot."

Table 4 provides examples of chemical agonists that activate these thermoTRPs.

All of these thermoTRPs are gated Ca^{2+} channels consisting of six transmembrane domains (TM1–TM6) flanked by large N- and C-terminal cytoplasmic domains (80). A schematic representation is shown in Figure 10 with the putative ion channel between TM5–TM6 in TRPM8, which is activated by menthol and other cold stimuli. In the case of TRP channels, it has been shown that they can oligomerize into tetramer assemblies, which presumably modulate the calcium ion gating processes (85–87).

Much of the knowledge gained on TRP activation by chemical stimuli has been derived by genetic expression of putative receptor domains and measurement of Ca^{2+} flux intensity by fluorometric imaging assays. Behrendt et al. used this technique to screen 70 odorants and menthol-related substances for activity on the recombinant cold-menthol receptor TRPM8

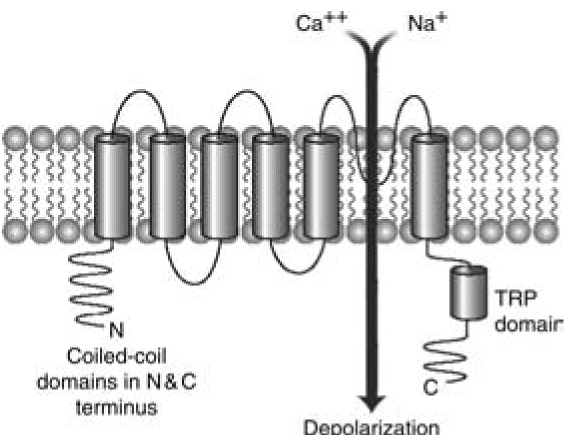

Figure 10 TRPM8 receptor channel.

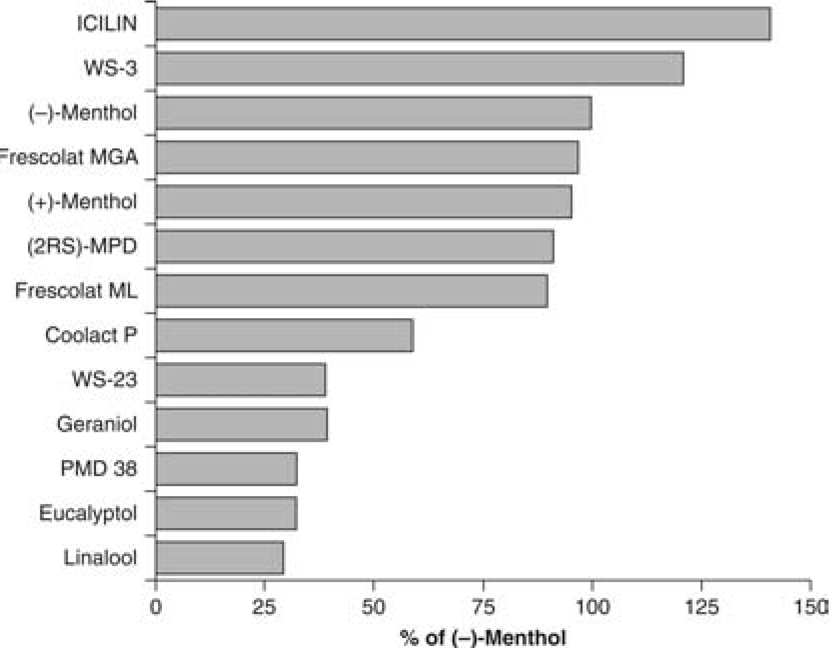

Figure 11 Efficacy of coolants on the TRPM8 receptor (by Ca^{2+} fluorometric assay). *Source*: From Refs. 72, 73, 88.

(mTRPM8), as expressed in HEK293 cells (88). The percentage efficacy of the most active candidates as compared to menthol is shown in Figure 11.

Although the fluorometric assay technique does not always translate into the human perception scale, it is already being used in industry to screen for promising new coolants (89).

In conclusion, from peppermint to menthol and to a plethora of new novel cooling compounds, we are now beginning to understand the importance of cooling substances even in the genetics of life. From early menthol-camphor-based over-the-counter (OTC) pharmaceuticals, which created famous trademarks such as Vicks® Vaporub and Mentholatum® in the early 20th century, to improved modern toothpastes, gums, breath fresheners, and cosmetic lotions, we expect this is just the beginning of even greater things for the future.

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66 Oral Cosmetics

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INTRODUCTION

As a living tissue, the skin requires nourishment to stay healthy. This nourishment cannot be provided by topical preparations only, but it largely comes from foods that are rich in vitamins, minerals, essential fatty acids, and other nutrients, which are crucial for optimal skin health and wellness. Skin functioning relies on specific nutritional needs, as evidenced by the development of skin disorders in response to various nutritional deficiencies (1,2). Supplementation with vitamins, minerals, and other dietary constituents was shown to improve skin conditions (3–5).

Cosmetics are preparations for topical use, i.e., products intended to be rubbed, poured, sprinkled, or sprayed on, introduced into, or otherwise applied to the human body for cleansing, beautifying, promoting attractiveness, or altering the appearance. Among the products included in this definition are skin moisturizers, perfumes, lipsticks, fingernail polishes, eye and facial makeup preparations, shampoos, permanent waves, hair colors, toothpastes, and deodorants as well as any material intended for use as a component of a cosmetic product (6). Cosmeceuticals are topical preparations specifically designed to improve the appearance of aging skin (7). The term "oral cosmetics" is used in the present chapter with respect to dietary supplements, which claim to have a beneficial, physiological effect on skin, hair, or nails, i.e., these are preparations for oral use only, such as capsules, tablets, liquids, or granulates. In this chapter, we will focus on oral antiaging preparations.

AIM OF ORAL COSMETICS

Intrinsic and Extrinsic Aging

Human skin represents the body's barrier to the external environment preventing it from mechanical damage, noxious substances, invading microorganisms and radiation. Moreover, the skin plays an important role in homeostatic regulation, controlling water retention, sensory perception, and immune surveillance (8,9). Aging leads to several changes in the skin and its appendages (i.e., hair and nails). These changes can be broadly categorized as either intrinsic aging (chronobiological) or photoaging (actinic aging). Oral cosmetics can be formulated to slow down this aging process.

Intrinsic aging is, by definition, inevitable and thus not subject to manipulation through changes in human behavior. Skin that ages intrinsically is smooth and unblemished and characterized by some deepening of skin surface markings (small wrinkles). Histologically, such skin manifests epidermal and dermal atrophy, reduced numbers of melanocytes, Langerhans cells, fibroblasts, and increased cell architecture disorders (10,11). Telomere shortening combined with metabolic oxidative damage is believed to play a major role in the intrinsic aging process (11,12).

Conversely, extrinsic aging is engendered by factors that originate externally and are introduced to the human body, such as smoking, excessive alcohol consumption, poor nutrition, and chronic exposure to the sun (13). Sun exposure is considered to be the most significantly deleterious to the skin; 80% of facial aging is believed to be due to chronic sun exposure (11). Leathery surface of the skin with blotches, yellowing, and deep wrinkles comprise the clinical presentation of photoaged skin. A marked decrease in collagen,

glycosaminoglycans, and proteoglycans is observed. Losses in tone and elasticity, epidermal atrophy, and distinct alterations in collagen and elastic fibers are also associated with photoaged skin. In aged skin, collagen is characterized by thickened fibrils, organized in ropelike bundles that appear to be in disarray in comparison to the pattern observed in younger skin. Alterations in elastic fibers are so strongly associated with photoaged skin that "elastosis," an accumulation of amorphous elastin material, is considered pathognomonic of photoaged skin (9,11,14–17). Compounds, which stimulate the synthesis or inhibit the degradation of connective tissue components (e.g., collagen, keratin, and glycosaminoglycans), may slow down the aging process or even rejuvenate the dermis and its appendages. Such compounds are potential candidates to be used in oral cosmetics.

Photoaging

Extensive research in the area of photoaging over the past decade has resulted in an improved understanding of the molecular mechanism of the aging process. Ultraviolet (UV) light penetrates into the skin; depending on its wavelength, it interacts with different cells that are located at different depths. UV light of the shorter wavelengths (UVB, 280–320 nm) is mostly absorbed in the epidermis and predominantly affects epidermal cells, i.e., keratinocytes, while longer-wavelength UV light (UVA, 320–400 nm) penetrates deeper and can interact with both epidermal keratinocytes and dermal fibroblasts. Melanin pigmentation of the skin absorbs UV light and thus protects skin cells from the detrimental effects of UV exposure. Once UV light has reached the cells of the skin, the different wavelengths exert their specific effects. UVA light mostly acts indirectly through generation of reactive oxygen species (ROS). "ROS" or "pro-oxidants" is a collective term for oxygen-derived species, i.e., oxygen radicals (e.g., superoxide anion, hydroxyl radical) and certain non-radicals (e.g., peroxides) that easily convert into radicals (18). ROS exert a multitude of effects such as lipid peroxidation, activation of transcription factors, and generation of DNA damage. While UVB light can also generate ROS, its main mechanism of action is the direct interaction with DNA via induction of DNA damage (12,19–21).

The skin's enzymatic antioxidant defense includes an enzymatic and a nonenzymatic system (Table 1). Copper-zinc superoxide dismutase (SOD), manganese SOD, catalase (CAT), and the selenoenzyme glutathione peroxide (GPX) have a direct antioxidant function, i.e., SOD converts superoxide anion into hydrogen peroxide, whereas CAT and GPX degrade hydrogen peroxide into water. Nonenzymatic antioxidants are classified into two groups, namely, endogenous (e.g., glutathione, α-lipoic acid) and dietary antioxidants such as vitamins and polyphenolic compounds (e.g., flavonoids). Cases of increased ROS generation and/or a depletion of the antioxidant levels will cause oxidative stress defined as a disturbance in the balance favouring ROS generation and leading to potential tissue damage (21,22). The use of oral supplements, which contain antioxidants (e.g., polyphenols, vitamin E and A), or compounds, which stimulate the enzymatic antioxidant system (e.g., selenium compounds to stimulate GPX activity), may protect the dermis against oxidative stress and prevent tissue damage.

Immune Function and Inflammation

In the last two decades it has become clear that the skin is an essential part of the immune system (23). Reduced immune function and inflammation can alter skin condition and

Table 1 Primary Components of the Human Antioxidant Defense System

Enzymatic	Nonenzymatic
Direct antioxidant function: SOD GSHPx CAT	*Endogenous antioxidants*: α-lipoic acid, glutathione, melatonin, coenzyme Q
Indirect antioxidant function: Glutathione-S-transferase GSSG reductase	*Dietary antioxidants*: Vitamin C and E Polyphenolic compounds

Abbreviations: SOD, superoxide dismutase; CAT, catalase; GSHPx, glutathione peroxidase; GSSG, Diglutathione.

functioning. Sunburn is a well-known acute effect of sun exposure and is clinically visible as erythema triggered by inflammation. After a certain threshold of UV exposure is reached, delayed and prolonged vasodilatation allows the passage of lymphocytes and macrophages into the tissue, which induces inflammation. Increased dietary intake of antioxidants or oral anti-inflammatory compounds was suggested to reduce UV irradiation-induced eythrema (3), i.e., these compounds are useful in the formulation of oral cosmetics claiming specifically photoprotection.

ACTIVE COMPOUNDS IN ORAL COSMETICS

Screening of Active Compounds

Clearly, the oral route of administration requires other product characteristics compared with a classical, topical cosmetic, and key issues in the development of an oral cosmetic are toxicology, bioavailability (absorption and distribution), and metabolization of its components. In vitro studies and animal studies are useful to study the mechanism of action, but studies in humans are required to document the efficacy of oral cosmetics to validate product claims. Observational studies do not have control over product exposure (e.g., longitudinal study) and are therefore limited to identifying associations between a dietary ingredient and skin benefits; i.e., such studies cannot provide a sufficient basis to determine whether a significant correlation between a product and a benefit reflects an underlying rather than a chance relationship. Intervention studies are more reliable since the investigator can control exposure of the study population to the investigated product. Nevertheless, these clinical trials should be randomized, placebo controlled, and double blinded to minimize bias of the study results. In addition, any change in dietary habits should be avoided, and the intake of other food supplements should be controlled during the complete study. The use of validated bioengineering methods combined with both clinical observations by the investigator and observations by the participant using standardized questionnaires are highly recommended to evaluate the efficacy of the treatment. Intervention studies to study the effect on the dermis are lengthy (5–12 months), and the use of a placebo with an identical appearance (e.g., galenic form, color, taste, and odor) compared with the active product is essential to determine the seasonal influence and the subjective effect on the investigated parameters. A parallel study design (i.e., separate groups of volunteers administered different products) is preferred since seasonal influence may bias the results from crossover studies (i.e., one group of volunteers administered different products).

Vitamins

Vitamin A (retinol and β-carotene derivatives), C (ascorbic acid), and E (tocopherols) are dietary antioxidants of particular interest when formulating oral cosmetics. Combined oral supplementation with vitamins C and E as well as carotenoids provides significant antioxidant activity in human skin with demonstrated UV protection and enhancement of cutaneous immune response (8,24).

In a randomized, placebo-controlled double-blind study, a food supplement (Seresis®, Pharmaton, Switzerland) containing a combination of carotenoids (β-carotene and lycopene), vitamins C and E, selenium, and proanthocyanidins was administered for three months in healthy females, and the effect on UV-induced matrix metalloproteinases (MMP-1, MMP-9) was investigated as a marker of photoprotection (24). In fact, the expressions of MMP-1 and MMP-9 are known as markers of the activity of the endogenous defense mechanism and are mainly induced by UVB irradiation. After supplementation and following UV irradiation, a significant difference was found between active treatment and placebo for MMP-1 with an increased level in the placebo group compared with a decreased level in the active-treated group. The changes in MMP-9 showed a similar but nonsignificant trend.

La Ruche and Cesarini investigated in a double-blind, parallel, placebo-controlled trial the photoprotective effect on the skin of combined intake of α-tocopherol (14 mg) and retinol (2700 μg) for three weeks in sixteen healthy subjects. Partial protection was observed compared with placebo at a low irradiation dose (suberythemal) against the formation of sunburn cells. No difference between placebo and active treatment was observed for the minimal erythema dose (MED) of UV, which was needed to induce erythema (25).

Combined oral intake of α-tocopherol, β-carotene, lycopene, and selenium for seven weeks was found to improve the epidermal defense against UV-induced damage in an open, single-arm study in 25 healthy volunteers (26). The individual UV sensitivity was measured as the actinic erythema threshold, and skin biopsies were collected to quantify lipoperoxides and to evaluate melanogenesis. After treatment with the antioxidant complex, a significant elevation of the actinic threshold, a general reduction of UV-induced erythema, and a reduction of lipoperoxide levels were observed, respectively.

Combined intake for 50 days of high doses of α-tocopherol (2 g/day) and ascorbate (3 g/day) were found to increase the MED compared with placebo in 40 healthy volunteers (27). These observations were confirmed with lower dosages, i.e., after eight days supplementation with 671 mg vitamin E and 2 g vitamin C daily, the MED increased compared with baseline in 8 of 10 subjects, whereas in the placebo group, the MED was unchanged in 6 of 10 subjects and decreased in 4 subjects. These studies indicate that combined supplementation of moderate to high doses of vitamin E and C exerts a photoprotective effect.

Several studies indicate that combined intake of a carotene mix such as β-carotene, α-carotene, cryptoxanthin, zeaxanthin, and lutein lowers the degree of erythema after exposure to UV irradiation and that this photoprotective effect is more pronounced when carotenes are combined with vitamin E (28,29). A recent study observed a decrease in sensitivity toward UV-induced erythema after about 10 to 12 weeks of dietary intervention with carotenoids and flavonoids (30). Overall, β-carotene exhibits some photoprotection, but carotenoids like lycopene still need more investigation. Because of concern about possible negative effects from large doses, most experts agree that getting carotenoids from foods is the safest.

Vitamin C is an essential cofactor of prolyl-4-hydroxylase, an enzyme that hydroxylates prolyl residues in procollagen, elastin, and other proteins with collagenous domains prior to triple helix formation (31). Hydroxyproline in elastin has no known function, but prolyl hydroxylation is essential for efficient collagen production. However, to our knowledge no clear benefit of oral vitamin C supplementation was demonstrated in humans on collagen synthesis in the skin or in a collagen-related process such as wound healing (32,33).

Biotin is an essential cofactor for several carboxylases, which catalyze vital steps in intermediary metabolism in the skin. Deficiency of biotin is known to manifest in various skin disorders, including dermatitis, scaling, and alopecia (34). Supplementation with large doses of biotin (2.5 mg) for six to nine months in subjects with documented brittle nails resulted in an increase of nail thickness and in a reduction of splitting of the nails (35).

Minerals

Few studies have been published, which investigate the effects of oral supplementation of minerals on aged skin. Zinc is an essential element of more than 200 metalloenzymes, including the antioxidative enzyme SOD, and has anti-inflammatory actions. Zinc is a component of enzymes required for DNA replication, gene transcription, and RNA and protein synthesis (36,37). Roughened skin and impaired wound healing have been reported in association with a mild zinc deficiency, implicating changes in the skin (38).

There is a popular belief that zinc deficiency can cause hair loss, but no such correlation is found in published data for alopecia areata (39) or telogen effluvium (40).

Césarini et al. (26) demonstrated that significant photoprotection can be provided by four- to seven-week supplementation with a specific antioxidant combination of vitamins, lycopene, and selenium. Selenium is present in the form of selenocysteine in the active center of the antioxidative enzyme GPX. Selenomethionine was shown to protect skin cells from UV-induced damage, DNA oxidation, and lipid peroxidation (41). The effect on skin health and skin aging of supplements containing a combination of lycopene, lutein, β-carotene, vitamin E, and selenium was investigated in a placebo-controlled study in 39 healthy volunteers. Roughness and scaling significantly improved after 12 weeks supplementation in the active group compared with that in the placebo group (42).

Lassus published in 1993 an open study concerning the effect of silicon supplementation on the skin and hair in 50 women with biologically aged skin and fragile hair or brittle nails (43). The study showed that combined treatment of oral and topical colloidal silicic acid had a beneficial effect on biologically aged skin structure and on the condition of hair and nails. The dermal thickness increased significantly after 90 days of supplementation. In addition, the hair was significantly thicker and less fragile, and nail brittleness had improved. However, no

evidence was presented to support the fact that the colloidal silica was actually absorbed in the gastrointestinal tract; i.e., it is not clear if the observed effects on the skin are the result of the oral or the topical treatment with colloidal silicic acid. In fact, it was clearly demonstrated in other studies that polymerized forms of orthosilicic acid, such as colloidal silica, are not bioavailable (44). Furthermore, seasonal changes may have biased the observed effects on the hair, skin, and nails since no placebo control was used in this study.

Polyphenolic Compounds

Polyphenolic compounds are widely distributed in higher plants and are an integral part of the human diet. In the last decade, the antioxidant activity of flavonoids and other polyphenols such as proanthocyanidins have been studied in detail (45,46).

Silymarin is a mixture of polyphenolic flavonoids derived from the seeds of the milk thistle plant *Silybum marianu* and has been shown in several animal studies to exhibit antioxidant, anti-inflammatory, and immunomodulatory properties, which may contribute to preventing or reducing photoaging (47), especially since silybin (most active component) was demonstrated to be available in skin after oral intake.

Pycnogenol® is the registered trademark of a standardized extract obtained from the bark of French maritime pine, which contains a mixture of procyanidins, also called proanthocyanidins, and phenolic acids, which are potent radical scavengers (48,49). Proanthocyanidins can also be found in grape seed, grape skin, bilberry, cranberry, black currant, green tea, black tea, blueberry, blackberry, strawberry, black cherry, red wine, and red cabbage. Pycnogenol has been suggested to support collagen skin density as it displays physical affinity to collagen and elastin and protects it against proteolytic degradation (50,51).

The efficacy of an oral supplement containing vitamins C and E, carotenoids, selenium, zinc, amino acids and glycosaminoglycans, and blueberry extract and Pycnogenol, respectively, was tested in a double-blind, placebo-controlled study in 62 women. After a six-week supplementation, skin elasticity and skin roughness improved in the active group compared with that in the placebo group (8).

Phytoestrogens

Phytoestrogens are polyphenolic nonsteroidal plant compounds with estrogen-like biological activity, which are classified in four main groups based on their chemical structure, i.e., isoflavonoids, flavonoids, stilbenes, and lignans (52). Since phytoestrogens are structurally similar to estrogen 17-β-estradiol, they may exhibit selective estrogen-modulating activities.

For women, particularly in the postmenopausal years, acceleration of chronologic aging is enhanced by the loss of estrogen, which causes a rapid loss of collagen during the first five years after menopause. It is assumed that phytoestrogens such as soy isoflavones may mimic the effects of estrogen in skin and reduce skin changes in postmenopausal women. A double-blind study in 26 middle-aged women indicated that oral intake of 40-mg soy isoflavone aglycones per day improved the extend of fine wrinkles at the lateral angle of the eyes after a 12-week supplementation compared with baseline. However, no significant differences in fine wrinkles were found between the active treatment group and the placebo group after supplementation, which may indicate that the observed improvement in the active group was biased by seasonal changes (53).

It is well documented that systemic hormonal replacement therapy (HRT) with estrogens, or combinations of estrogen-glucocorticoid, in postmenopausal women improves the gross appearance of their skin, resulting in decreased slackness, wrinkling, and roughness. At the microscopic level, HRT seems to affect mostly dermal collagen, increasing its content and augmenting dermal thickness (8,54,55). It should be noted, however, that HRT has been correlated with increased cancer risk (56,57). Furthermore, HRT is a drug therapy and certainly cannot be categorized as an oral cosmetic.

Glycosaminoglycans

It was suggested that extracts derived from marine fish cartilage have a repairing effect on photodamaged skin. In an open study, 10 females with sun-damaged skin were treated with 0.5 g/day glycosaminoglycans derivatives (Imedeen®, Soeberg, Denmark) for 90 days (58). After 90 days of treatment, all signs of sun damage had improved, and brittleness of hair and nails was normalized in all cases. These clinical observations were confirmed by changes in skin

thickness and elasticity; however, the obtained results may have been biased by seasonal changes since a control group was missing in the study. In a second double-blind, placebo-controlled study, 30 females of the same age range and with similar signs of sun damage were treated with 0.5 g/day glycosaminoglycans derivatives or placebo for 90 days. The results in the glycosaminoglycan-treated group corresponded to those in the first study, whereas no response to treatment was observed in the placebo treatment group (58). Kieffer et al. showed that after three months of treatment with glycosaminoglycans derivatives (Imedeen) there was no significant improvement in photoaging of the skin compared with placebo or baseline. The study was continued for another nine months in an open design i.e., without the use of a control group. After one year of treatment, a significant improvement was found compared with baseline in the investigator's evaluation of fine lines and overall photoaging, and, respectively, in density measurements by ultrasound, transepidermal water loss, and skin smoothness (59).

Eskelinen and Santalahti (60) studied the effect of oral intake of natural cartilage polysaccharides (Vivida®, Helsinki, Finland) on sun-damaged skin in 15 women aged 40 to 60 years. After 90 days of treatment, significant improvements compared with baseline were found in the active group, respectively, for the clinical evaluation of skin condition (e.g., dryness, thinning, and wrinkles), epidermal and dermal thickness by ultrasound, and the erythemal index, whereas no changes were observed in the placebo group.

Choline-stabilized Orthosilicic Acid

Choline-stabilized orthosilicic acid (ch-OSA) is a specific complex with high bioavailability (61), and the effect of ch-OSA on connective tissue (e.g., bone and skin) was evaluated in animal studies and in placebo-controlled clinical studies. Ch-OSA is a specific concentrated form of orthosilicic acid with choline as stabilizing agent. Physiological concentrations of orthosilicic acid were found to stimulate collagen type I synthesis in skin fibroblasts in vitro (62). Choline is a precursor of phospholipids such as phosphatidyl choline, which is an essential component of cellular membranes.

Supplementation of young animals with low doses of ch-OSA resulted in a significant higher hydroxyproline content in the dermis (63) and increased femoral density (64,65). Oral intake of ch-OSA for 20 weeks in 50 women with photoaged skin resulted in a significant positive effect on skin microrelief and skin mechanical properties compared with that in placebo group, suggesting a regeneration or de novo synthesis of collagen fibers in the dermis. Assessment of hair and nail brittleness on a visual analogue scale indicated a significant improvement in the ch-OSA group, whereas no change was observed in the placebo group (66).

The effect of ch-OSA on hair was further investigated in a randomized, double-blind, placebo-controlled study in 48 women with fine hair. Hair morphology and tensile properties were evaluated before and after treatment with validated methods. Oral intake of ch-OSA had a positive effect on tensile strength, including elasticity and break load, and resulted in thicker hair (67). The authors suggested that the observed increase in cross-sectional area of the hair shaft after ch-OSA supplementation may be explained by a stimulation of the collagen synthesis by fibroblasts in the dermal papilla, which determine the volume of the hair follicle.

Polyunsaturated Fatty Acids

Common food sources of *n*-3 polyunsaturated fatty acids (PUFA) are cod liver oil, fish oil, and marine animals with a high amount of fat, such as mackerel, salmon, and menhaden. A few studies have assessed the photoprotective effects of dietary intakes of fish oil. Orengo et al. (68) observed a small but statistically significant increase in the MED after intake of a fish oil–enriched diet for four weeks, showing that a relatively low dose (2.8 g eicosapentaenoic acid and 1.2 g docosahexaenoic acid) of fish oil is photoprotective. In another study (69), fish oil consumption (10 g daily) was also found to reduce UV irradiation-induced erythema, but the susceptibility of the skin to lipid peroxidation increased because of the unstable nature of *n*-3 fatty acids.

Dietary fatty acids were reported to be capable of changing the fatty acid composition of membrane phospholipids of immune cells, which may modulate the function of these cells. A few studies are published, which evaluate the effect of PUFAs in delayed-type hypersensitivity (DTH) skin tests using a panel of antigens, which have generally been accepted as an important

means to monitor the cell-mediated immunity in vivo. However, variable results were obtained in these studies with respect to the effect on DTH skin reactivity, which illustrates that more research is needed to document the immunomodulating activity of PUFAs (70–72).

The most important benefit of oral *n*-3 PUFA intakes from fish oil may be ascribed to their anti-inflammatory effects. These effects of *n*-3 PUFAs have been reported to be the result of their competition with n-6 PUFAs as a substrate for cyclooxygenase and lipoxygenase, resulting in the formation of less-active prostaglandins and leukotrienes. Interference with inflammatory cascades in the skin may occur through reductions in the synthesis of pro-inflammatory lipid mediators or through reductions in the production of cytokines. Moreover, *n*-3 PUFAs are unstable and may preferably be damaged by free radicals, thereby protecting other structures from attack by free radicals. Nevertheless, to protect the skin against excessive formation of free radicals and lipid peroxidation, appropriate amounts of antioxidants should also be consumed.

Multicomponent Supplements

Murad and Tabibian conducted a randomized, single-blind study in 72 women to evaluate the effect on skin roughness of a five-week supplementation with a combination of glucosamine, amino acids, minerals, and various antioxidant compounds. Women without supplementation were used as a control group, i.e., placebo supplementation was missing in this study. A statistically significant reduction in the number of visible wrinkles and fine lines was observed in the active group but not in the control group. There was no significant change in epidermal hydration in either the control or the active study group (73).

Skovgaard et al. investigated in a placebo-controlled study the effects of a novel dietary supplement (Imedeen Prime Renewal®, Soeberg, Denmark) on skin in hundred postmenopausal women. The supplement contained soy extract, fish protein polysaccharides, white tea extracts, grape seed and tomato extract, vitamins C and E, as well as zinc and chamomile extract. The clinical grading of skin condition and the density of the skin measured by ultrasound structure improved after six months of supplementation in the active group but not in the placebo group (74).

The effect of oral intake of a combination of marine proteins with zinc, copper, vitamins C, E, B3 and B5, α lipoic acid, pine bark extract, red clover extract, tomato extract, and soya extract (DermaVite®, Florida, U.S.A.), respectively, was tested in a placebo-controlled study in 40 women with aged skin (75). There was a significant increase in skin thickness and elasticity after six months of supplementation with the active preparation but not in the placebo group. A significant improvement in global evaluation of skin condition using a visual analogue scale was also observed in the active group after six months of supplementation, whereas no change was observed in the placebo group.

Sixty-two women aged 45 to 73 years participated in a double-blind, placebo-controlled trial to evaluate the efficacy of a proprietary combination of vitamins C and E, carotenoids, selenium, zinc, amino acids, glycosaminoglycans, blueberry extract, and Pycnogenol (see description in this chapter in the sect."Polyphenolic Compounds"), respectively. Compared with that of placebo, it was found that skin elasticity and skin roughness improved significantly after 6 and 12 weeks of supplementation (8).

LEGISLATION CONCERNING ORAL COSMETICS

Oral cosmetics are dietary supplements (food supplements), i.e., the legislation on food supplements should be followed. Considerable differences in such legislation exist between different countries with respect to the maximal doses, the chemical forms of vitamins and minerals, plant species, and product claims, which are allowed to be used in and for dietary supplements. In the European Community, the legislation on both food supplements and health claims was recently harmonized for its member states when the European Commission (EC) Directive on Food supplements (i.e., Directive 2002/46/EC) and the Regulation on Nutrition and Health Claims (i.e., Regulation 1924/2006) came into force.

CONCLUSION

Sun avoidance and the use of sunscreens are well established as primary components in antiaging regimens, although these are still underappreciated by many people. Clearly, sun

avoidance is not easy to manage and is often impossible. Consumer-driven demand has led to the development of products to counteract the signs of aging skin. Functional foods positioned as beauty enhancers are a recent concept in Western countries. Bearing this in mind, the development of novel or more active cosmetics (cosmeceuticals) or dietary supplements, which specifically target the skin, hair, and nails (oral cosmetics) is one of the most exciting and promising ways in which the future of cosmetology and dietetics may address human health needs and well-being (76). Considerable research and firsthand experience of physicians have shown that using topical creams in conjunction with dietary supplements leads to superior results compared with using either skin care or supplements alone. It may come as a surprise to many consumers that only few ingredients in topical skin care products have the capacity to penetrate far enough into the dermis to ameliorate deep wrinkles. Therefore, using a combination of topical and oral cosmetics will likely be the favored recommendation in the near future to develop efficient antiaging therapies.

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67 Hair Conditioners

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INTRODUCTION

Despite myriad claimed benefits, the primary purpose of a hair conditioner is to reduce the magnitude of the forces associated with combing or brushing of the hair (1), especially when wet (2,3). This is generally accomplished by the deposition of conditioning agents that lubricate the hair fiber, diminishing surface friction and, therefore, combing forces (4).

In general, deposition of a conditioning agent also causes the hair to feel softer and more moisturized. Another secondary benefit is the reduction or prevention of flyaway hair (5), especially by cationic conditioners (6). Besides making the hair more manageable, increasing the ease of combing also improves the ability to align the hair fibers in a more parallel configuration, which can result in an increase in hair shine, even if the shine of the individual fibers is not increased (7). Some ingredients can also form a film on the hair surface that provides color retention benefits for color-treated hair (8).

A number of other benefits have sometimes been claimed or implied for conditioners, including repair of damaged hair, strengthening of hair, repair of split ends, vitamin therapy, etc. Some of these are marketing hype or are based on laboratory conditions or concentrations not found under actual usage conditions. In this chapter, we will confine ourselves to a discussion of only the observable conditioner benefits presented above. The chapter will begin with a discussion of the relationship between hair damage, conditioning, and the state of the hair surface. This will be followed by a discussion of the major classes of conditioning agents currently in use. Finally, we will end with a brief discussion of the auxiliary ingredients necessary for the production of a commercial conditioning product.

CONDITIONING AND THE HAIR FIBER SURFACE

Hair Damage

In previous chapters, it has been shown that hair fibers consist of a central cortex that comprises the major portion of the fiber, surrounded by 8 to 10 layers of overlapping cells termed "cuticle." The cortex is responsible for the tensile properties of the hair (9,10), while the state of the cuticle affects a variety of consumer-perceivable properties including hair feel, shine, combability, etc.

A major function of conditioners is to protect the hair's structural elements, especially the cuticle, from grooming damage. This type of stress, characterized by chipping, fragmenting, and wearing away of cuticle cells, is probably the single most important source of damage to the hair surface (11–13).

A rather extreme example of combing damage can be seen in Figure 1, which shows the results of an experiment in which a tress of virgin hair was washed with a cleaning shampoo and then combed 700 times while wet. Since hair is more fragile when wet (3) and combing forces are higher (2), combing under these conditions insures maximum damage. It can be seen that damage to the cuticle was extensive with many cuticle cells lifted from the surface, while others were completely torn away by the combing process.

The ability of conditioning agents to protect the hair from the above type of damage can be seen in Figure 2, which shows the results of an experiment in which a tress was washed with a high-conditioning 2-in-1 shampoo and then combed 700 times while wet. In this case, because the conditioning agents in the shampoo reduced combing forces, the hair surface is seen to be intact with evidence of only minor chipping and fragmenting of cuticle cells. This

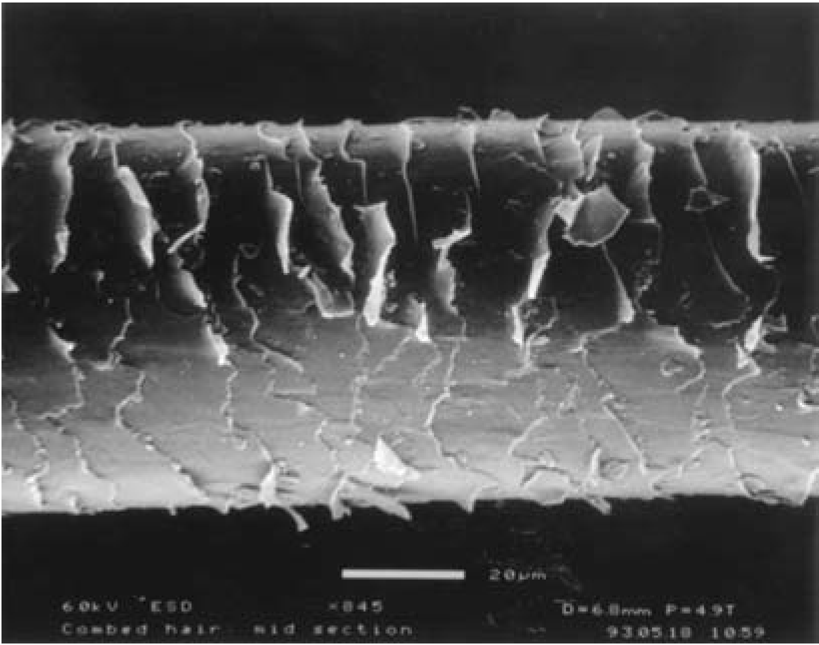

Figure 1 Typical SEM of hair taken from a tress washed with a cleaning shampoo and then combed 700 times while wet. Note raised and chipped cuticle cells and areas where cells have been completely torn away. *Abbreviation*: SEM, scanning electron micrograph.

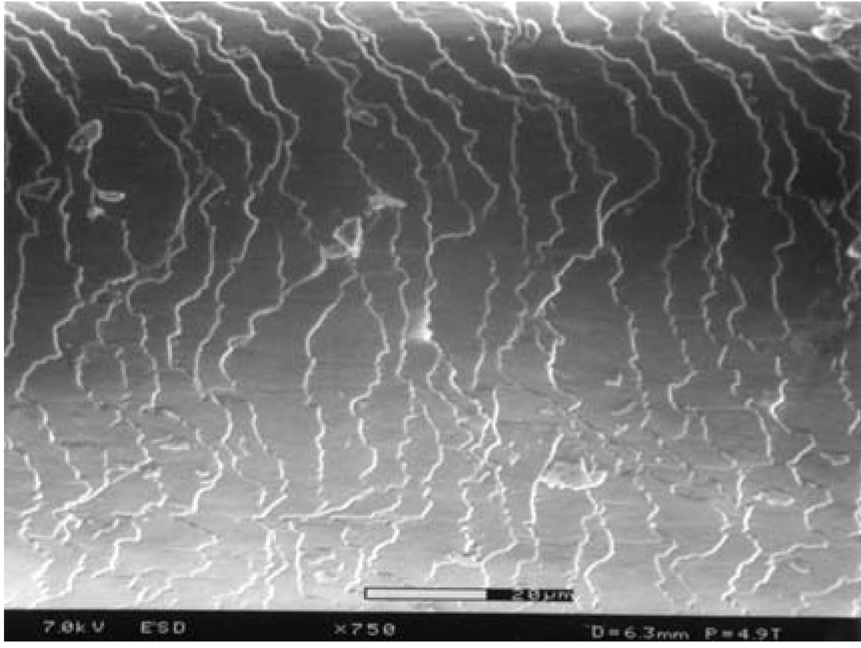

Figure 2 Typical SEM photo of hair taken from a tress washed with a high conditioning 2-in-1 shampoo and then combed 700 times while wet. Note the minimal damage compared with that in Figure 1. *Abbreviation*: SEM, scanning electron micrograph.

demonstrates the important role conditioners can play in maintaining the integrity of the hair fiber.

Heat produced by the use of appliances can also cause hair damage. Many styles require the use of blow dryers and/or curling irons, which can produce temperatures of 200°F to 400°F (14). Steam can be released from the hair fiber causing bubbling and buckling of the cuticles, especially if the hair is not completely dry while being curled. In addition to minimizing combing forces, to protect the hair from this type of damage, certain conditioning polymers can provide added protection in the presence of heat, resulting in increased characteristic life of the hair fiber (15).

Hair Damage and the Cuticle Surface

The susceptibility of a hair fiber to grooming damage and the type of conditioner most effective in preventing this damage is affected to a large degree by the nature and state of the hair surface. It is therefore helpful to precede a discussion of conditioning agents with a presentation on the hair surface and how it affects conditioner requirements and deposition.

Virgin Hair Surfaces

Hair that has not been chemically treated is termed "virgin hair." The cuticle surface of virgin hair in good condition is hydrophobic (16,17), in large part, as a result of a layer of fatty acids covalently bound to the outermost surface of the cuticle (epicuticle) (18,19). As a result of its protein structure, however, the hair surface has an isoelectric point near 3.67 (20), which insures that the surface will contain negatively charged hydrophilic sites at the ordinary pH levels of hair care products. This mix of hydrophobicity and hydrophilicity affects, of course, the types of conditioning agents that will bind to the virgin hair surface.

The situation is further complicated by the fact that the negative charge density on virgin hair increases from root to tip. This is primarily a result of oxidation of cystine in the hair to cystine S-sulfonate and cysteic acid as a result of exposure to ultraviolet (UV) radiation in sunlight (21,22). The tip portions of the hair, being older than the root portions, will have been exposed to damaging (11) UV radiation for a longer period of time and will therefore be more hydrophilic, again affecting the nature of species that can bind to these sites.

In addition to greater UV damage, the tips of hair are also subject to greater combing damage. One reason for this damage is simply that, being older, the tip portions will have been exposed to more combing. In addition, the surface friction of hair tips is higher (C. Reich, unpublished data) so that combing forces increase as one moves from root to tip. Finally, the ends of hair are subject to unusually high combing stress as a result of entangling during the combing process (2). This eventually results in destruction of the covalently bound lipid layer and a feeling of dryness at the tips. Because of this damage, the tip ends of hair require more conditioning than the rest of the fiber. Without sufficient conditioning, the cuticle layer is eventually lost, resulting in a split end. An example is seen in Figure 3, which clearly shows the exposed cortical cells.

Chemically Treated Hair Surfaces

Chemical treatments, such as perming, bleaching, and permanent dyeing, can all cause significant damage to the hair fiber (3,11,23–25). In addition to causing tensile damage, all these treatments, which include oxidative steps, modify the surface of the hair, introducing negative charges as a result of oxidation of cystine to cysteic acid (3,11,23,24,26). This can result in transformation of the entire fiber surface from a hydrophobic to hydrophilic character.

All of the above treatments also increase surface friction considerably (3,4,27,28), resulting in a significant increase in combing forces. The result is that the hair feels rough and dry and is subject to extensive grooming damage. Because of this damage, treated hair generally requires more conditioning than virgin hair. By using a conditioner, one can prolong the health of the hair fiber. It has been found that cuticle cells on damaged, chemically processed hair are in better condition when a conditioner is used as part of the grooming

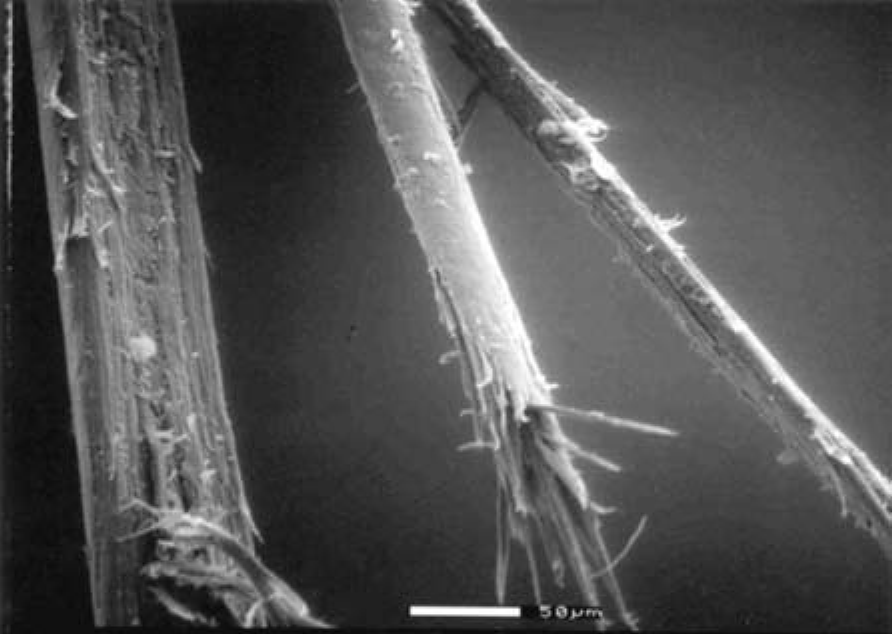

Figure 3 SEM photo of a split end. Note the exposed cortex and the complete loss of cuticle cells on the fiber surface. *Abbreviation*: SEM, scanning electron micrograph.

process (29). Therefore, using a conditioner is particularly meaningful in improving the condition and health of chemically processed hair fibers.

COMMERCIAL CONDITIONERS

The commercial hair conditioners produced to deal with the above problems have appeared in almost every conceivable form, including thick Vaseline pomades, creams, gels, mousses, lotions, and spray mists.

Categorizing by application method, conditioners have been marketed as regular rinse-off conditioners, intensive treatment conditioners, and leave-in products. The first, regular rinse-off conditioners are normally applied after shampoo, followed by a rinsing step. This is the most common form of conditioner sold.

Intensive treatment conditioners are used similarly to the above products, but are not meant for daily application. They are used, instead, for intensive treatment and a higher degree of conditioning. These products generally contain a higher level of active ingredients that are kept on the hair for a longer period of time prior to rinsing. Intensive conditioners are typically sold as thicker creams to provide the perception of higher conditioning.

Leave-in products usually are lighter and can potentially provide more significant benefits than the above rinse-off products since everything applied stays on the hair until the next shampoo. Leave-in conditioners come in various forms, such as detanglers, leave-in lotions, and sprays. They are marketed either for single application or multiple applications during the day.

Despite the wide variety of forms available, most commercial conditioners are oil-in-water emulsions in lotion form, having viscosities somewhere between 3000 and 12,000 centipoises. In addition, despite the different forms and positioning, most commercial conditioners contain the same general classes of conditioning agents with differences mainly in concentrations, numbers of different agents, and particular members of a conditioning class employed.

The major classes of conditioning agents used in commercial products are surveyed in the following sections. Example formulae taken from the patent literature are listed below for some of the various forms of conditioning products.

Hair conditioner (30)	
Ingredients	Weight (%)
Water	q.s. to 100
Stearyl alcohol	2.50
Stearamidopropyl dimethylamine	1.00
Mineral oil	0.50
Cyclomethicone	0.25
Propylene glycol	0.50
Distearyl dimonium chloride	0.75
Hydroxyethylcellulose	1.00
Citric acid	0.20
Polyvinylpyrrolidone	0.10
Formalin (preservative)	0.10
Fragrance	0.20

Deep hair conditioner (31)	
Ingredients	Weight (%)
Water	q.s. to 100
Cetyl alcohol	6.00
Stearamidopropyl dimethylamine	1.50
Mineral oil, heavy	0.50
Propylene glycol	1.00
Distearyl dimonium chloride	1.00
Citric acid	0.20
Germaben II (preservative)	0.50
Fragrance	0.40

Conditioning spray (32)	
Ingredients	Weight (%)
Trimethylolpropane triisostearate	1.00
Methyl myristate	1.00
Cetyl alcohol	1.00
Monoalkyl trimethyl ammonium chloride	0.2
Preservative	0.1
Perfume	0.1
Denatured ethyl alcohol	q.s. to 100

Conditioning styling gel (33)	
Ingredients	Weight (%)
Sodium PCA (50% aqueous solution)	2.00
Glycerin	1.50
Hydrolyzed collagen	0.50
Carbomer 940	0.35
SD alcohol 40	25.00
Nonionic surfactant	0.50
Fragrance	0.10
Water	q.s. to 100

KEY INGREDIENTS OF HAIR CONDITIONERS

Cationic Surfactants

Cationic surfactants in the form of quaternary ammonium compounds (quats) are the most widely used conditioning agents in commercial products (34–36). Among the reasons for this popularity are their effectiveness, versatility, availability, and low cost.

Important examples of these quats include stearalkonium chloride, cetrimonium chloride, and dicetyldimonium chloride. Different counter anions, such as chloride, bromide, and methosulfate, have been used with these materials.

$$C_6H_5CH_2-N^+(CH_3)_2-CH_2(CH_2)_{16}CH_3\ Cl^-$$

Stearalkonium Chloride

$$H_3C-N^+(CH_3)_2-CH_2(CH_2)_{14}CH_3\ Cl^-$$

Cetrimonium Chloride

$$H_3C-N^+(CH_3)(CH_2(CH_2)_{14}CH_3)-CH_2(CH_2)_{14}CH_3\ Cl^-$$

Dicetydimonium Chloride

Because of the positive charge on the quats such as the ones described above, they are substantive to hair and bind to negative sites on the hair surface. Treatment with these quats results, therefore, in a hydrophobic coating on the fiber that render the hair softer and easier to comb (37). Buildup of static charge (flyaway) is also greatly reduced as a result of this surface modification (6).

Another consequence of the positive charge on quats is that deposition increases with increasing negative charge on the hair surface. This is seen in Table 1, which shows the results of an experiment in which hair tresses were treated with 1% stearalkonium chloride and then rinsed. Compared with the roots, 22% more quat was found to bind to the tips of virgin hair, while deposition of stearalkonium chloride on bleached hair was found to be more than twice that on untreated fibers.

This result is important since, as was discussed above, damaged portions of the hair, which generally carry a greater amount of negative charge from either environmental damage or chemical treatment, require a greater amount of conditioning. The fact that cationic surfactants can supply this increased conditioning makes them effective on a wide variety of hair surfaces. This is a major factor in the widespread use of these types of conditioning agents.

Research conducted at TRI/Princeton has shown that the type of deposition and degree of penetration into the hair fiber depends on the size or molecular weight of the compound. The interaction between cationic conditioners and the hair fiber mainly occurs at the surface; however, low–molecular weight materials may penetrate the interior via intercellular diffusion. Cetrimonium bromide (CETAB), e.g., can penetrate the cuticular sheath as well as cortex (39).

Conditioner Properties and Hydrophobicity

Many important properties of quaternary ammonium conditioners are related to the degree of hydrophobicity of the lipophilic portion of the surfactant. Thus, increasing the length of the

Table 1 Binding of Stearalkonium Chloride to Human Hair[a]

Type of hair	Quat deposition at roots (mg/g hair)	Quat deposition at tips (mg/g hair)
Virgin hair	0.649	0.789
Bleached hair	1.62	1.83

[a]Data taken from Ref. 38.

alkyl chain of a monoalkyl quat and, therefore, making it more hydrophobic leads to increased deposition (40–45) on hair. Cetrimonium chloride, as a result, deposits on hair to a greater extent than laurtrimonium chloride. Increasing the number of alkyl chains also increases the deposition so that tricetylmonium chloride exhibits greater deposition than dicetyldimonium chloride, which, in turn, is more substantive than the monocetyl quat.

This dependence of deposition on degree of hydrophobicity indicates that van der Waals forces play an important role in deposition of quaternary ammonium conditioners (45). This conclusion is consistent with the entropy-driven deposition demonstrated by Ohbu et al. (46) and Stapleton (47) for a monoalkyl quat and a protonated long-chain amine.

Increased hydrophobicity also correlates with increased conditioning by quats (40–43,48). Thus, cetrimonium chloride provides light to medium conditioning, while dicetyldimonium and tricetylmonium chlorides provide heavier conditioning. Detangling and wet combing, in particular, improve significantly from monocetyl to dicetyl to tricetyl quats; differences in dry combing and static charge among these compounds are not as significant.

Increased conditioning with increased hydrophobicity is probably due to, in part, the increased deposition of quat on hair. Data from Garcia and Diaz (49), however, indicate greater improvements in wet combing from heavier conditioning quats even when present on the hair in much lower amounts than less hydrophobic species. The degree of hydrophobicity of a quat must, therefore, play a direct role in the conditioning efficacy of these compounds (37).

Note that on some types of hair, the greater substantivity of higher conditioning quats can lead to buildup with repeated use and result in limp, unmanageable hair. This is especially true, e.g., for untreated, fine hair. Different quats or mixtures of conditioning agents are, therefore, suitable for different uses or different types of hair. A tricetyl quat might be used, e.g., in an intensive conditioner meant only for occasional use.

The length and number of alkyl chains of quats also determines water solubility of these compounds. Monoalkyl quaternaries up to cetrimonium chloride are water soluble, e.g., distearyldimonium chloride is water dispersible, while tricetylmonium chloride is insoluble in water (43).

Compatibility with Anionics

The quaternium compounds normally used in commercial conditioners are not generally found in shampoos because of its incompatibility with common anionic detergents (44). Introducing hydrophilic groups into the quat can increase compatibility with anionics. An example is the class of ethoxylated quaternaries, termed "ethoquats." Typical members of this class are polyethylene glycol (PEG)-2 cocomonium chloride, where x + y equal 2 and R is a

$$
\begin{array}{c}
(CH_2CH_2O)_xH \\
| \\
R-N^{+}-CH_3 \quad Cl^{-} \\
| \\
(CH_2CH_2O)_yH
\end{array}
$$

Ethoxylated quaternary

C12 alkyl chain, and PEG-15 stearmonium chloride, where x + y equal 15 and R is a C18 chain.

Both of these quats are compatible with typical anionic detergents. As would be expected from the above discussion, however, introducing hydrophilic groups decreases the conditioning efficacy of these materials (40,43). They are, therefore, suitable only in light conditioning formulations. Furthermore, conditioning shampoos based on ethoquats would not be expected to be very effective as a result of low deposition of the detergent-soluble ethoquat complex.

Other detergent-soluble quats have been produced. These include alkylamidopropyl dihydroxypropyl dimonium chlorides (50), lauryl methyl gluceth-10 hydroxypropyl dimonium chloride (51), and even a hydrolyzed ginseng-saponin quaternary derived from Korean ginseng saponin (52). Although certain advantages have been claimed for these surfactants, particularly low irritation, they all suffer from much the same conditioning limitations as the ethoquats.

Other Cationic Surfactants
In addition to the above examples, numerous other cationic surfactants are in use or have been proposed for commercial products. One example of a compound that has been receiving increasing use is the behentrimonium (C22) quat. This quat exhibits significantly reduced eye and skin irritation compared with the corresponding C18 conditioner due to the longer fatty chain length. In addition, superior conditioning and thickening properties have been claimed (53).

Another interesting example is hydrogenated tallow octyl dimonium chloride (54). This material is quite substantive and provides high conditioning as a result of its two hydrophobic chains. Unlike conventional dialkyl quats, however, this particular conditioner is soluble in water as a result of branching (2-ethylhexyl) in the octyl moiety. This solubility makes the compound much easier to formulate into a commercial product.

Several patents (55–61) have disclosed immidazoline-based quats containing the immidazoline ring and fatty chains. Some patents have claimed a softening effect on fabrics or hair. Conditioner compositions using these types of quats have also been disclosed (62,63).

Concern for the environment has led to the synthesis of ester quats that exhibit increased biodegradability and environmental safety. One such example is dipalmitoylethyl hydroxyethylmonium methosulfate, an ester quat based on a partially hydrogenated palm radical (64).

Other cationic surfactants used in conditioners include quats derived from guerbet alcohols (48) (low to high conditioning depending on length of the main and side alkyl chains), distearyldimonium chloride (high conditioning), and the quaternized ammonium compounds of hydrolyzed milk protein, soy and wheat protein, and hydrolyzed keratin (varying conditioning efficacy depending on alkyl chain length).

Amines
Amines with fatty chains, such as stearamidopropyl dimethylamine, can also be found in many commercial conditioners. These types of materials become cationic after protonation at the low pH normally employed in conditioning products and therefore act as both cationic emulsifiers and conditioning agents. Neutralization is normally required to decrease the pH and convert the neutral compounds to cationic. Different acids may have different effects on the viscosity of the final product.

Lipophilic Conditioners

Quaternary ammonium surfactants in commercial products are almost never used alone. Instead, they are employed in combination with long-chain fatty conditioners, especially cetyl and stearyl alcohols (36). These fatty materials are added to boost the conditioning effects of the quaternary compounds (51). In one study, e.g., addition of cetyl alcohol to CETAB nearly doubled the observed reduction in wet combing forces on hair (65). In another study, using a novel hydrodynamic technique, Fukuchi et al. (66) found that addition of cetyl alcohol to a behentrimonium chloride formulation resulted in a significantly reduced surface friction.

Several workers have studied combinations of cationic surfactants and fatty alcohols. Under the right conditions, these mixtures have been found to form lamellar liquid crystal mesophases and gel networks (67–71) that can greatly increase viscosity and, at the same time, confer stability upon emulsions. As a result of reduced repulsion between cationic head groups when long-chain alcohols are interposed, liquid crystal formation has been observed even at low concentrations (70,71). The ready formation of these extended structures between quats and cetyl and stearyl alcohols, along with the low cost, stability, and compatibility with cosmetic ingredients of the latter are important reasons why these alcohols are so ubiquitous in conditioning formulations.

Long-chain fatty compounds are generally solids at room temperature, requiring heating to incorporate into a product. Care should be taken in manufacturing formulations so that the cooling rate is not so rapid when it interferes with liquid crystal formation. In addition, it has been claimed that improved freeze-thaw stability is conferred upon conditioners when using certain combinations of ethoxylated branched-chain fatty alcohol ethers or esters as stabilizers (72).

Other lipids found in commercial products include glycol distearate, triglycerides, fatty esters, waxes of triglycerides, liquid paraffin, etc.

Polymers

Cationic Polymers

There are numerous cationic polymers that provide conditioning benefits, especially improved wet combing and reduced static charge. Important examples of these polymers are polyquaternium-10, a quaternized hydroxyethylcellulose polymer; polyquaternium-7, a copolymer of diallyldimethylammonium chloride and acrylamide; polyquaternium-11, a copolymer of vinylpyrrolidone and dimethylaminoethyl methacrylate quaternized with dimethyl sulfate; polyquaternium-16, a copolymer of vinylpyrrolidone and quaternized vinylimidazole; and polyquaternium 6, a homopolymer of diallyldimethylammonium chloride.

By virtue of their cationic nature, the above polymers are substantive to hair. The particular conditioning effectiveness of any these materials depends on the polymer structure. In one set of studies, deposition on hair was found to be inversely proportional, roughly, to cationic charge density (73,74). This has been explained by the observation that the higher the charge density, the lower the weight of polymer needed to neutralize all of the negative charge on the hair. Once deposited, however, multiple points of electrostatic attachment make these polymers harder to remove, especially if charge density is high (38,75). Care must be taken, therefore, in formulating conditioners containing these materials to avoid over-conditioning as a result of buildup with continued use.

As with the preceding monofunctional cationics, deposition of polyquaterniums increases on treated or damaged hair (38,75). Unlike common monofunctional quats, however, the first four of the above polymers are compatible to varying degrees with anionic surfactants (75–78). As a result, such polymers are used more often in shampoos than in stand-alone conditioners, although they find some use in leave-in conditioners.

Polyquaternium-10 (PQ-10) and polyquaternium-7 (PQ-7) are two of the most frequently used polymers in commercial shampoos. Both of these polymers form negatively charged complexes (73, 75) with excess anionic surfactant, resulting in reduced deposition because of repulsion by the negatively charged hair surface. The magnitude of this effect depends on the particular anionic employed and anionic surfactant-polymer ratio. In all cases, however, conditioning from shampoos is significantly less than from stand-alone conditioners.

Despite reduced deposition, Hannah (79) has reported that polyquaternium association complexes formed with sodium lauryl sulfate resist removal from hair. Buildup and a heavy, coated feel on the hair can therefore result from conditioning shampoos containing polyquats unless they are carefully formulated.

In addition to providing conditioning benefits, some polyquaternium materials have been shown to improve adhesion of the cuticle scales thereby increasing resistance to scale uplift when the hair is stressed. The same effects were observed for at least one quat—CETAB (39).

Other Polymers

In recent studies, other polymeric materials, including amphiphilc polymers (80–82), amphoteric polymers (83), block copolymers (84–86), graft polymers (87, 88), and dendrimers (89), have been investigated for use as conditioning agents, stabilizers, and deposition agents. In part probably because of cost, commercial products containing those novel polymers are rare. However, these research activities may indicate a future trend toward the use of polymers with more complicated structures in personal care applications.

Silicones

The use of silicones in hair care products has increased considerably in the past two decades, although their first incorporation into commercial products dates back to the 1950s. Different types of silicones find use as conditioning agents in a wide variety of products including conditioners, shampoos, hair sprays, mousses, and gels (90). One of the most widely used silicones is dimethicone, which is a polydimethylsiloxane. Other important silicones are

$$H_3C-\underset{CH_3}{\overset{CH_3}{SiO}}-\underset{CH_3}{\overset{CH_3}{(SiO)_x}}-\underset{CH_3}{\overset{CH_3}{Si}}-CH_3$$

Dimethicone

dimethiconol, which is a dimethylsiloxane terminated with hydroxyl groups; dimethicone copolyol, which is a dimethylsiloxane containing polyoxyethylene and/or polyoxypropylene side chains; amodimethicone, which is an amino-substituted silicone, and silicone quats, which contain permanently quaternized ammonium groups. In general, amodimethicones and silicone quats condition better than dimethicones, which condition better then dimethicone copolyols (91). Presumably this is due to differences in substantivity from rinse-off products. Because of the increased substantivity, care should be taken with amodimethicones and silicone quats to make sure they do not build up over time. Likewise, many dimethicone copolyols are soluble in water and therefore may not be as effective in rinse-off products.

CH_3 CH_3 CH_3 CH_3
$H_3C—SiO—(SiO)_m—(SiO)_n—Si—CH_3$
CH_3 CH_3 $(CH_2)_3$ CH_3
$O(C_2H_4O)_aH$

Dimethicone copolyol

CH_3 CH_3 CH_3
$HO—SiO—(SiO)_x—(SiO)_y—H$
CH_3 CH_3 $(CH_2)_3$
$NHCH_2CH_2NH_2$

Amodimethicone

Many silicones used in hair care products are insoluble and must therefore be emulsified. To increase ease of product manufacture, many suppliers offer silicones as preformed emulsions in addition to the pure material. Emulsions can vary in charge (anionic, cationic, or nonionic), size (microemulsion or macroemulsion), and how they are made (mechanical or emulsion polymerization). The factors affecting deposition of silicones from such emulsions have been reported by Jachowicz and Berthiaume (92,93) and by Hoag et al. (94).

Silicone emulsions can also vary in particle size. Typically, the smaller the size of the silicone particles, the more stable the product emulsion is. Additionally, it has been claimed that reducing the particle size also improves conditioning performance (95). If a preformed silicone emulsion is not used, particle size of the silicone droplets can be controlled by combining the correct amount of heat and shear when making the product.

Conditioning Properties of Silicones

Silicones used in hair care products possess a range of unique properties including lubricity, low intermolecular forces, water insolubility, and low surface tension. These properties permit the silicones to spread easily on the hair surface, thereby forming a hydrophobic film that provides ease of combing and imparts a smooth, soft feel to the hair without greasiness.

The relative conditioning efficacy of silicones compared with other conditioners was demonstrated by Yahagi (96), who found that dimethicone lowered frictional coefficients and surface energy of virgin hair to a greater extent than a series of cationic surfactants, including distearydimonium chloride, a very effective conditioning agent. Dimethicones with molecular weights greater than 20,000 were found to be most effective in reducing surface tension.

Nanavati and Hami (97) measured conditioning on slightly bleached European hair treated with dimethicone fluids and dimethiconol gums. Both types of silicones were found to significantly reduce combing forces on hair. Ease of wet combing was roughly the same for the two silicone treatments, while dimethiconol was found to be more effective in reducing dry combing forces.

Interestingly, under the treatment conditions employed (exposure to silicone solutions for 30 seconds followed by drying without rinsing), deposition of all silicones studied was found to nearly double if tricetylmonium chloride was present in the treatment solution.

Reduction in combing forces was also roughly doubled when silicones were deposited in the presence of quat. This latter effect was found to be synergistic; i.e., it depended on deposition of both silicone and quat, and its magnitude was greater than the sum of the individual conditioner contributions.

Wendel et al. (98) used electron spectroscopy for chemical analysis (ESCA) to demonstrate that the presence of amino groups in silicones considerably increases substantivity of these materials. This is a result of the positive charge developed by these groups at the pH commonly found in commercial products.

Comparison of conditioning effects of a series of silicone emulsions on bleached and virgin hair was carried out by Hoag et al. (94). Most of the silicones were dimethicones or amodimethicones, while emulsions were anionic, neutral, or cationic in nature. Diluted emulsions were applied directly to the hair and combing forces measured both before and after rinsing. Prior to rinsing, reduction of combing forces by most emulsions was greater than 80%. This number decreased after rinsing as a result of partial removal of deposited silicone. Unsurprisingly, the least change in ease of combing was found for cationic emulsions, especially those containing amodimethicone. Combing forces on virgin hair increased less than on bleached hair after rinsing, indicating that the silicones were more substantive to this type of hair. This is also unsurprising considering the hydrophobic nature of these conditioning agents.

Further effects of amodimethicones can be seen in work reported by Berthiaume et al. (99) who studied a series of amodimethicone emulsions in a prototype conditioner formulation. Deposition on hair from the conditioner was found to increase with increasing amine content in the silicone. This increased deposition was found, in half-head tests, to correlate with conditioning efficacy, including wet and dry combing, softness, and detangling. A microemulsion in the test series that provided high conditioning was also shown to significantly reduce the color fading caused by shampoo of temporarily dyed hair.

Other Silicones

One silicone that is widely used in conditioners to help improve wet combing is cyclomethicone, which refers to a class of cyclic dimethyl polysiloxanes ranging from trimer to hexamer. Cyclomethicone is volatile and will not remain on dry hair, especially after blow-drying. It helps other conditioning agents to disperse, however, and form films on hair. It also helps improve wet combing and provides transient shine. In addition, cyclomethicone is widely used as a solvent to reduce the viscosity of silicone gums with much higher molecular weights.

Because of its high refractive index, close to that of hair, phenyl trimethicone is commonly used in leave-in conditioners to enhance the shine of hair fibers. To improve substantivity higher molecular weight versions (Si-Tec PTM 1000, and International Specialty Products) and versions that incorporate amino groups (DC 2-2078 fluid, Dow Corning) have been produced.

Newer silicones include dimethicone copolyol phosphates, which are anionic functional silicones and fluorocarbon-modified organosilicones. The copolyol phosphates are able to complex with tertiary amines of cationic hair conditioners and form effective emulsifiers and conditioners (100). The fluorocarbon-modified silicones are very hydrophobic like dimethicone; however, they are claimed to have a lighter and more lubricious feel (100).

Interesting block copolymers with silicone blocks and organic segments have been developed for personal care applications (101). DC CE8401 from Dow Corning Co. is a commercially available example. This material has a unique structure. In contrast to traditional silicone copolyols that have a rake structure, it is a block copolymer containing silicone and polyether segments in its backbone.

Other examples of silicones include blends of these materials, having different molecular weights (102), different functional groups (103,104), and silicones with other hydrophobic oils (105). Those silicone blends have been reported to improve overall conditioning benefits.

2-in-1 Shampoos

Silicones find important application as the primary conditioning agents in 2-in-1 conditioning shampoos. On their introduction in the latter part of the 1980s, these shampoos represented a major advance in hair care technology, providing a significantly higher degree of conditioning

than was then the norm for conditioning shampoos and, at the same time, leaving a desirable soft, smooth feel on the hair.

Conditioning from 2-in-1 shampoos is expected to occur primarily at the rinsing stage, when the shampoo emulsion breaks, releasing the silicone for deposition on hair. This separation of cleaning and conditioning stages permits the shampoo to perform both functions efficiently.

The conditioning agent used most frequently in 2-in-1 shampoos is dimethicone. This silicone can provide good performance in shampoo formulations without excessive buildup on the hair (106). With advances in technology, newer formulations are now employing easier-to-process silicones, such as dimethicone emulsions, amodimethicones, dimethiconols, and copolyols as well as combinations of these different types to deliver the desired level of conditioning as well as improved product aesthetics.

The level of conditioning from 2-in-1 shampoos is lower than that from stand-alone conditioners. This is especially true for treated hair since the greater the degree of negative charge on the hair surface, the lower the substantivity of a hydrophobic material like dimethicone. Many 2-in-1 products contain polyquats, which might be expected to increase conditioning on damaged hair. In shampoos with high levels of anionic detergent, however, polyquat performance on treated hair may be no better than dimethicone as a result of formation of the negatively charged polymer complexes discussed in the section "Cationic Polymers."

Yahagi (96) studied the performance of dimethicone, amodimethicone, and dimethicone copolyols in 2-in-1 shampoos. Ease of combing was found to be similar for hair treated with shampoos containing dimethicone or amodimethicone. Unsurprisingly, soluble dimethicone copolyols did not perform well; insolubility, or at least dispersibility, was required for adequate silicone deposition. In the latter case, dimethicone copolyols were found to provide a somewhat lower level of conditioning than the other two silicones studied, especially once blow-drying was begun. Yahagi also studied silicone effects on foam volume. In these studies, dimethicone was found to significantly reduce foam volume in a model shampoo formulation, while amodimethicone and dimethicone copolyol had a minimal effect on foam.

Auxiliary Ingredients

A number of ingredients, besides conditioning actives, are added to commercial conditioners for functional, esthetic, and marketing purposes (107). These include fragrances, dyes, preservatives, thickeners, emulsifying agents, pearlizers, herbal extracts, humectants, and vitamins. Some of these are discussed in the following sections. The literature also contains many examples of such additives (36,108–112).

Preservatives

Preservatives are necessary to insure the microbiological integrity of a conditioning product. If the product contains high concentrations of ethyl alcohol (generally 20% or above), additional preservatives are not needed and the product is described as self-preserving.

For other products, a wide variety of preservatives are available; in general, combinations of different preservatives provide the broadest possible protection. Every commercial product that is not self-preserving must be carefully tested over time for adequacy of preservation. Most of the preservatives used in personal care products are described in the *Cosmetic Preservatives Encyclopedia* (110).

Thickeners

As described in the section "Lipophilic Conditioners" thickening as a result of liquid crystal formation in those products containing common quats and fatty alcohols. Cationic conditioning polymers can also act as thickeners. Many formulations may require additional thickening agents. Hydroxyethylcellulose, a nonionic cellulose ether compatible with cationic surfactants and stable over a wide pH range, is the most common thickening agent added to conditioning products (36). In addition to providing increased viscosity, this material stabilizes viscosity over time.

Polyamides may also be used to thicken formulations. A commercial product, Sepigel (which contains polyamide, laureth-7, and isoparaffin) can be used to emulsify and thicken lotion or cream conditioners. Other thickeners are described in reference (111).

Polyacrylate-based thickeners such as carbopol have been widely used in personal care products. However, in the past these thickeners have not always been compatible with cationic surfactants. Recently, new thickeners based on polyacrylate chemistry have been commercialized to address this issue. Structure Plus polymer (National Starch & Chemical Company) and Carbopol Aqua CC polymer (Noveon Inc.) are two examples that are used at low pH and show good cationic surfactant compatibility.

Humectants

Many conditioners contain humectants whose purpose is to attract moisture. Examples are propylene glycol, glycerin, honey, chitosan, and hyaluronic acid. These materials are not expected to be very effective in rinse-off products.

Emulsifiers

As discussed in the section "Lipophilic Conditioners", the fatty alcohol/quat combinations found in common conditioners confer stability on product emulsions. If necessary, other emulsifiers may be added to improve stability. Information on emulsions and emulsifiers may be found in the literature (112,113) as well as from manufacturers' technical bulletins. Most emulsifiers used in conditioners are nonionic, including ethoxylated fatty alcohols, ethoxylated fatty esters, and ethoxylated sorbitan fatty esters.

CONCLUSION

The foregoing sections have surveyed the action and properties of a diverse assortment of commercially available conditioning agents. The availability of a large selection of conditioning materials enables the formulator to tailor products to a wide variety of people having differing conditioning needs and preferences. Thus, a person having short, straight hair in good condition might desire a conditioner primarily to control flyaway. Such a need could be satisfied by one of the ethoquats, which provide light conditioning benefits together with very good static control. A person having long, heavily bleached hair, on the other hand, would require improved hair feel, ease-of-combing, and manageability. These benefits could best be provided by a trialkyl quat.

Those people sensitive to hair feel might prefer a product containing a silicone as a secondary conditioner. Other people might prefer the convenience of a 2-in-1 shampoo. In many cases, both 2-in-1 shampoos and stand-alone conditioners are used to condition the hair.

There are a number of ways in which one might satisfy the conditioning needs of a target population. It is anticipated that the information in this chapter will help the formulator to quickly choose the best conditioning system for a given purpose. It is also hoped that the material in this chapter will help the formulator to effectively evaluate new conditioning agents and even to work with synthetic chemists as well as suppliers to design new conditioning compounds to solve particular problems.

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68 Measuring Hair

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THE STRUCTURE OF HUMAN HEAD HAIR

Desmond Morris referred to humans as "Naked Apes" (1). While the body hair on most humans is vellus, we grow hair on our heads that is far longer than the more abundant coat on most other mammals. Human head hair typically ranges from 20 to more than 100 μm in diameter (2), and some people can grow their hair to lengths of more than 5 ft.

The hair shaft is composed of columnar cortical cells that are surrounded by the overlapping cuticle scales. In some hairs, there may also be a more porous area in the center called "the medulla." A transmission electron micrograph (TEM) of a horizontal cross-section through the hair follicle at the level of the reticular dermis is shown in Figure 1. The various layers of the follicle and the hair can be clearly seen. Working in from the glassy collagen layer of the dermis (D) that surrounds the follicle, the outer root sheath (ORS), Henley's (he) and Huxley's (HU) layer of the inner root sheath, the innermost cuticle of the inner root sheath (CL), and the cuticle (CU) and cortical (CO) layers of the hair shaft itself can be seen. This particular hair does not show clear evidence of a medulla.

Human head hair has 6 to 10 layers of cuticle when it emerges from the scalp (3). A cross-section of a hair stained with silver methenamine is shown in Figure 2. Each cuticle cell is connected to the cortex, and the cells overlap from the root to the tip at an angle of about 5° (4), causing the well-known directional difference in hair friction (5). Cuticle cells are flat and approximately square, being about 50 μm on a side and about 0.5-μm thick (4). Each cuticle cell is composed of a cell membrane complex (CMC) and three distinct internal layers of differing sulfur content—α-layer, exocuticle, and endocuticle. The CMC has two 3-nm thick β-layers on either side of an 18-nm thick δ-layer. The upper β-layer, which faces out from the hair, has an outer surface of 18-methyl eicosanoic acid (18-MEA) (6) that is covalently attached to proteins by thioester bonds (7,8).

Cortical cells are roughly cylindrical being 50 to 100 μm long and 3 to 6 μm in diameter. They have longitudinal flutings and may separate into smaller fingerlike structures. The cells are closely packed together in the hair shaft so that the fluted surfaces interlock putting their cell membrane complexes in contact (9).

The mechanical properties of hair are dominated by the keratin microfibrils in the cortex, while the optical and surface properties are dominated by the cuticle and particularly by the state of the 18-MEA on the surface.

DETERMINATION OF HAIR DIMENSIONS

In order to determine tensile properties such as the elastic modulus, it is necessary to accurately measure the cross-sectional dimension. Determining the cross-sectional dimensions of a hair is not always straightforward. Not only is hair a thin fiber, it is not necessarily uniform in cross section. While Caucasian hair is generally considered elliptical in shape, significant variations from ellipticity can occur. With African-American hair, the problem is compounded by the high elliptical ratio and the presence of many nonuniform shapes. There is now laser micrometer that can be used for this purpose. Dia-stron makes an instrument that can be interfaced with their automated tensile testers. Several measurements must be made along the fiber, and the fiber must be rotated to ensure measurement of the major and minor axes of the ellipse. Perhaps the most accurate method of determining the cross-sectional area of a hair is to section it and measure the diameters directly from the micrographs (10).

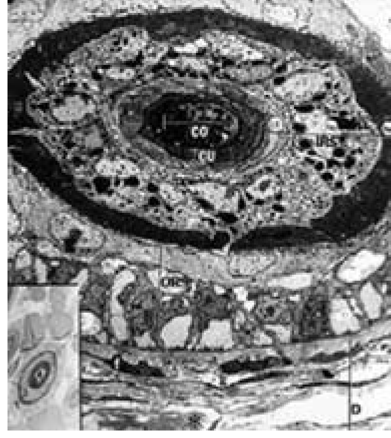


Figure 1 TEM of cross-section of human hair. *Abbreviation*: TEM, transmission electron micrograph. *Source*: Micrograph courtesy of Raymond Boissy.

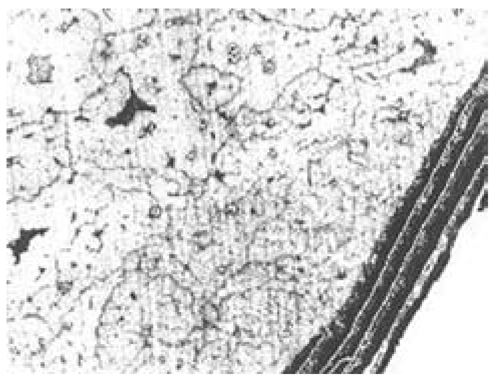


Figure 2 Cross-section of a hair stained with silver methenamine.

TENSILE PROPERTIES OF HAIR

The mechanical behavior of hair is frequently studied in extension by obtaining a stress/strain curve. Stress/strain curves for hair can be obtained by using tensile meters such as Instron or Dia-stron.

Figure 3 shows stress/strain curves for the adjacent sections from the same hair fiber in extension in water and at 50% relative humidity (RH). The curves can be characterized by three

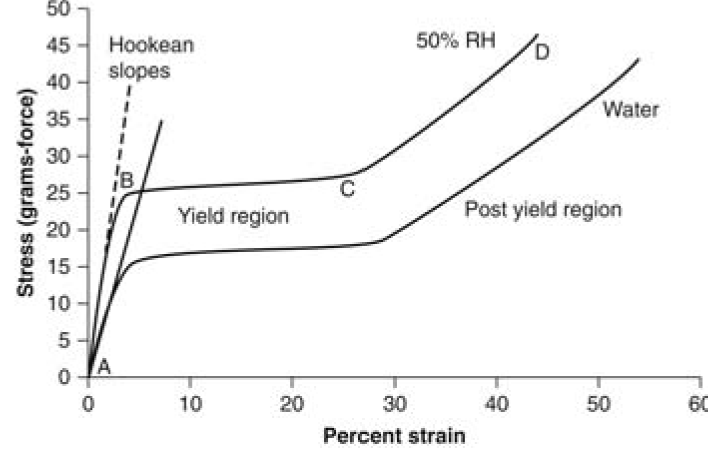


Figure 3 Stress/strain curve for hair at 50% RH. Data obtained using a Dia-stron miniature tensile tester.

different regions. The curve in the first region (A-B) is approximately linear and a slope can be determined. This part of the curve is often called "the Hookean region," and it extends to about 102% of the equilibrium length of the fiber (2% strain). The slope of the Hookean region is considerably higher in dry hair. Between 2% and 4% strain, the curve "turns over" into the yield region (B-C). In the yield region, very little increase in force is required to increase extension. In the post-yield region (C-D), which typically begins between 25% and 30% deformation, the force again increases markedly with strain. For the data presented in Figure 3, under the specified testing conditions, the slope in the post-yield region was about one-fifth of that in the Hookean region of the dry fiber. It is also observed that there is little difference in post-yield slopes between the wet and dry sections of the hair.

Published papers on the mechanical properties of keratin fibers date back to the 1920s and to the work of Speakman (11) who first reported on the effect of water on keratin mechanical properties. Since that time, extensive research on hair and wool has led to an interpretation of each region of the stress/strain curves of keratin fibers in terms of changes occurring in the molecular structure. Much of the seminal work in this area was carried out by Max Feughelman, and his book on the subject gives an excellent and detailed overview of the physical properties of keratin fibers (12).

The curve in the Hookean region (A-B) can be used to calculate Young's modulus of elasticity, E. The elastic modulus is the stress divided by the strain, so the cross-sectional dimensions of the fiber must be known accurately. The sample calculation given below was done assuming a circular hair with a diameter of 60 μm using typical numbers for a virgin hair at 50% relative humidity.

Sample calculation of the elastic modulus:

- $E = \text{stress/strain} = \Delta F^*L/(\Delta L^*A)$.
  - ΔF is change in force corresponding to the length change ΔL.
  - L is length of fiber.
  - A is the cross-sectional area.
- Assuming circular hair is 60 μm in diameter.
  - $A = 2.83 \times 10^{-5}\ \text{cm}^2 = 2.83 \times 10^{-9}$ m
- A 10-cm length is extended to 10.2 cm.
  - 2% extension
- Force change is 22 grams force = 0.216 N.
  - 1 gram force = 980 dynes = 9.8×10^{-3} N
- $E = (0.216 \times 10\ \text{cm})/(0.2\ \text{cm} \times 2.83 \times 10^{-9}\ \text{m}^2)$
- $E = 3.8 \times 10^9\ \text{N/m}^2$
- $1\ \text{Pal} = 1\ \text{N/m}^2$, $1\text{N} = 105$ dynes, $1\ \text{m}^2 = 10^4\ \text{cm}^2$
- $E = 3.8 \times 10^9\ \text{Pa} = 3.8 \times 10^9\ \text{N/m}^2 = 3.8$ GPa
- In older papers, E is called Y and is reported in dynes/cm^2.
- $E = 3.8 \times 10^{10}\ \text{dynes/cm}^2$

E is typically about 1.5 to 2.0 GPa for wet hair and 3.5 to 4.0 GPa for hair at 50% to 65% RH. The mechanical properties of hair or wool in the Hookean region and the effect of water on mechanical properties (Fig. 3) can be explained by the two-phase model proposed by Feughelman (13–15). Feughelman's model considers the mechanical properties of the fiber to be determined by a water-impenetrable phase, C, the microfibrils, and a water-permeable phase, M, the matrix. The microfibrils consist of α-helical proteins (keratins) aligned parallel to the fiber axis (16–19), and the matrix is composed of keratin-associated proteins (20), which are packed around the microfibrils. The composite is modeled as a fixed spring in parallel with a spring and viscous dashpot in series; the spring contributes about 1.4 GPa to the Young's modulus and is contained in the water-impenetrable microfibrils. The main resistance to extension of the microfibrils probably comes from the hydrogen bond network in the α-helical proteins (21). The matrix contributes viscous forces, which decay with time, causing stress relaxation. The viscosity of the matrix decreases greatly as the water content of the fiber increases. The two-phase model of keratin fibers accounts for the effects of water on the mechanical properties, the fact that increasing strain rate increases Young's modulus, the stress relaxation behavior in the Hookean region, and the behavior of wet, dry, and permanently set fibers in torsion (15,21).

The Yield Region

Somewhere around 2% to 3% strain, the stress/strain curve “turns over” into the yield region. Past this point, the stress does not increase markedly until about 25% to 30% extension. The mechanical properties of a fiber extended into the yield region can be recovered by relaxing the fiber in water for a few hours if the fiber is not held too long in extension and the extension is carefully confined to the yield region. This fact is of great practical importance in designing protocols to measure the effect of treatments on hair strength, as we will see. X-ray diffraction results have demonstrated that there is a progressive loss of a helical content and a concomitant increase in β-sheet as a fiber is extended through the yield region (22). By the end of the yield region, about 30% of the original α-helix has been unfolded reversibly. Mechanical behavior of keratin in the yield region can be accounted for by application of a Burte–Halsey model (23). The fiber is considered to contain a continuum of units, which can exist in a short state, A (α-helix), or an extended state, B (β-sheet), with an energy barrier between the states. The yield region corresponds to a phase transition between state A and state B at constant stress. This first-order phase transition, producing a length change at constant stress and temperature, is thermodynamically equivalent to the transformation of water to steam, producing a volume change at constant temperature and pressure.

Data from hair strained into the yield region are usually reported as either force at a given extension such as 15% or 20% or the work to extend a hair to a given extension, which is obtained by integrating the area under the stress/strain curve. Hu (24) measured the work to extend hairs to 15% and compared results for Caucasian, Asian, and African-American hair. Results for Caucasian hair, in terms of W_{15} as a function of hair diameter, are shown in Figure 4.

Table 1 shows the correlation equations for the work to extend to 20% for hair from each ethnic group. The work to extend African-American hair by 20% was found to be about two-thirds of that required for either Asian or Caucasian in agreement with other studies showing that hair of African origin is not as strong as either Asian or Caucasian hair (25).

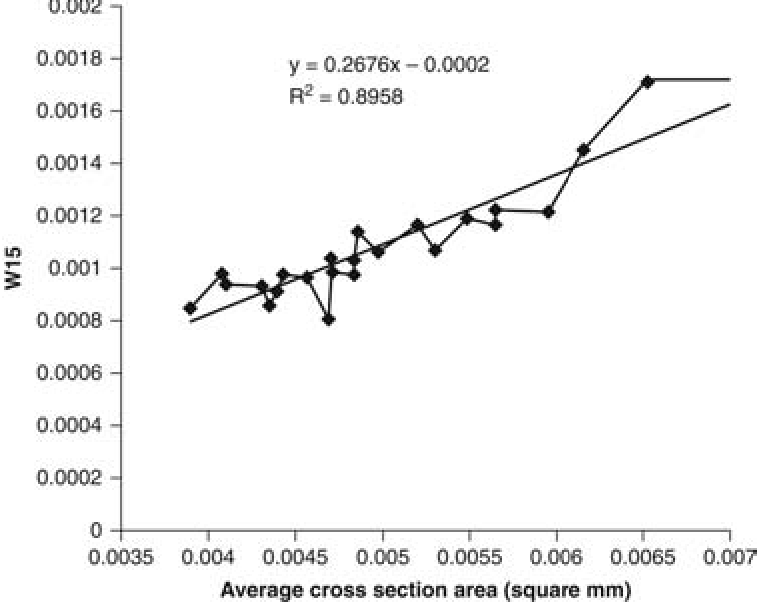


Figure 4 Work (in Joules) to extend hair by 15% versus cross-sectional area.

Table 1 Correlation of Work of Extension to Cross-section Area (A) for Different Ethnic Groups

Hair type	Correlation equation	R^2
Caucasian	W20 = 0.2676A − 0.0002	0.90
African-American	W20 = 0.1844A + 0.0002	0.83
Asian	W20 = 0.2961 − 0.0003	0.91

The Post-Yield Region

The C-D region in Figure 3 is known as the post-yield region. Speakman (11) found the post-yield slope to be independent of the water content of the fiber. This is borne out by the essential equivalence of the slopes (for dry and wet hair) of the C-D region shown in Figure 3. The increased stiffness in the post-yield region apparently results from a covalently bonded network involving cystine. The post-yield slope has been shown to be dependent on the disulfide content of the fibers (26–28).

Tensile Testers

In the past, stress/strain measurements on hair were mostly made using one of the models of the Instron® tensile tester (2,5,29,30). The Instron is a robust and versatile instrument but is more than a bit of overkill for measuring hair mechanics. In recent years, the Dia-stron miniature tensile tester (MTT) has been widely used. The instrument can be equipped with an automated sample head (31) to allow running of up to 100 hairs in one setup and can be interfaced with a laser micrometer system to automatically measure the dimensions of the hair fiber.

Software with the instrument can automatically record the stress/strain curve and report various parameters to the operator, including elastic gradient, work of extension, and breaking load. Evaluation of hair tensile properties has come a long way from the days of tediously measuring data points off a chart recorder with a ruler.

Tensile Measurements of Hair Damage

The most common use of hair tensile properties is for the evaluation of the effects of treatment on hair strength to determine the level of "damage" produced by a given treatment. The mechanical properties of wet hair are greatly affected by treatments that lead to a reduction in the number of disulfide bonds. Measurement of breaking strength may show differences between treatments if they are large and large number of hairs are run, but one must try to select hairs of approximately the same diameter for measurement, if possible. For this reason, many workers have relied on the fact that mechanical properties of hair extend into the yield region, but not beyond, and can be recovered by soaking in water. Beyak et al. (29) extended hair by 20% and measured the force. After recovery, treatments were investigated using each hair as its own control. The average change between tests for 25 untreated control hairs was only –0.33%. In contrast to this, a five-minute "cold wave" treatment led to a 12.6% decline in the force at 20% extension.

Tate et al. (32) and Robbins and Crawford (33) also used mechanical measurements in extension to study hair damage. The study by Robbins and Crawford revealed the interesting fact that significant damage to the cuticle can occur with little or no effect on the tensile properties of hair.

Gamez-Garcia described the use of short-term relaxation measurements from small deformations to assess the effect of oils, emulsions and solutions of salts, amino acids, and proteins on stress recovery in hair fiber (34). The author analyzed the relaxation curves and showed that the curves had a short-term (on the order of minutes) component and a long-term (of the order of hours) component. The medium in which the fiber is immersed was found to have a strong effect on short-term relaxation.

Hu (24) investigated the effects of heat and relaxer treatment using both breaking stress and the work to 15% extension (Fig. 4). Heat treatments were for five minutes. Relaxer treatment was with a commercial relaxer according to label directions. Data are summarized in Table 2 shown below.

Table 2 Effect of Treatment on W15 and Break Stress

Treatment	Change W15 (%)	Change break stress (%)
60°C	1.9	−5.2
115°C	−.02	−9.9
130°C	−3.5	−10.6
160°C	−5.9	−10.5
Relaxer	−25.7	−37.5

It does appear that breaking stress is more affected by heat at low temperature, and the effect of heat on breaking stress was significant at 130°C, while W15 was not significantly affected. This may be due to loss of disulfide bonds that are not extended before post-yield region is reached. To obtain this kind of result for breaking strength, the hairs must be carefully prescreened to be of approximately the same diameter.

MECHANICAL FATIGUE BEHAVIOR

Kamath et al. described an apparatus for studying the mechanical fatiguing of hair (25,32). The instrument subjects the fibers to an impact-loading mode of fatiguing at a constant load and rate of one cycle per second for up to 100,000 cycles. The strain in hair was kept within the Hookean range and the fatigue data were interpreted in terms of the following equation:

$$F(x) = A(x)^{\mathrm{n}}$$

where $F(x)$ is the cumulative probability of failure, x is the number of cycles to failure, and A is constant. The exponent n and the number of cycles required for half of the specimens to fail (h_f) were employed to quantify the damaging effect of grooming treatments on hair. The exponent n was found to vary from approximately 0.5 ± 0.052 for untreated hair to 0.11 ± 0.098 for hair after three perming treatments. A concomitant change in half-life parameter, h_f, ranged from more than 100,000 cycles to 3000 cycles. This technique was also found to be useful in evaluating fiber damage as a result of bleaching and perming.

DYNAMIC MECHANICAL ANALYSIS

Dynamic mechanical analysis has not been employed frequently in hair studies. This was probably due to the fact that the old generation instrumentation was difficult to use, and the experiments were very time consuming.

During the last 20 years, several new instruments were introduced. They include the dynamic mechanical and thermal analyzer (DMTA) (Rheometric Scientific, Piscataway, New Jersey, U.S.) and the dynamic mechanical analyzer (Perkin Elmer DMA7), characterized by high sensitivity, broad dynamic range, and high force control.

The dynamic mechanical experiment gives information on both storage and loss modulae (or tan delta) and can provide a complete characterization of viscoelastic properties of hair fibers. The measurements can be performed as a function of time, temperature, or frequency in both stretching and bending modes of fiber deformation. For characterization of hair and hair-care products, the bending mode of operation is of particular interest because it is probably a predominant mode of deformation for "in vitro" hair on the scalp.

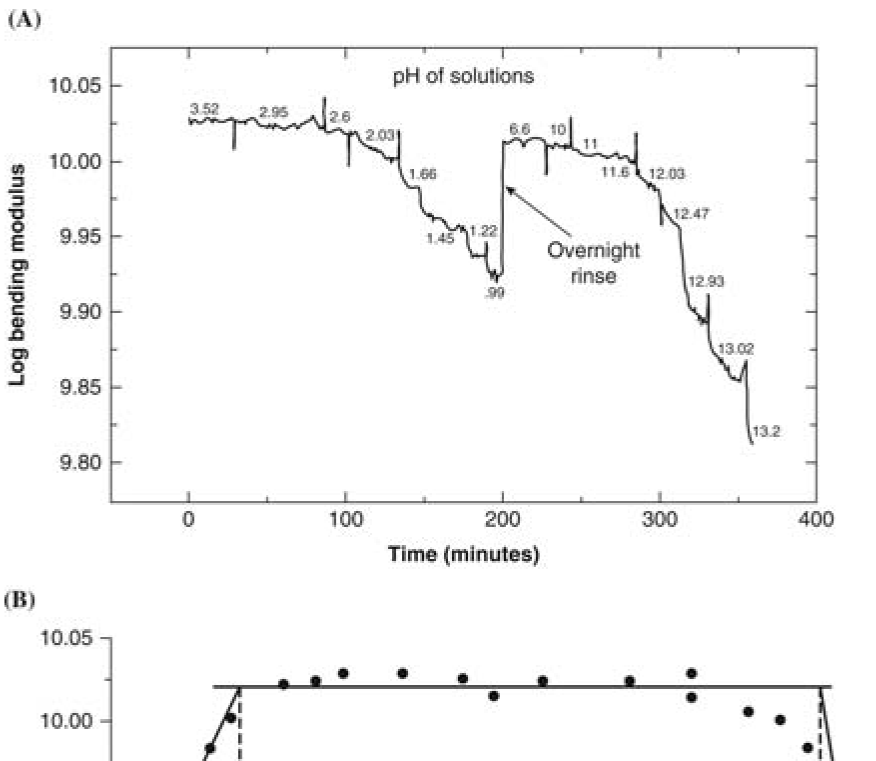
An example of the use of dynamic mechanical measurements in hair studies is the pH dependence of storage modulus shown in Figure 5. The data were obtained through the use of the DMTA equipped with a humidity controller and an online treatment attachment (M. Zielinski, unpublished data).

Figure 5A presents the actual DMTA trace, presented as the logarithm of bending modulus as a function of the experiment's time, obtained for a 40-fiber assembly mounted in a frame for a single cantilever bending measurement. The active length of fibers was 1 mm, with an amplitude- and frequency-held constant at 128 μm peak-to-peak and 3 Hz, respectively. The pH was adjusted with HCl and NaOH by using solutions at 22°C continuously flowing over the hair sample.

Figure 5B shows the averaged modulus data from Figure 5A plotted as a function of pH. The results demonstrate a relative constancy of bending modulus in the pH range from 3 to 10, and its decrease in both very acidic ($\mathrm{pH} < 2$) and very basic ($\mathrm{pH} > 12$) solutions. A similar analysis can be performed for hair exposed to chemical treatments, surfactants, or polymer solutions.

Flexabrasion Testing

Swift has pointed out that the mechanism of hair breaking on the head is probably different from simple breakage in tension (35,36). A method called "the flexabrasion test" may be



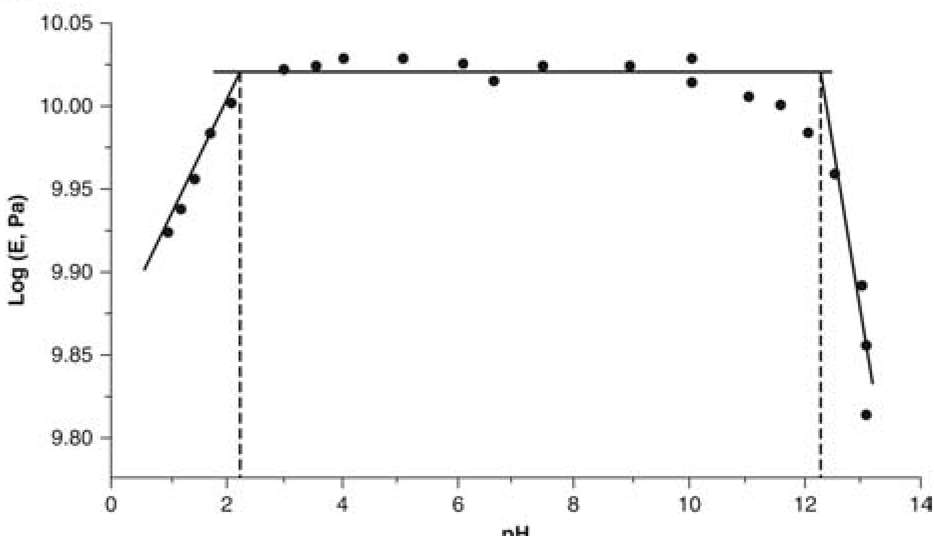


Figure 5 Dynamic mechanical analysis. (**A**) DMTA trace obtained by employing an online treatment procedure (with HCl and NaOH solutions) and shown as the logarithm of the bending modulus. (**B**) Bending modulus of wet intact hair as a function of pH. *Abbreviation*: DMTA, dynamic mechanical and thermal analyzer.

more relevant to the actual consumer experience. In this test, weighted hairs are pulled back and forth across a fine wire by a reciprocating motor as illustrated in Figure 6 on the next page.

The parameter measured is the number of cycles required to break 50% of the hairs. This number has a very high variance from hair to hair. Swift reported data were obtained using adjacent sections of the same hair for treatment and control to reduce the variance. By using three sections from each hair, a control, a damaging treatment and an intervention could be studied. Some of Swift's (36) data are presented in Table 3.

Data from this method are the basis of some rather extreme sounding claims for large increases in hair strength. It is not the tensile strength that is increased by the treatment but the resistance to fraying under repeated abrasion. This method will obviously reflect the presence of treatments that can reduce the friction between the wire and the hair.

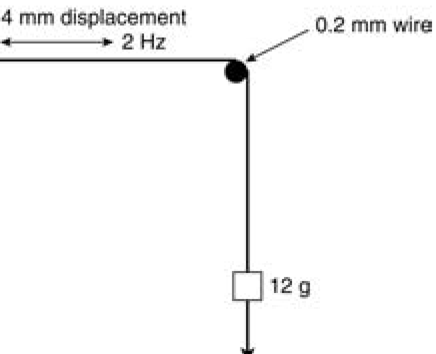


Figure 6 Illustration of the flexabrasion test.

Table 3 Effect of Conditioner on Bleached Hair by Flexabrasion

Conditioner	Bleached	Bleached + conditioner	Difference	Increase (%)	*p*
Leave on	1066.5	1621.5	555.1	52.1	0.04
Rinse off	649.7	1548.9	699.2	82.3	0.01

Torsion and Bending Measurements

Forming a curl from straight hair involves a combination of twisting and bending deformations. Response to torsion or bending stress is highly dependent on hair diameters, as both the bending and torsional moments of inertia depend on the fourth power of cross-sectional dimensions. For example, the resistance to bending of an elliptical hair is given by E^*I_b, where E is bending modulus and I_b is the bending moment of inertia, and by $(\pi/64)ab^3$, where a and b are the major and minor semidiameters of the ellipse. The torsional moment of inertia is given by $I_t = (\pi/4)(a^3b + b^3a)$, while the resistance to twisting is given by $G^*(\pi/4)(a^3b + b^3a)$, where G is the shear modulus. Because of this extreme dependence on cross-sectional dimensions, the cuticle may contribute more to torsion or bending than to extension, especially with very fine hairs.

The shear modulus of a fiber can be determined using a torsion pendulum. In this method, a small cylindrical weight is hung from the hair. Application of torque to the weight causes it to rotate back and forth. If a small white strip or a small mirror is attached to the weight as illustrated in Figure 7, the amplitude and period of the torsional deformation as the weight rotates can be easily determined. The shear modulus can be determined from the following equation:

$$G = (Ml/\omega 2)/I_t$$

where l is the fiber length, I_t is $(\pi/4)(a^3b + b^3a)$ as explained above, and M is moment of inertia of the weight. Another parameter that can be determined from a torsion pendulum is the log

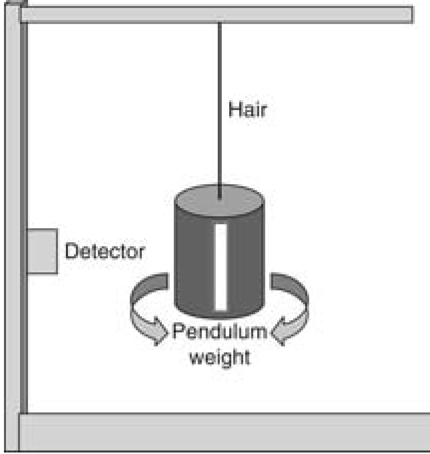


Figure 7 A schematic of a torsion pendulum for the study of hair.

decrement (δ). Log decrement is related to the log of the change in amplitude between one cycle and the next. Log decrement will be increased by treatments or conditions that either increase frictional loss or decrease the storage of elastic potential energy as the fiber twists. Pesuad and Kamath (37) have recently described such a device in detail.

Bogaty (38) first pointed out how important the behavior of hair under torsional and bending strains is to formation and maintenance of hairstyle. He reported that permanent waving decreased the torsional rigidity of hair in the wet state but actually increased it slightly at 65% RH. Harper and Kamath (39) reported similar results for bleached hair. At low RH, the shear modulus of bleached hair was higher than that for untreated hair, but above 70% RH the shear modulus of bleached hair was found to be lower than for untreated hair.

Wolfram and Albrecht (40) carried out torsional measurements on hair using a torsion pendulum. They concluded that the cuticle is very stiff in the dry state and may make a significant contribution to the torsional rigidity, especially for fine hairs. However, in the wet state, the cuticle was found to be so plasticized as to make no contribution to mechanical behavior. On the other hand, Harper and Kamath (39) and Yasuda et al. (41) reported that the cuticle makes a significant contribution to the shear modulus of dry hair.

Bending is also a key component of a hairstyle, but bending measurements are generally not simple to perform. Scott and Robbins (42) described a method for measuring the bending stiffness of hair using a balance. A long hair is draped over a small wire with small weights attached to each end. The bending stiffness can be calculated from the distance between the two ends. It is also possible to measure bending strength by a three-point beam deflection method. This method has been applied to measuring the stiffness of beard hairs (43). Another approach is cantilever beam method as applied by Yasuda et al. (41). The balanced fiber method has the disadvantage of requiring a relatively long fiber but is simpler to use. Wortman and Kure (44) used a similar fiber method to study bending relaxation during permanent waving of hair.

SPECTROSCOPY

Fluorescence Spectroscopy

Fluorescence spectroscopy is employed to measure the wavelength dependence of the intensity of emitted light as a function of the excitation wavelength. Hair and skin are characterized by strong fluorescence because of the presence of tryptophan, kynurenine, tyrosine, and phenylalanine aminoacids in the structure of keratin. Tryptophan has the strongest absorption ($\lambda_{max} = 280$ nm, $\varepsilon_{max} = 4500$ M^{-1} cm^{-1}) and a high-quantum yield of fluorescence. Its fluorescence band, excited at 290 nm, has a maximum in the range from 330 to 350 nm, which is dependent on the extent of hair pigmentation.

The technique has been employed as a sensitive analytical technique to study reactions accompanying hair photo and thermal degradation (45–47). A fluorescence instrument used to study hair is typically equipped with remote fiber optics, which allows for recording the spectra directly from hair fibers. Experiments described in the literature consisted of irradiating hair with UV/visible light and recording emission spectra in a wavelength range where fluorescence emission occurs. Photodegradation studies have shown that tryptophan undergoes photodecomposition that can be quantified by the measurements of the emission intensity at 300 to 550 nm for hair before and after exposure (Fig. 8). The peaks at 350 , 420 , and 465 nm have been assigned to tryptophan, *N*-formylkynurenine, and kynurenine, respectively (45,47). The intensity of emission of all these chromophores is shown to decrease as a result of photoirradiation, with tryptophan emission undergoing the largest change. The phenomenon was shown to occur both in natural outdoor conditions and as a result of artificial light irradiations in a weatherometer. This technique can also be used to determine the extent of hair photoprotection, in terms of tryptophan damage, by incorporating photofilters in hair-care formulations such as conditioners, mousses, shampoos, and hairsprays.

IR and Raman Spectroscopy

Raman and infrared (IR) spectroscopies provide alternative ways to detect vibrational states of molecules. While transitions producing IR bands are due to vibrations of groups with a permanent dipole, those corresponding to Raman bands are due to changes in the polarizability of nonpolar groups as a result of nuclear motion.

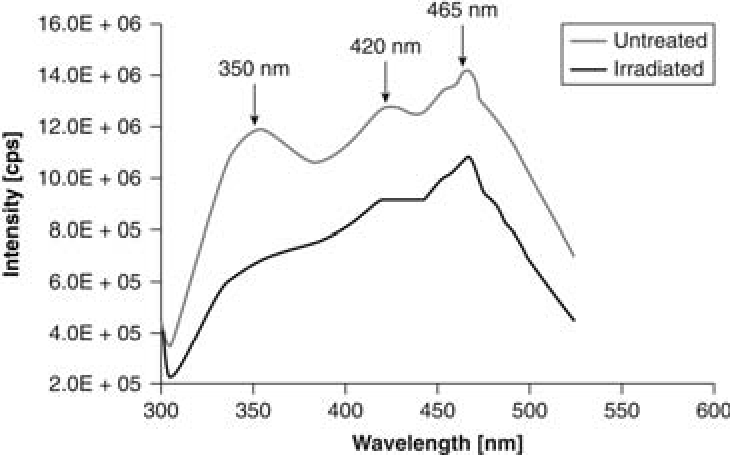


Figure 8 Comparison of the fluorescence spectra of untreated and piedmont hair irradiated for 48 hours in a weatherometer.

In recent publications on hair, fourier transform (FT)-IR spectroscopy was combined with a microscope (referred to as FT-IR microscopy), allowing researchers to examine hair shaft areas in the size range 10 to 100 nm. Bramanti et al. (48) examined micro regions of hair for anagen, catagen, and telogen hair. The data from bulb and shaft areas were analyzed in terms of relative intensities of amide II (1540 cm^{-1}) and amide III (1238 cm^{-1}) vibrations of the protein component of hair structure and O-P-O vibrations (1080 cm^{-1}) of nucleic acids. It was shown on the basis of simple spectra and their derivatives that there was a gradual change in the ratios of absorbance values for nucleic acids/proteins (A_{1080}/A_{1238}) for inferior bulb, central bulb, and suprabulbar and shaft regions for anagen, catagen, and telogen hair. It was suggested that these ratios can be used as reproducible parameters to differentiate the anagen, catagen, and telogen hair phase and to estimate the degree of hair aging.

Other reported applications of FT-IR microscopy included an estimation of the extent of hair oxidation by analyzing the intensity of a peak at 1041 cm^{-1} corresponding to cysteic acid formation in the oxidation of cystine (45).

In a typical Raman experiment, the sample is irradiated with an intense beam of light at a specified frequency u. The emitted light consists of radiation with an unchanged frequency of u (light scattering and refractive index), Raman bands with frequencies at $u + u'$ (Stokes band), and $u–u'$ (anti-Stokes band).

The main advantage of Raman spectroscopy in studies of biological molecules is the low intensity of the water spectrum. A strong IR spectrum of water overlaps the regions where biomolecules have IR absorption bands. The intensity of water spectrum in Raman spectroscopy is relatively weaker, which makes it useful for studies of proteins, etc.

For hair, Raman measurements were reported for unpigmented and bleached hair, employed to minimize the fluorescence effects predominant in more pigmented fibers (49,50). The spectra of untreated hair show a number of bands not observed in the IR analysis. They correspond to disulfide bonds (510 cm^{-1}), tyrosine (646 cm^{-1}, 853/827 cm^{-1}), phenylalanine (1003 cm^{-1}), and tryptophan (1554 cm^{-1}).

The Raman spectra could be used to assess the incurred damaged associated with hair bleaching, permanent waving, and photoirradiation:

- For bleaching, a decrease in intensity of the 510 cm^{-1} band with a concomitant increase in the intensity of a band at 1045 cm^{-1} (SO_3, cysteic acid) was observed.
- For perming, reduced hair showed a peak reduction at 510 cm^{-1} and the appearance of a peak at 2568 cm^{-1} (mercaptan).
- For photodamaged hair, analysis showed a decrease in intensity of the disulfide band with the appearance of vibrations corresponding to sulfur in various oxidation states, including a thiosulfonate bond. In addition to this, there was an increase in the intensity of a mercaptan band and a change in the amide I region corresponding to a disordered protein.

Near Infrared Spectroscopy

Near infrared spectroscopy (NIR) refers to the portion of the IR spectrum in the wavelength range from 1000 to 2200 nm (10,000 –4500 cm^{-1}). The observed bands correspond to overtones and combination of characteristic bond vibrations. The technique is typically employed to study bands corresponding to O-H from water, C-H from hydrocarbons, and N-H for proteins. It may also cause transitions of highly delocalized electronic systems, such as those present in the structure of melanin.

Several authors evaluated NIR spectroscopy for studying hair (51,52). Pande et al. (52) found the technique well suited for measuring relative moisture content of hair in situ. The NIR spectra of hair conditioned at 50% RH and dehydrated by heating to 110°C for 90 minutes are shown in Figure 9. It was concluded that peaks at 1450 and 1935nm are due to water, while the peaks at 1740 cm^{-1} are related to methylene C-H stretch, and the bands at 2051 and 1984 nm are due to protein. The 1900-nm absorption was further used to quantify the amount of water in hair at 50% RH after drying and as a result of moisture regain. The technique proved to be sensitive to detect small differences in the kinetics of moisture regain observed for hair treated with a hair-care product such as conditioner.

Another application described by Pande et al. (52) is the measurements of melanin bleaching during oxidative coloring of hair. Such measurements are possible because the synthetic hair dyes have no effect on the reflectance of hair beyond 750 nm, while natural melanin strongly absorbs in NIR range from 1000 to 1300 nm. The data revealed that the bleaching effect can be quantified in terms of melanin absorption and even small differences between bleaching products can be detected. It should be added that such measurements cannot be performed with a typical colorimeter because of interference from the synthetic dyes.

Integrating Sphere Spectrophotometry

Integrating Sphere spectrophotometry is employed to study the absorbance of light scattering samples such as dispersions of solids and liquids. In cosmetic research, one frequently deals with non-transparent materials, or chromophores incorporated in turbid formulations, or deposited on non-transparent substrates such as skin or hair. In such cases, one cannot employ routine UV-vis spectroscopy because the light scattering results in a very weak intensity of transmitted light. In order to include scattered light, a technique referred to as integrating sphere UV-vis spectroscopy is employed. In this method, the scattered light is focused by

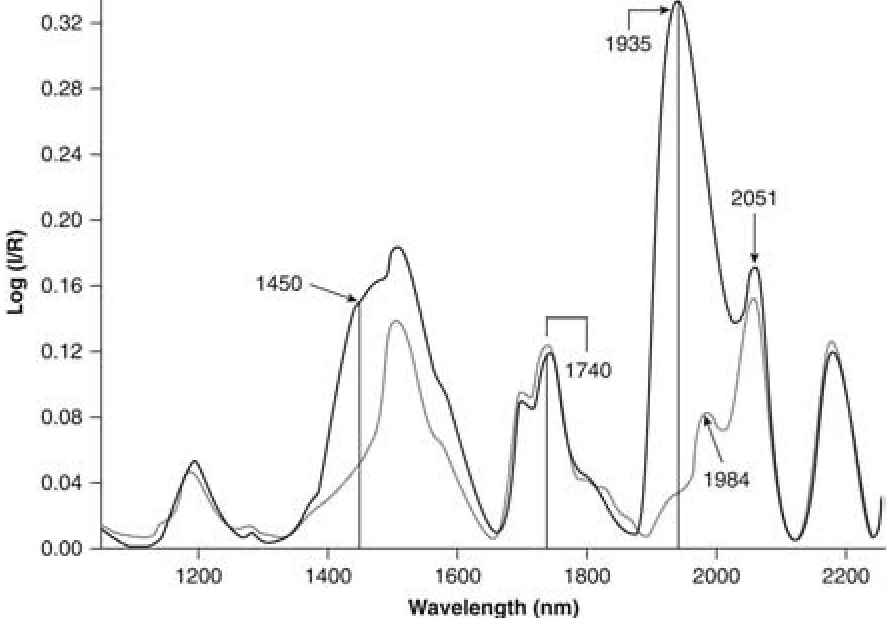


Figure 9 Near-IR absorption spectrum of unpigmented human hair (*fine line*) and the effect of water (*bold line*). *Abbreviation*: IR, infrared.

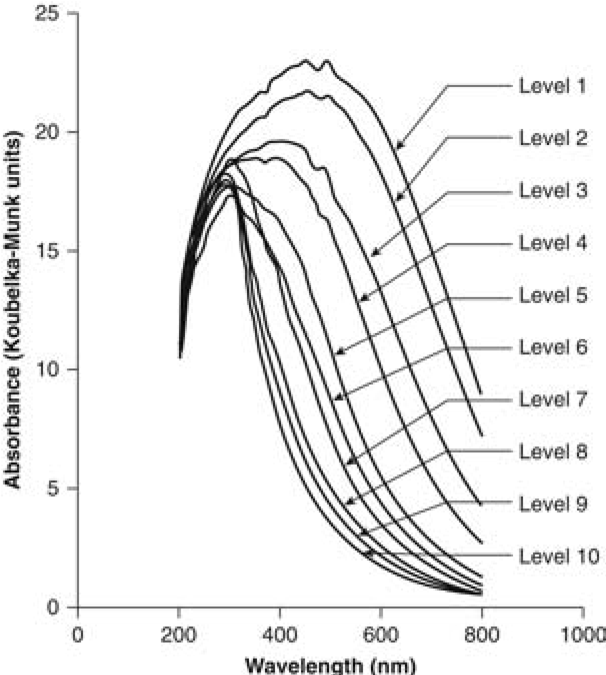


Figure 10 Absorbance (Koubelka-Munk units) as a function of wavelength for hair with various levels of pigmentation. The data were obtained by using integrating sphere UV-vis spectrophotometer in the reflectance mode. *Abbreviation*: UV, ultraviolet. *Source*: Courtesy of Johnson and Johnson Co.

reflecting from the walls of a $BaSO_4$-coated sphere. The spectra can be obtained by UV-vis spectrophotometers equipped with an integrating sphere, which operate in the transmission or reflectance mode (e.g., a Perkin Elmer Model 950). An example of the usage of the instrument in the transmission mode is testing skin-care sunscreen formulation to determine a sun protection factor (SPF) value. The tested product, which is typically a turbid formulation, is spread on the surface of a substrate (e.g., artificial skin), and the spectra are collected in the transmission mode and subsequently converted into Koubelka-Munk absorbance units. By using appropriate controls as well as the erythema action spectrum of sunlight, one can calculate the SPF value of a given product. In hair research, the technique is a quantitative tool to study hair coloration. Figure 10 presents a plot of absorbance (in Koubelka-Munk units) as a function of wavelength for hair with various levels of natural hair color classified by visual grading on the scale from 1 (black) to 10 (white). The data, such as those presented in this figure, can be further processed by, for example, mathematical spectral subtraction to derive information about the hair deposition of sunscreens, artificial hair color, color fading, etc.

X-ray Spectroscopy

The crystalline structure of keratins has been extensively studied in the past by X-ray diffraction (21,22,53,54). A new approach to this problem has been the use of synchrotron radiation, which can produce high quality X-ray diagrams in short experimental times (55).

High-resolution small angle X-ray scattering (SAXS) and wide angle X-ray scattering (WAXS) diffraction patterns were obtained by using high-intensity synchrotron radiation. SAXS diffractograms permitted the calculation of intermacrofibrillar, intermicrofibrillar, and inter-protafibrillar distances (88, 67.7, and 40 Å, respectively). WAXS gave the distance between individual helices (5.15 and 9.8 Å).

The key result was that cosmetic treatments, including perming, bleaching, or a combination of both, affect not only the distances between supramolecular elements of hair structure but also the distances between individual protein chains (55). Larger distances between micro-, macro-, and protafibrils are reflected in the increased swelling of chemically treated hair, a well-known phenomenon previously described. On the other hand, an increase in interhelical separation, probably as a result of interaction with water, was unexpected because of previously accepted models that assumed that the crystalline phase of hair structure was impenetrable by water or aqueous solutions of hair treatments.

ESR

Electron spin resonance (ESR) spectroscopy measures the transitions of an unpaired electron between energy levels produced by magnetic field (56). This is due to the phenomenon that an electron spinning at a given frequency can adopt two spin orientations in a magnetic field with each characterized by a different energy. It is possible to induce transitions between electronic spin energy levels by applying electromagnetic radiation with the frequency equal to the electron's precessional frequency. A typical magnetic field range employed in an ESR experiment is from zero to a few tesla units. ESR can only be employed for the detection of unpaired electrons such as free radicals and radical ions. The key parameters employed to characterize the ESR spectrum are (*i*) electron precessional frequency given by

$$\mathrm{Vprec} = 13.95\ [\mathrm{GHzT}^{-1}]gB_0$$

where B_0 is the strength of the magnetic field in tesla units (T), and (*ii*) g, which is referred to as the Lande factor. The values of g vary from 2.00220 for the ethylene radical to 2.0091 for the trichloromethane radical. A typical precessional frequency for a free electron at a field strength of 0.34 T is about 9500 MHz. The population difference between electrons in different spins states is larger than in NMR experiment for protons (it can be calculated from Boltzmann distribution), and therefore, ESR spectroscopy is more sensitive than NMR Spectroscopy. An ESR spectrum can be obtained for radicals at a concentration as low as 10^{-8} M at room temperature for a volume of a few 10ths of a milliliter in both the liquid and solid state. Also, the time scale of an ESR event is about 10^{-9} seconds; thus, it is faster than that in proton NMR, so the technique can provide information about processes that are too fast for NMR analysis.

As in NMR, an ESR signal of one electron can be split by a magnetic field of neighboring hydrogens according to Pascal's triangle rule. The separation between lines in a multiplet in the ESR spectrum is termed "hyperfine coupling" and is designated by symbol a (in Gauss units). For example, for a methyl radical, the ESR signal will be a quadruplet (with the intensity ratio of 1:3:3:1) at $g = 2.00255$ and $a = 23.0$ G.

In cosmetic chemistry, the application of this technique is limited to the studies of oxidation, antioxidants, and melanin chemistry. For melanins, the ESR spectrum consists of a featureless peak with a line width of about 4 to 6 G and a g value close to 2.004 (57). There is no hyperfine coupling, and the spin concentration is very small, about 4 to 10×10^{17} spins/g. The ESR method of melanin characterization is important because this natural polymer is considered to be a photoprotective and antioxidant agent for skin and hair.

Photoirradiation of hair was also investigated by ESR using spin trapping with DMPO (5,5-dimethyl-1-pyrroline *N*-oxide), which forms DMPO-OH adduct with brown, bleached, and red hair (58). The spectral evidence confirms the formation of oxyradicals during photoirradiation. Bleached and red hair (pheomelanin) was also found to produce more oxyradicals than black hair melanin (eumelanin).

MICROSCOPY

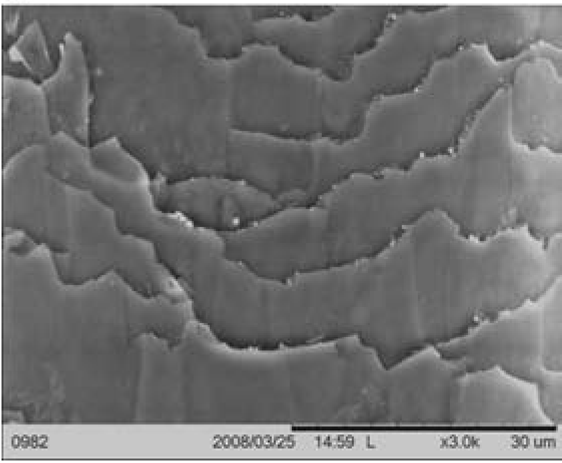
Optical Microscopy

Optical microscopy is employed for a variety of tasks in a cosmetic laboratory. It is very useful to evaluate hair geometrical shape and dimensions, detect the presence of surface deposits, or assess a degree of fiber damage in terms of cortex integrity, state of cuticles, or the presence of split ends. Traditional light microscopy, however, has limited resolution (approximately half of the wavelength of light) and is characterized by limited depth of field. New instruments address this problem by collecting images of the object at various focal lengths and subsequently computing reconstructed in-focus image.

Scanning Electron Microscopy

Electron microscopy requires high vacuum and metal coating of a nonconductive polymer for biological samples. The resolution of this technique is limited to a few nanometers. Scanning electron microscopy (SEM) is usually not sensitive enough to detect adsorbed polymers or a surfactant layer with molecular dimensions. Newer SEM instruments offer ease of use and are capable of producing good resolution images (below a magnification of 5000×) without metal coating. They can be also equipped with energy-dispersive X-ray fluorescence detectors, which

(A)



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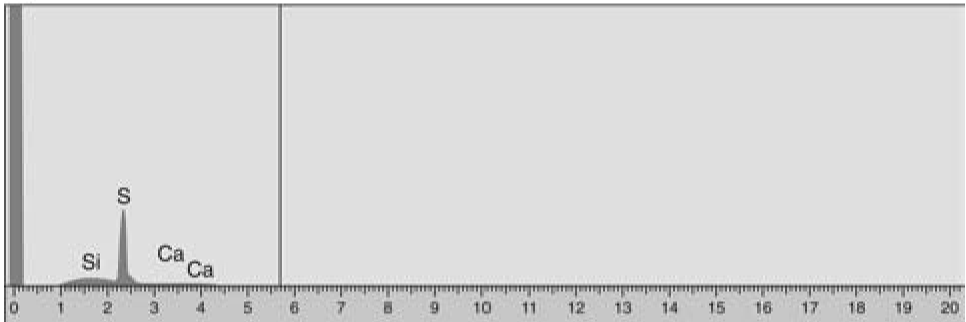


Figure 11 (**A**) SEM micrograph of hair at 3000× magnification and (**B**) results of EDS analysis of surface deposits concentrated near the cuticle edges. *Abbreviations*: SEM, scanning electron microscopy; EDS, energy dispersive spectroscopy. *Source*: Courtesy of Sunny Chen of Johnson and Johnson Co.

can display energies characteristic of the elements in the sample. The results are typically presented in the form of a histogram of signal strength as a function of energy (eV) with the detection limits ranging from 0.05% to 2%. Signal strength is related to relative concentration of a given element in the sample. Qualitative, semiquantitative, and quantitative bulk determination of elements for atomic numbers higher than 13 (all elements except H, He, Li, and Be) is possible. Energy-dispersive spectroscopy (EDS) can also provide elemental maps of the sample by identifying the elemental compositions of sample features as small as 1 μm.

Figure 11A presents an SEM image of hair (without metal coating) at a magnification of 3000× obtained by a benchtop Hitachi SEM instrument. The image illustrates the presence of granular surface deposits, which concentrate in the areas close to the cuticle edges. The use of EDS detector (Fig. 10B) indicates a high content of Si and Ca in the structure of the deposited material.

Atomic Force Microscopy

Scanning probe microscopy (SPM) can be used for imaging nonconductive surfaces of materials from the atomic to micron scale. Atomic force microscopy (AFM), and lateral force microscopy (LFM) fall under SPM designation of techniques, which also include scanning tunneling microscopy (STM), chemical force microscopy (CFM), and phase detection microscopy (PDM). These techniques can provide information about the topography and frictional and mechanical properties of a sample from the nanoscale to micron level. In both AFM and LFM, the probe, in the form of a sharp tip attached to a cantilever, scans the surface by using force on the order of 10 to 20 nN in the contact mode and 0.1 nN in the tapping mode. The latter is used to measure the surface characteristics of soft materials such as keratin fibers. In AFM, one obtains a topographical image by measuring the deflection of a soft cantilever, to

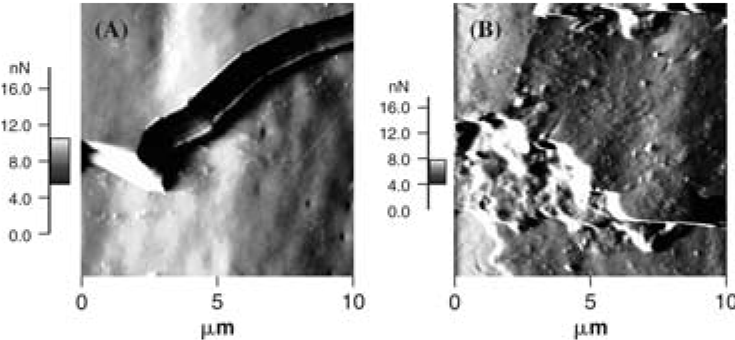


Figure 12 (**A**) Untreated hair (**B**) and hair treated with polyquaternium-28, as imaged by using error signal by LFM (64). *Abbreviation*: LFM, lateral force microscopy.

which the tip is attached, as the tip is rastered over the surface. The cantilever deflections normal to the surface are representative of topographical surface features. In LFM, one measures the torsional twisting of the cantilever as it is rastered over the surface. These lateral cantilever deflections result from drag forces between the tip and sample surface.

Both AFM and LFM have been used in cosmetic field to image hair fibers and to identify new morphological features under dry and wet conditions (59–67). O'Connor et al. (59) employed an atomic force microscope to quantitatively analyze the morphology of hair in air and water, the kinetics of hair hydration, and the effect of pH on hair morphology. Images were analyzed quantitatively by taking line cuts to illustrate height data versus position. They have shown that an average step height in the cuticle sheaths increased from 500 nm for dry hair to 1200 nm for wet fibers. By following the changes in geometrical dimensions of the cuticle height, they were also able to determine the rate constant of hydration and the effect of pH on swelling.

Goddard et al. (60,61) used an atomic force microscope in both modes (contact and tapping) to collect data on the distribution and configuration of adsorbed cationic polymers on the surfaces of hair and mica. They concluded that the hair surface is not smooth enough for quantitative analysis. Mica, on the other hand, has a model, well-characterized surface. Goddard's study of cationic polymers on mica demonstrated loop-and-trains configuration of adsorbed polymer chains, molecular weight dependence of the layer thickness, and polymer distribution as a function of charge density.

McMullen et al. (64) employed LFM to visualize deposition of cationic polymers on hair. Figure 12 presents a comparison of error signal image of untreated and co(vinylpyrrolidone—methacrylamidopropyl trimethylammonium chloride)-treated hair. The surface properties of hair treated with the polymer change significantly, with the polymer deposits taking the form of donut-shaped structures. The inside and outside diameters of the polymer deposits were determined to be 70 ± 11 nm and 202 ± 47 nm, respectively. Since the diameter of hair micropores was found to be 149 nm, it is plausible that the polymer may bind preferentially to the perimeter of the pores whose edges would presumably have a higher electric field than the more homogenous portion of the hair surface.

Confocal Microscopy

Confocal microscopy can be used to obtain specimen images that do not have out-of-focus areas. In a classical light microscope, the light illuminates a large portion of the sample, and if its geometry is not flat, a part of the image is always out of focus. Confocal light microscopy employs a focused beam of light with a reflected light passing through a pinhole in front of a detector, eliminating out-of-focus reflections. A focal plane image is generated by scanning the surface. Internal elements of a structure can also be scanned in a similar way, with the limitation being the opacity of the specimen.

Swift et al. (68) studied the penetration of fluorescently labeled proteins through intact and chemically modified hair fibers. The extent of penetration was assessed by imaging transverse sections of resin-embedded hair with a confocal laser-scanning fluorescence microscope. It was determined that the main sites for peptide deposition were endocuticle, cortex, nuclear remanants, intermicrofibrillar matrix, and cell boundaries that undergo massive swelling by water.

Corcuff et al. (69) used confocal microscopy to study the surface of dry, wet, and chemically modified hair. They claimed resolution of 0.25 versus 0.6 mm for conventional light microscopy. Their technique made possible the direct observation of sweat and sebum on hair surface and the quantitative assessment for periodic bulging of cuticles on swelling. They have also performed optical sectioning of hair samples at various depths to provide a three-dimensional reconstruction of the internal structure of hair stained with a fluorescent marker.

MICROFLUOROMETRY

The instrumental setup consists of a fluorescence illuminator, objective, interference filters, photomultiplier, and a scanning sample stage. The hair fiber was illuminated by a focused beam of light, and the fluorescence emission was monitored by a photomultiplier as the specimen was moved under the exciting light.

Weigmann et al. (70) employed microfluorometry to study deposition, substantivity, and buildup of various components of cosmetic formulations on hair. They used the sodium salt of fluorescein as a marker and assumed that the deposited film thickness, resulting from the precipitation of polymers, surfactants, and polymer-surfactant complexes, is directly proportional to fluorescence intensity. Various distributions of emitted light intensity were observed, including honeycomb patterns, which may be indicative of hair damage. Multiple treatments of hair with shampoos containing cellulose (and fluorescent marker) showed gradual increases in fluorescence intensity and an uneven distribution of surface deposits.

SURFACE ANALYSIS

Dynamic Electrokinetic and Permeability Analysis

Electrokinetic measurements have been applied in wool and hair research for some time. Recent developments, however, have made possible simultaneous measurements of electrokinetic and permeability parameters of fiber plugs in order to obtain information about the interactions of various cosmetic raw materials with hair (71–75). The technique has been termed "dynamic electrokinetic and permeability analysis" (DEPA).

The DEPA instrument consists of a streaming potential cell, conductivity meter, pressure transducer, test and treatment solution reservoirs, flow interruptor, an electronic balance, and several electric and manual valves. The most important features of the design are

- online positioning of test and treatment solution reservoirs, permitting fiber treatment within the streaming potential cell;
- the pulse mode of flow for test and treatment solutions;
- simultaneous measurement of the streaming potential, conductivity, and flow rate (permeability of the plug); and
- special software allowing flexible programming of the experimental procedures, such as the control of pressure and the timing of treatment and test cycles.

A typical experiment yields information about the electrokinetic characteristics and permeability of untreated fibers and the kinetics of sorption/desorption of cosmetic actives as a result of one or multiple treatments of hair. Figure 13 presents the results of an experiment in which hair was treated with 0.5% solutions of anionic surfactant (SLES-2), cationic surfactant cetryltrimethyl ammonium chloride, and cationic polymer cationic guar gum. The first five data points in each figure correspond to untreated hair. They are followed by a 5-minute treatment period, first measurement period of 30 minutes, a second 5-minute treatment period, and the second measurement period of 30 minutes.

Figure 13A presents the time dependence of ξ-potential and demonstrates an increase in hair-negative ξ-potential as a result of binding of SLES-2 to hair. It also shows a reversal of the surface charge for hair treated with quaternary ammonium surfactant and cationic polymer. While surfactants are rinsed off the hair after prolonged treatment with the test solution, a layer of cationic guar gum is stable and imparts a permanent positive ξ-potential to hair.

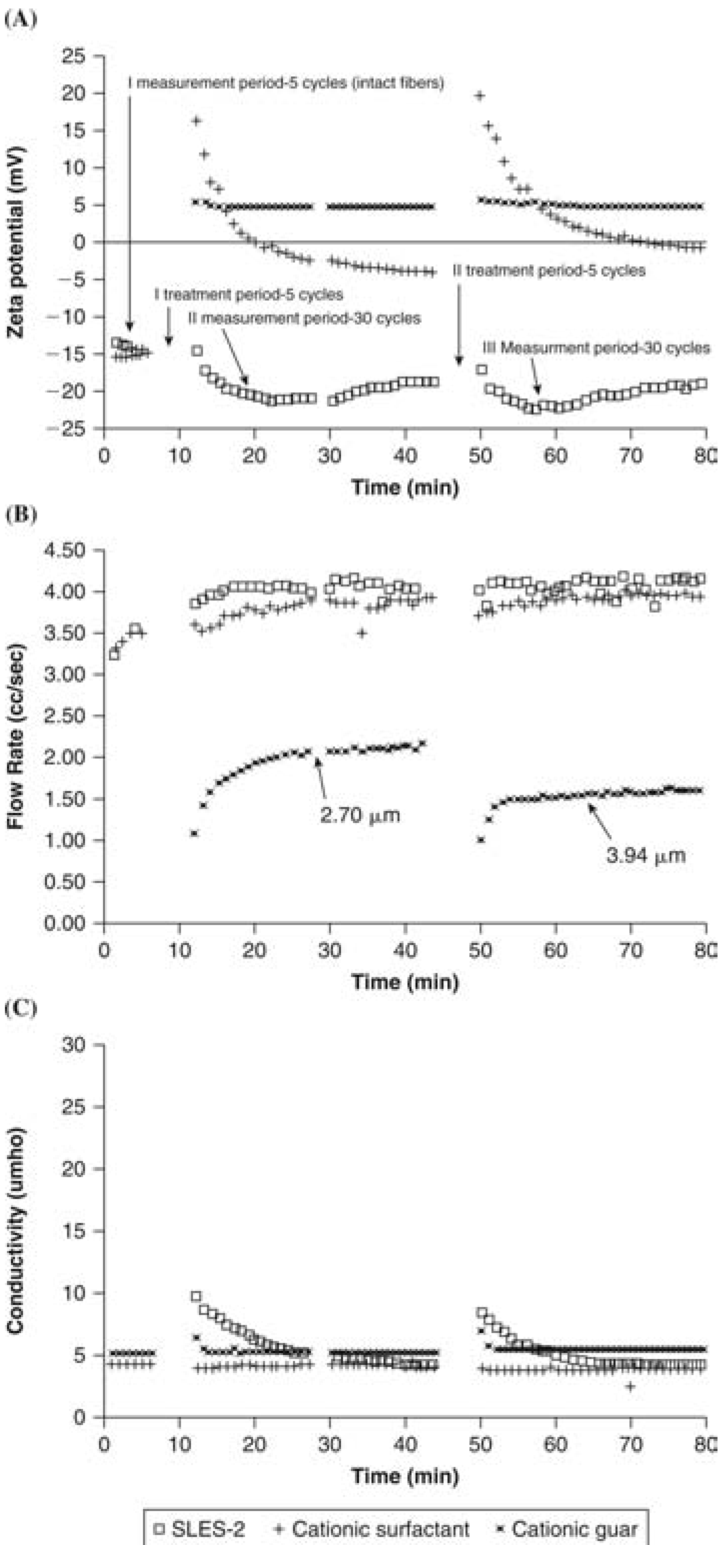


Figure 13 (**A**) ξ-potential (**B**), flow rate (**C**), and conductivity as function of time for hair treated with different classes of materials such as anionic surfactant (sodium laureth-3 sulfate), cationic surfactant (cetyl trimethylammonium chloride), and cationic polymer (cationic guar gum).

The flow rate data (Fig. 13B) show that only in the case of cationic guar gum there is a formation of a thick layer of the polymer (2.7 and 3.94 μm after the first and second treatment, respectively) on the surface of hair. This is probably due to the presence of microgels of the polysacchride in the treatment solution.

The hair conductivity is slightly reduced after quat (cetryltrimethyl ammonium chloride) treatment, a behavior typical for all cationics (Fig. 13C). A small delay in conductivity decrease for the polymer is related to the reduced flow and the resulting prolonged presence of the excess treatment solution in the plug. In contrast to this, SLES-2 slowly desorbs from hair and gives rise to increased conductivity of the plug even after extended rinsing with the test solution.

Similar data can be obtained not only for simple single component systems but also for complex, multicomponent solutions such as shampoos, conditioners, hair dyes, or any other finished cosmetic product.

The main criteria of product assessment are changes in the ς-potential, permeability, and conductivity relative to an untreated control. On the basis of these parameters, one can make conclusions about the deposition of cationic, anionic, nonionic surfactants, and polymers on hair. One can also obtain information regarding emulsions, substantivity of various treatments, their removability upon shampooing, buildup on consecutive treatments with the same formulation, and rate of desorption of residual surfactants or polymers (71–75).

The technique can also be employed to quantify the "sealing effects" produced by surfactants, polymers, and oils on dyed hair (71) or on fibers subjected to reactive treatments such as perming or bleaching (74).

A significant advantage of DEPA is that it performs the measurements on fiber assemblies rather than on single fibers, giving an average value of the assessed parameters. Also, the experimental protocols can be planned so that they simulate any sequence of operations performed on real hair, such as a combination of treatments including shampooing, conditioning, or perming.

Wettability

The use of wettability measurements to study the effect of chemical and physical treatments on the surface of hair has been described in detail by Kamath et al. (76). They basically employed a high-sensitivity balance and measured the wettability forces by immersing hair fibers into water or other liquids. An advance in this area, reported by the same authors, was the development of liquid membrane wettability scanning. In this process, a fiber is passed through a liquid membrane, and the measured wettability force is calculated from the following equation (77):

$$F = P\gamma_{LV}(\cos q_a - \cos q_r)$$

where P is the fiber perimeter, γ_{LV} is the surface tension of the membrane liquid, and q_a and q_r are the contact angles in the advancing and receding modes.

A typical experimental procedure consists of obtaining wettability scans along the length of fibers before and after treatments with solutions of conditioning actives, such as cationic cellulose or protein. Determining the value of q_r in a separate experiment, and assuming that it does not change along the fiber length, allows one to obtain a plot in which $\cos q_a$ is a function of distance. The wettability traces usually show a lot of variation along the length of a fiber so that an average value is usually employed for comparisons between intact and modified hair. It was shown that unoxidized hair, characterized by a value of $\cos q_a = -0.22 \pm 0.17$, becomes more hydrophilic after a single treatment with a solution of a cationic polymer as evidenced by an average value of $\cos q_a = -0.08 \pm 0.15$. This method was also employed to assess the effect of multiple treatments.

In current laboratory practice, automated wettability instruments are employed. They are capable of detecting a point of contact with the liquid during the fiber movement in the advancing direction, reverse the direction of movement to measure receding wettability forces, detect the point of separation from the liquid, average the forces during the fiber scan, and calculate contact angles using previously determined fiber perimeter (which is accomplished by employing wettability data for a given fiber obtained by using hexane as a solvent). Figure 14A,B shows wettability traces (wettability force as a function of distance) for virgin hair (Fig. 14A) and for bleached hair (Fig. 14B). On the basis of these measurements, the advancing contact angles were found to be 101.6° and 41.2° for intact and commercially bleached hair, respectively.

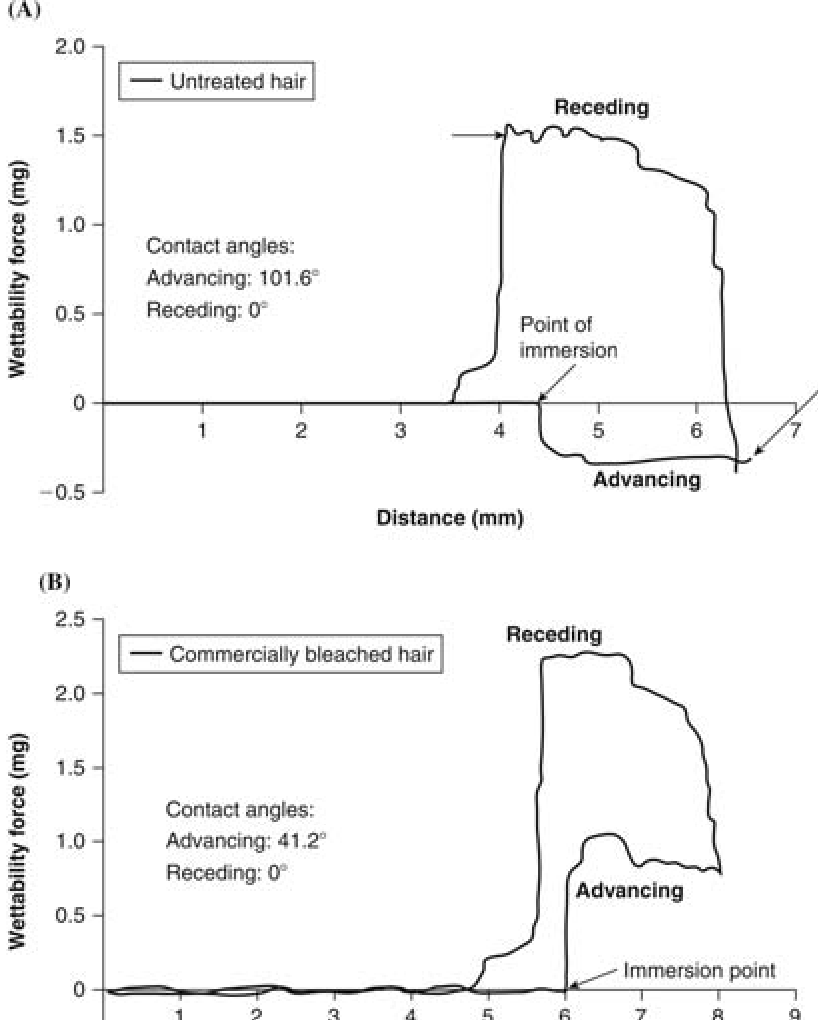


Figure 14 Wettability trace for (**A**) intact and (**B**) bleached hair.

Profilometry-FT and Fractal Analysis

Profilometry has been recently employed to study the geometrical properties of hair surface (78,79). This technique in which the surface of hair is scanned longitudinally using a wedge-shaped stylus has a resolution similar to that of optical microscopy.

A typical experimental result is a hair surface profile exhibiting a large number of random peaks and valleys ranging in size from a fraction of a micron to millimeters. The traditional way of handling these data is to calculate roughness parameters such as average roughness depth, average roughness, or geometric average roughness (78). However, these parameters are not constant and increase with an increase of the scan length, a consequence of self-similarity or fractality of hair surface profile (79).

Hair profilometric traces can be subject to Fourier transform (FT) in which the height versus length dependence is converted into intensity versus frequency spectra, where frequency is termed "spatial frequency" and is defined as number of crests per unit length. The analysis of the averaged FT spectra of hair surface showed no preferred frequencies of height variation and allowed for calculation of the fractal dimensions. They were found to be 1.31 for the high-frequency (small spatial dimension) end and 1.63 for the low-frequency (large spatial

dimension) end. This leads to the conclusion that hair is "smoother" in the probing scale from 0.5 to 5 mm than in the scale from 5 to 100 mm.

Combing Measurements

The use of quantitative combing measurements has been well established in the characterization of hair-care products. The technique has been developed over the years by Newman et al. (80), Tolgyesi et al. (81), Garcia et al. (82), and Kamath et al. (83). It is widely used in research, development, and claim substantiation.

The method consists of passing a comb through a hair tress, with a well-defined geometry, and measuring force as a function of distance. These measurements can be performed on dry or wet fibers. The parameters used for comparing product performance include the maximum combing force or combing work. The data are typically reproducible within ±20% for wet-combing measurements and ±50% for dry-combing measurements.

Jachowicz et al. recently reported a modification of the method aimed at increasing its sensitivity (84,85). The method, termed "spatially resolved combing analysis," employs special frames that allow the application of a treatment to selected areas of the fibers while shielding the remaining portions, thereby providing internal reference sections. The treatments may include thermal exposure, wet applications of cosmetic formulations or raw materials, and physical modification of hair by photoirradiation. The combing curves of hair treated in such a way, obtained by using a tensile tester such as Instron or Dia-Stron, show positive or negative peaks depending on whether the treatment results in an increase or a decrease in friction of the hair surface.

Figure 15 illustrates the application of this method to the analysis of two different conditioners on hair. Their affinity to hair is assessed by performing combing measurements

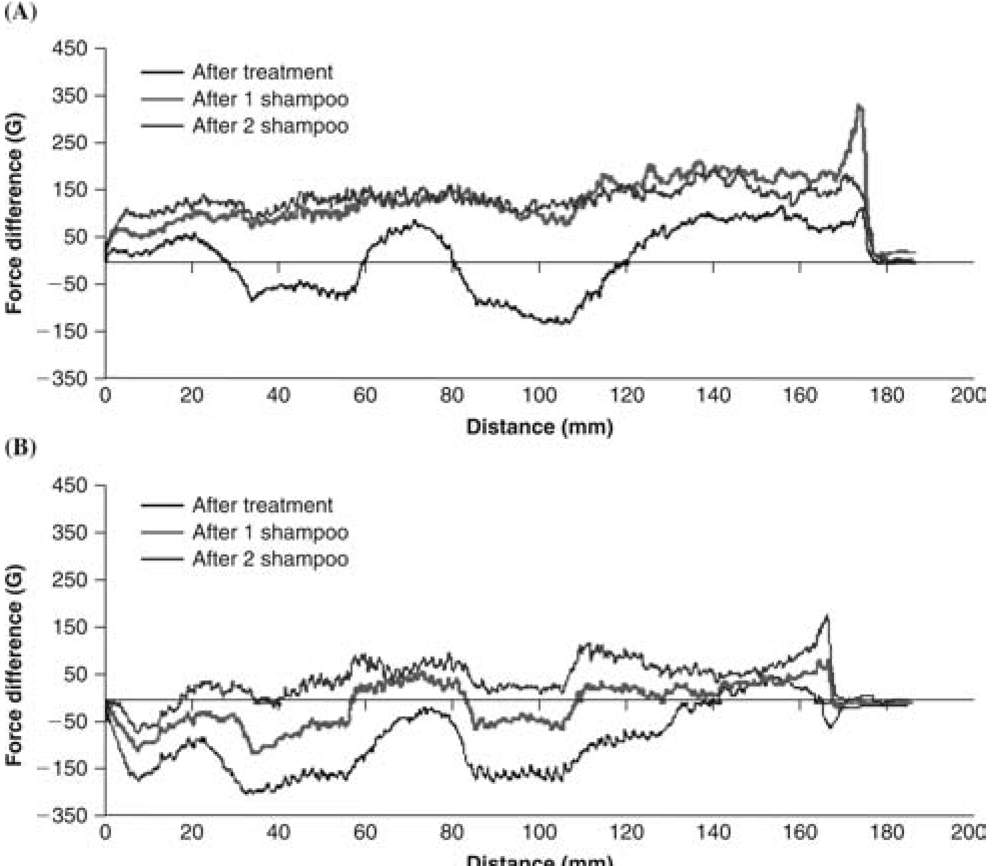


Figure 15 Differential combing curves for hair treated with (**A**) conditioning agent 1 and (**B**) conditioning agent 2 and subsequently subjected to two shampooings.

after hair treatment and after two subsequent shampooings. The presented traces are differential combing curves obtained by subtracting a curve for untreated hair from the combing trace obtained after a given treatment.

Figure 15A shows the traces of combing curves for hair treated through a two-window frame with a cationic conditioning agent, which is characterized by low affinity to hair in terms of its resistance to shampooing. Unlike a conditioning treatment, shampooing is applied to the whole tress, including untreated and conditioner-treated portions of a hair. Significant decreases in combing forces are evident in treated sections of hair, with the effect nearly completely eliminated by a single shampooing. In contrast to this, Figure 15B gives the traces obtained for a high-affinity conditioning agent, showing reductions in combing works after treatment in the window areas. The effect persists after one and two shampooings, suggesting that the conditioning agent (Fig 15B) remains adsorbed on hair surface. Notably, combing forces corresponding to untreated (shampooed only) portions of hair gradually increase probably as a result of lipid removal or adsorption of anionic surfactants from a shampoo.

Other uses of this technique include the studies of the effect of chemical treatments on hair and the analysis of hair adsorption by cationic polymers, proteins, and complexes.

TEXTURE ANALYSIS

A new tensile meter′ referred to as texture analyzer can measure the force in both the compression and tensile modes while monitoring probe displacement in relation to the sample (Texture Technologies Corporation, Scarsdale, New York, U.S.). It was developed primarily for quantitative characterization of food products in terms of texture parameters such as hardness, springiness, tackiness, and resilience.

The instrument was recently adopted for conducting quantitative analysis of hair and hair-care products by employing a procedure referred to as dynamic hairspray analysis (Fig. 16) (86–88). It involves the use of hair samples shaped into omega loops by applying a temporary wet set. Both the instrument and the sample are housed in a constant humidity chamber that can maintain relative humidity in the range from 30% to 95% at ambient temperatures. The mechanical measurements of hair loops are carried out by oscillating a plastic probe between the fiber surface and the calibration height of a few centimeters. After touching the surface of hair and sensing a trigger force (1–2 G), the probe produces an additional 1- to -4-mm deformation (6–25%) of the loop before rising to the calibration height. One-millimeter deformation is typically within the elastic limit of both untreated and resin-modified hair. On the other hand, 4-mm deformation (25%) usually results in irreversible breaking of polymer-fiber or polymer bonds in polymer-treated hair and is employed to study flexibility of styling products.

Experimental data for polymer-treated hair at high deformation of 25% are presented in a plot of force as a function of distance (Fig. 17) for the first deformation (Fig. 17A) and the first 10 consecutive deformation cycles (Fig. 17B). The data in the figures correspond to a brittle

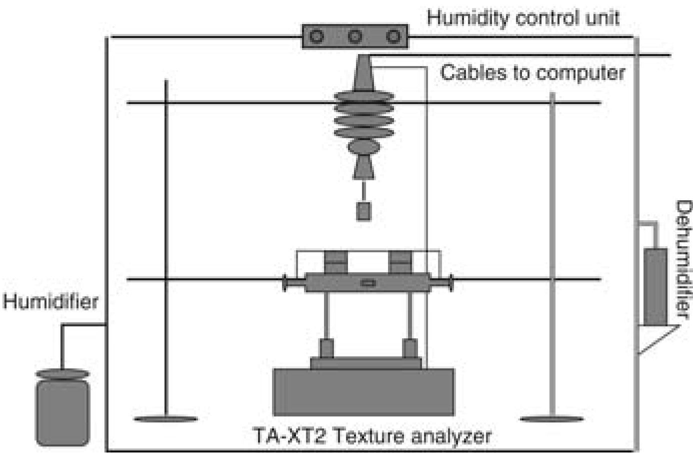


Figure 16 The experimental setup to study mechanical properties of hair-care polymers. It consists of a texture analyzer and an environmental chamber. Hair sample is in the form of omega loop placed under the probe of the texture analyzer.

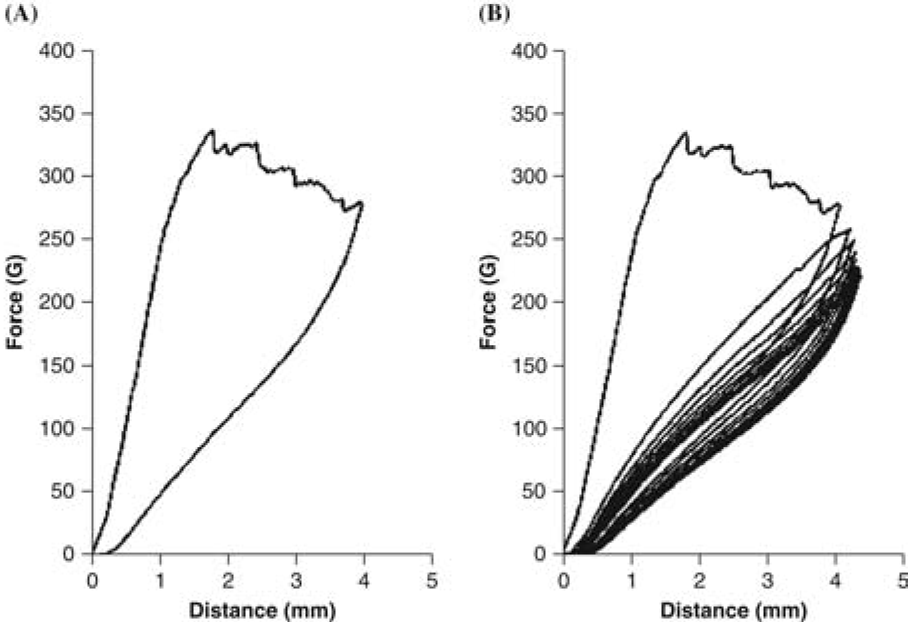


Figure 17 Force as a function of deformation for hair in the form of omega loops treated with a fixative polymer.

polymer characterized by an elastic response in the deformation range from 0 to 1 mm. It is in this deformation range that the ratio of modulae, E_{10}/E_1, is calculated (modulus is calculated as the slope of the dependence of force as a function of distance in the linear portion of the curve). E_{10} and E_1 are the modulae of the 10th and the first deformation, respectively. The ratio E_{10}/E_1 can be used as a measure of sample (hair treated with a polymer) flexibility. To further characterize the flexibility of the polymer used as a hair treatment, a parameter F_{10}/F_1 can be calculated as the ratio of the maximum force in the 10th deformation, $F_{10,}$ to the maximum force in the first deformation, F_1. As illustrated by the curves in Figure 17, at a deformation of about 2 mm in the first cycle, the polymer bonds between fibers break, resulting in a reduction of maximum force (F) in subsequent deformations. The measurements such as those presented in Figure 17 can also be used to calculate plasticity parameter of treated hair.

By using a setup shown in Figure 16, the drying of a fixative was also investigated by applying low (1%) intermittent deformations to ω-loop shaped hair tress. First, the instrument determined the properties of untreated hair; then, the fibers were treated with a fixative, and the instrument measured the changes in both tackiness of a fixative solution on the hair surface as well as mechanical stiffness of the fiber assembly as a function of drying time. The experimental procedure yields parameters such as the stiffness of untreated and resin-modified hair, duration of tack, maximum value of tack force, and time of drying. The kinetic measurements of the stiffness change can also be performed at 90% RH, resulting in information about the resistance of fixative resins to high humidity.

Other applications of this instrument include the characterization of hair (especially ethnic hair) in terms of textural parameters, analysis of skin softness, and the measurements of tactile properties of skin products. This new tensile meter can also be employed for the characterization of the textural (rheological) behavior of cosmetic formulations such as shampoos, creams, waxes, and pomades.

OPTICAL PROPERTIES AND LUSTER MEASUREMENTS

Hair luster is an important property readily assessed by a visual observation and frequently invoked in claim substantiation and advertising. It is largely dependent on the cleanliness, uniformity, and extent of damage to the hair surface. Hair luster can be affected by chemical treatments that reduce hair gloss by damaging cuticles, dissolving lipids, or changing hair

color. It can be also modified by application of shampoos, hair conditioners, or special shiner formulations.

The key papers in this area were published 30 years ago by Stamm et al. (89). Recent developments include the use of computerized goniophotometers to quantify light-scattering effects produced by single fibers (90–95) or aligned fiber tresses (96,97). The principle behind these measurements is the same as in earlier work: a light source illuminates the sample at an incidence angle, and the light intensity is recorded for different receptor angles, providing a light-scattering curve. Rotating light-scattering photometers or optical multichannel analyzers can be employed for luster measurements.

The usual criterion of gloss is the sharpness of specular reflection, which can also be quantified by defining various luster parameters given by the formula (90,93):

$$L = S/D(W_{1/2})$$

where L is luster or shine, D is the integrated diffuse reflectance, S is the integrated specular reflectance, and $W_{1/2}$ is the width of a specular peak at half height.

A different experimental approach to luster measurements was taken by Maeda et al. (96) who obtained pictures of illuminated natural hair wigs on model heads and analyzed them by using a color image processor. The data obtained by scanning across highlighted and dark areas could be presented in a format similar to a photogoniometric-scattering curve with the ability to resolve reflected light into three color signals R, G, B (red, green, and blue), or L, a, b parameters. A similar approach was taken by McMullen et al. (98,99) who employed image analysis to measure luster of hair simulated by light reflected from a curved hair tress. Hair samples were mounted side by side in a special sample holder in the form of a cylinder and illuminated by a uniform beam of polarized white light. Digital images of hair tresses were captured with a high-resolution camera and analyzed by scanning across highlighted and dark areas of the resultant image using image analysis software. Plots, similar to goniophotometric-scattering curves, were used to calculate luster values according to previously published work (89,93).

By employing all these methodologies, researchers demonstrated small variations in shine as a result of the application of shampoos with and without substantive ingredients on untreated dark brown Asian hair (90,93). Other researchers showed the effect of special shine formulations on damaged hair (91). Luster measurements were also employed to demonstrate a cuticle-abrading effect of multiple combings resulting in a shift of maximum in a light-scattering curve. Other investigations based on luster measurements documented gloss variation between root and tip sections of hair and the effect of humidity. Nagase and coworkers (100–101) and Okamoto et al. (102) have emphasized the importance of internal structure of hair to optical properties and demonstrated the presence of "glittering" patches on the hair surface caused by internal damage due to blow-drying.

Lim et al. (97) measured luster of hair tresses mounted on cylinders, similar to those reported earlier (98), with a video camera in an experimental setup called SAMBA. The authors did not provide the results of calculations of fundamental parameters, and the data interpretation is given in terms of "percentage of luster increase". It was reported that the correlation of instrumental readings and consumer evaluation ratings of luster was good.

The image analysis procedure was employed to assess the luster of natural white, light blonde, light brown, medium brown, and dark brown hair and revealed an increase in luster indices in proportion to an increase in fiber pigmentation (98,99). Figure 18 presents images of reflected light from natural white, light blonde, light brown, and dark brown hair. These images were obtained by selecting the exposure values in such a way as to visualize the details of the specular reflection band.

The light distribution curves are presented in Figure 19, and they are consistent with the visual representation of the images shown in Figure 18.

For example, one can clearly see two specular reflection bands for natural white and light blonde hair, which are evident by two peaks in the light distribution curves. The peak at 16 mm gets progressively smaller with an increase in the extent of fiber pigmentation, which indicates that it is due to reflection from the back-face of the hair fibers. The narrowest light distribution curve was obtained, as expected, for dark brown hair. Hair luster parameters, calculated according to equations published by Stamm et al. (89) and Reich and Robbins (93), are presented in Table 4.

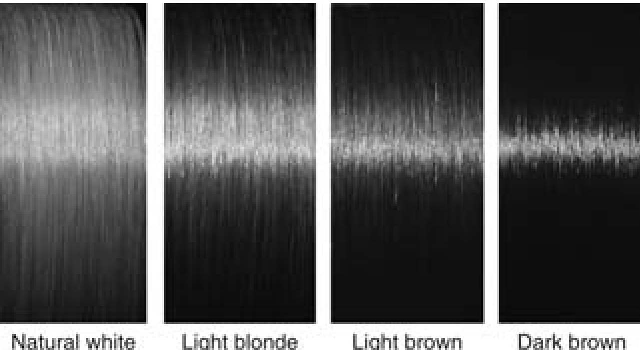


Figure 18 Images of reflected light from natural white, light blonde, light brown, and dark brown hair.

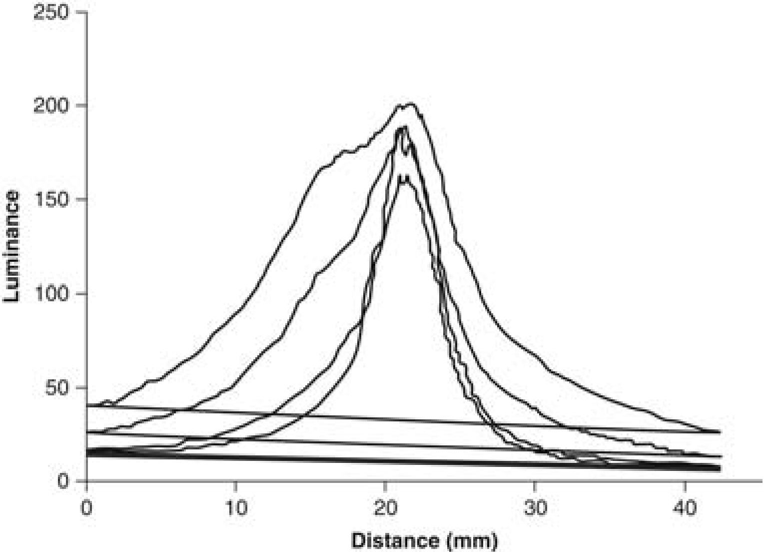


Figure 19 Light distribution curves for images of hair presented in Figure 18.

Table 4 Luster Parameters for Various Hair Types

Hair type	$W_{1/2}$ (mm)	L_{Stamm}	$L_{\mathrm{Reich\text{-}Robbins}}$
Dark brown	5.28 ± 0.44	0.72 ± 0.020	0.67 ± 0.006
Medium brown	5.44 ± 0.16	0.72 ± 0.006	0.67 ± 0.005
Light brown	10.46 ± 0.30	0.70 ± 0.001	0.32 ± 0.008
Light blonde	14.91 ± 0.23	0.65 ± 0.005	0.19 ± 0.006
Natural white	22.78 ± 0.24	0.32 ± 0.013	0.06 ± 0.002

The calculations carried out by both formulas indicate lower luster values for fibers containing less melanin pigment, i.e., the highest luster for dark brown hair and the lowest for natural white hair. Also, $W_{1/2}$ follows the same trend, consistent with visual perception, pointing to an increase in the width of reflected light distribution for less-pigmented fibers.

Cosmetic oils such as phenyl trimethicone, amodimethicone, and castor oil were also found to increase luster of hair as a result of change in contrast between the specular and diffuse reflection (98). Styling resins such as butyl ester of PVM/MA copolymer, vinyl caprolactam/PVP/dimethylaminoethyl methacrylate copolymer, and isobutylene/ethylmaleimide/hydroxyethylmaleimide copolymer were shown to increase hair gloss by a similar

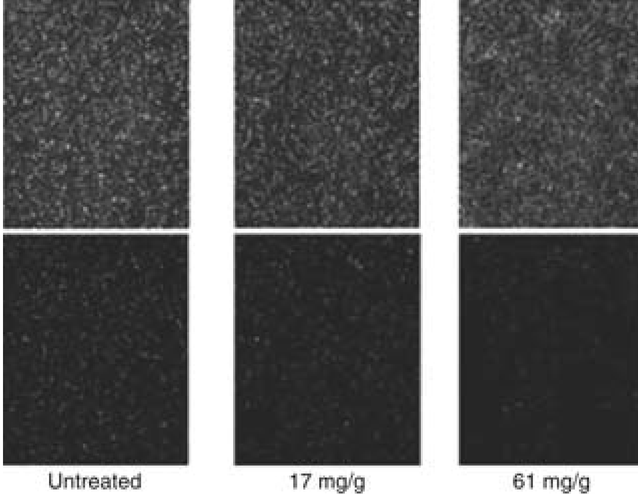


Figure 20 The effect of sebum on the luster of African hair. First row shows unprocessed images, while the second row presents corresponding images after image thresholding.

mechanism as evidenced by calculated higher values of Stamm and Reich–Robbins luster parameters (98). On the other hand, an effect of hair dulling by deposition of micronized ZnO at various concentrations as well as by synthetic sebum was also discussed (98).

African hair provided an interesting substrate for optical analysis because of the curls that are naturally present providing multiple reflection patterns (99). It is important to note that unlike straight hair, which exhibits one specular reflection band that coincides with the band on the cylinder mount, homogenous illumination of African hair with a collimated light beam results in many reflection centers of equal intensity in all regions of the sample. To quantify the multiple reflection patterns, the authors used the image analysis software, which allowed them to tally the number of reflection sites as well as to characterize the shape of the reflection. Figure 20 provides images obtained for untreated hair along with hair treated with 17 and 61 mg of artificial sebum per gram of hair. Visual inspection of the images reveals a perceived decrease in luster with increasing concentrations of sebum. Further, a decrease in luster is coupled with a decrease in the number of reflection sites. Figure 20 includes corresponding images in which all of the reflection sites have been isolated on a black background using an image threshold technique. This is accomplished by looking at a histogram corresponding to the colors present in the image and isolating the bright white light that corresponds to the reflection centers. Image file types usually have a scale from 0 to 255 to represent the colors in the image, with 0 representing the darkest colors (black) and 255 the brightest (white). By isolating values that fall in the range from 225 to 250, we can look at the brightest reflections on an entirely black background (Fig. 19), allowing us to count the total number of reflections.

As shown in Table 5, the number of reflections decreases with increasing concentrations of sebum, which was also clearly evident after visual inspection of Figure 19. It should also be added that the reflection sites could be also characterized in terms of perimeter, roundness, and compactness by using the tools of image analysis.

Table 5 Quantification of Reflection Sites on African Hair Treated with Sebum

	No. of reflections	Black (%)	White (%)
Untreated	742	98.34	1.66
17 mg/g	536	99.02	0.98
61 mg/g	273	99.67	0.33

EVALUATION OF PERMANENT WAVES

Permanent waving involves breaking disulfide bonds in hair with a reducing agent followed by reformation with a neutralizer (103,104). Evaluation of permanent waving chemistry can be carried out either by study of reduction and reoxidation rates or by measuring the permanent set achieved in the hair. Reduction rates in hair can either be determined by amino acid analysis (105–107) or by methods based on chemical stress relaxation. In chemical stress relaxation methods, a hair is stress relaxed in buffer at fixed extension until a constant level of force is reached. Addition of a reducing agent causes the stress supported by the hair to decrease as disulfide bonds are broken by reduction (108–113). Kinetics of the reaction can be followed, and some information on reaction mechanisms can be deduced. Wickett (109) introduced the term "single fiber tensile kinetics" (SFTK) to describe chemical stress relaxation.

The effect of temperature and pH on reduction with sodium thioglycolate (NaTGA) at pH 9.0 is shown in Figure 21.

The curves from pH 9.0 at 39°C and pH 10.0 at 22°C clearly show the two shapes of SFTK curves typically observed with this method. At pH 9.0, TGA follows pseudo first-order kinetics (108,109). In this model, one assumes that the reagent is in large excess, that the reaction is slow compared with diffusion, and that all stress supporting S-S are equally reactive. Then, the rate of change in S–S bonds is given by

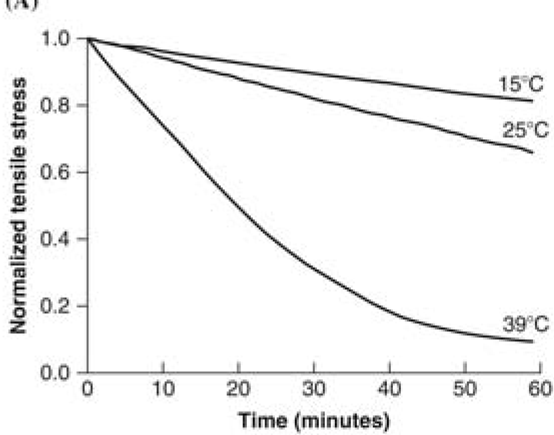
$$d(\text{S–S})/dt = -kC_0(\text{S–S})$$

where C_0 = the concentration of reducing agent and k is the reaction rate constant. If each S–S is assumed to support an equal amount of stress then the force, $F(t)$, at any time t, is given by

$$F(t) = F(0)\exp(-kC_0t)$$

And, plots of $-\ln(F(t)/F(0)$ versus t will be linear with a slope of kC_0.

(A)



(B)

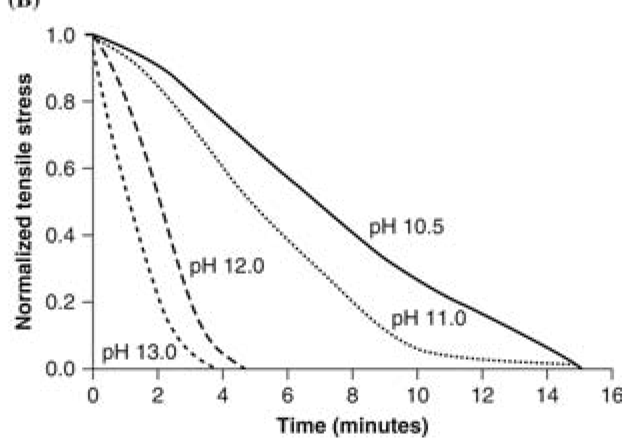


Figure 21 (**A**) Effect of temperature on SFTK reaction kinetics with NaTGA at pH 9.0. (**B**) Effect of pH on NaTGA SFTK curves at 22°C. *Abbreviations*: SFTK, single fiber tensile kinetics; NaTGA, sodium thioglycolate.

Figure 22 Pegboard for permanent wave evaluation.

The model that fits curves shaped like those for pH 10.5, as shown in Figure 21B, is more complex and assumes that diffusion is slower than reaction until some reaction has occurred, greatly speeding up diffusion. In this model, there is sharp front or moving boundary of reducing agent working its way into the hair. This model has been discussed in detail in other papers (104,109,112).

An effective way to measure the efficacy of permanent waves is the pegboard method (114), which is based on a uniform pegboard made of plastic that is 5.5-cm long and 1-cm wide and contains 14 removable pegs at a height of approximately 2 cm. The distance between each peg is about 3/10 cm. Two grams of hair are interlaced between the two peg rows without tension and secured at each end with rubber bands (Fig. 22).

The hair must be wound evenly and smoothly without any twisting. After winding, the hair is thoroughly saturated with waving lotion and the pegboard is covered and placed in a constant temperature bath at 25°C for a prescribed time. The pegboard is then removed from the bottle, rinsed thoroughly with water for 30 seconds, and then saturated fully with a neutralizer again for a prescribed time and finally rinsed with water. The rubber bands are removed from each end, and the pegs are carefully removed. The curled hair is then immersed in water for at least five minutes, and the length of the waved swatch is determined. Waving efficiency is calculated as given below.

$$100\% - [100 \times (B–A)/C–A] = \% \text{ waving efficiency}$$

A is the distance between the first and sixth peg (2.7 cm), C is the length of straight hair (14.8 cm), r, and B is the length of the curled hair swatch. Substituting these constants the equation becomes

$$100\% - [100 \times (B–2.7)/12.1] = \% \text{ waving efficiency}$$

CONCLUSION

There are a great many methods for evaluation of hair and hair-care products in the hair cosmetics laboratory. We have reviewed several of them, but there are many that we could not review in this chapter. The authors hope that the reader will find this review useful and informative and apologize for the fact that some other methods were not included.

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69 The Normal Nail

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ANATOMY

The nail plate, also abbreviated as "nail," is a hard keratin plate, slightly convex in the longitudinal and the transverse axes. It is set in the soft tissues of the dorsal digital extremity, from which it is separated by the periungual grooves (proximal, lateral, and distal) (Fig. 1) (1–5). It stems from the nail matrix located in the proximal part of the nail apparatus. The nail plate and matrix are partly covered by a skin fold called "the proximal nail fold" (PNF). The lunula, also known as "half-moon," is a whitish crescent, visible at the proximal part of some nails and more specifically those of the thumbs and big toes. It corresponds to the distal part of the matrix. From the latter, the nail plate grows toward the distal region sliding along the nail bed to which it adheres closely and from which it only separates at the distal part, called "hyponychium." The latter and overhanging free nail provides a crevice, which is a reservoir for microbes.

Two other structures deserve our attention:

1. The cuticle, which is the transparent horny layer of the proximal nail groove, adheres to the nail surface and acts as a seal between the nail plate and the PNF. Its disruption allows water, foreign bodies, bacteria, and fungi to penetrate under the PNF, which favors paronychia (periungual inflammation).
2. The onychodermal band or better known as the onychocorneal band, which is "orangey," is located in the distal region of the nail. It can be partly blanched by pressure, thus exsanguinating the region. It provides a zone of rugged attachment of the nail-to-nail bed. As for the cuticle, disruption of the onychocorneal attachment will severely affect the nail function, leading to onycholysis (detachment of the nail from its bed).

The upper surface of the nail plate is smooth and has discrete longitudinal ridges becoming more obvious with age (Fig. 2) and in some pathological states. This is a frequent cause of nail brittleness.

The under surface is corrugated with parallel longitudinal grooves that interdigitate with the opposite ones of the nail bed surface, enhancing the adhesion of the nail plate to the nail bed. The most important adhesion is located in the distal, central part of the nail.

HISTOLOGY

The Nail Plate

The nail plate is made up of parallel layers of keratinized, flat, and completely differentiated cells, called "onychocytes." The latter are, in contrast with the corneocytes, firmly adherent and not desquamated. Nuclear remnants can be observed, but they disappear completely, near the distal free edge.

Three zones (characterized by different staining affinities) can be identified at the distal part of the nail: the upper (or dorsal) nail plate, which makes up one-third of the nail; the lower nail plate, which makes up two-thirds of the nail; and the subungual keratin. The latter corresponds to the thick, dense, horny layer of the hyponychium (Fig. 3) (6,7).

In electron microscopy (Fig. 4) (8), the nail plate cells appear to be made of a regular weft of keratin filaments within an interfilamentous matrix. In the upper (or dorsal) nail plate, cells are flat, their cellular membranes are discreetly indented, and they are separated from each other by ampullar dilatations. At the surface, those cells are piled up like roof tiles, which give

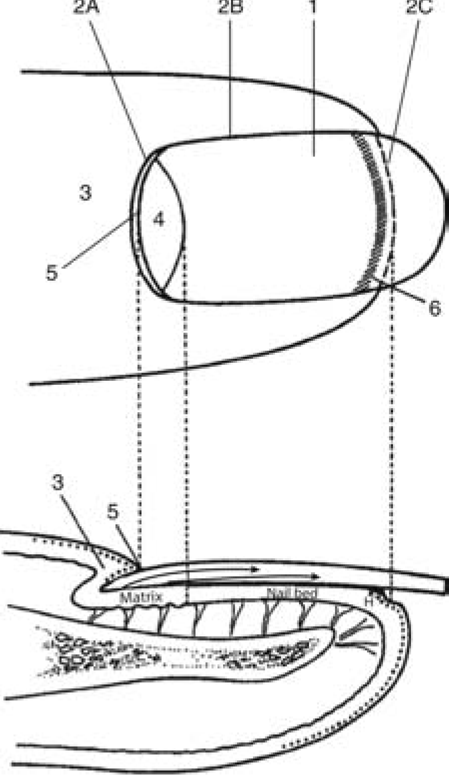

Figure 1 The normal nail: (1) nail plate, (2) nail grooves [(2**A**) proximal nail groove, (2**B**) lateral nail groove, (2**C**) distal nail groove], (3) proximal nail fold, (4) lunula, (5) cuticle, (6) onychodermal band. Small dots represent the stratum granulosum. *Abbreviation*: H, hyponychium.

Figure 2 Obvious longitudinal ridges on the nail surface noticed in older people.

the nail surface its smooth aspect. In the lower nail plate, cells are thicker, their cellular membranes are anfractuous, and they interpenetrate through extensions, making real anchoring knots that seem to be partly responsible for nail elasticity.

Recently, the histological structure of the nail plate has also been studied by synchrotron X-ray microdiffraction (9). Three transversal layers (characterized by different orientations of

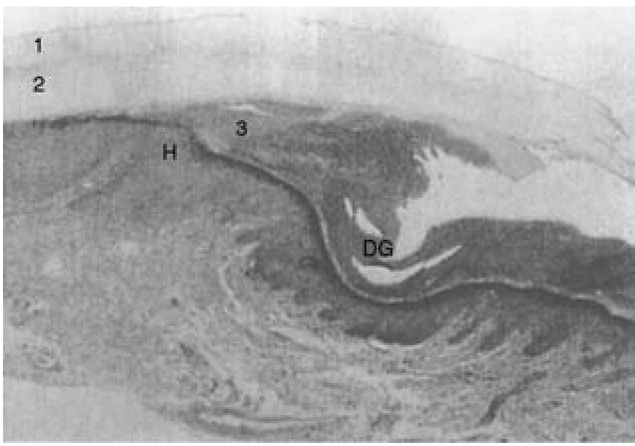

Figure 3 Longitudinal section of the distal part of the nail apparatus: (1) upper or dorsal nail plate, (2) lower nail plate, (3) subungual keratin. *Abbreviations*: H, hyponychium; DG, distal groove.

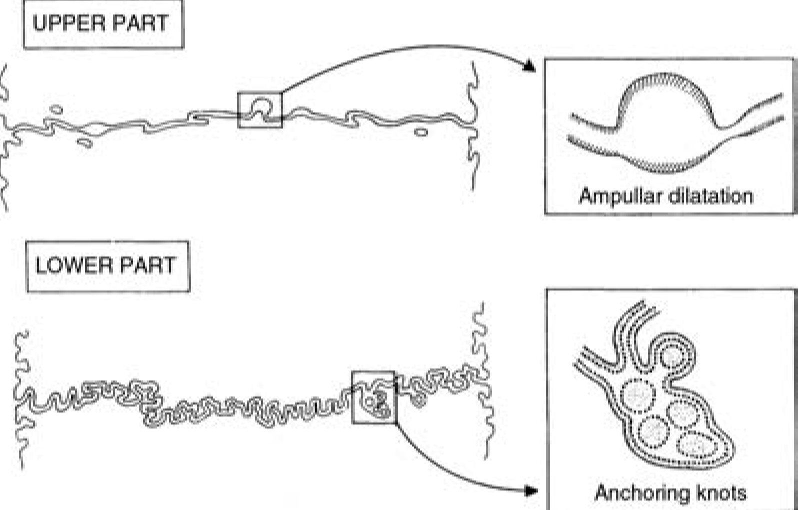

Figure 4 Schematic drawing of the cell membranes in the dorsal and ventral part of the nail plate, as observed in electron microscopic examination. *Source*: From Ref. 8.

the keratin molecules) are also identified. The outer or dorsal nail plate, which makes up the one-fourth of the nail, contains epidermal-type keratin filaments, perpendicular or parallel to the nail growth axis. The intermediate nail plate, accounting for approximately two-thirds of the nail, is the only one containing hairlike type α-keratin filaments, perfectly orientated perpendicularly to the growth axis. The very thin (one-twelfth of the nail plate only), ventral nail plate is made up of epidermal type keratin filaments, perpendicular or parallel to the nail growth axis.

In the latter study (9), and in those previously mentioned, it should be pointed out that the denominations given to the three parts of the nail are different, which could lead to confusion. In Figure 5, the correspondence between the different terms used is shown.

Other Nail Structures

A longitudinal section of the nail apparatus enables us to visualize most characteristics of the other ungual structures (Fig. 1). From the proximal to the distal region, the following are identified:

- The PNF (Fig. 6). Its dorsal part is in continuity with the epidermis of the digit back. Its vascularization is noticeable. The capillary loops are parallel to the skin surface, which allows their in vivo examination, under a special microscope with epi-illumination.

LIGHT AND ELECTRON MICROSCOPY (6,8)

	DENOMINATION (Thickness)	ULTRASTRUCTURAL CHARACTERISTICS
NAIL PLATE	Upper Naill Plate (1/3)	Flat cells, Discreetly indented cellular membranes, Ampullar dilatations
NAIL PLATE	Lower Nail Plate (2/3)	Thick cells, Anfractuous cellular membranes, Anchoring Knots
	Subungual Keratin	Polyhedral cells Desmosomes

X-RAY DIFFRACTION (9)

	DENOMINATION (Thickness)	KERATIN (K) Filaments TYPE-ORIENTATION
NAIL PLATE	Outer or Dorsal Nail Plate (1/4)	Epidermal type k ⊥ or // to the nail growth axis
NAIL PLATE	Intermediate Nail Plate (2/3)	Hard K ⊥ to the nail growth axis
NAIL PLATE	Ventral Nail Plate (1/12)	Epidermal K ⊥ or //

Symbols: ⊥, perpendicular; //, parallel.

Figure 5 Nail Plate in Transversal Section.

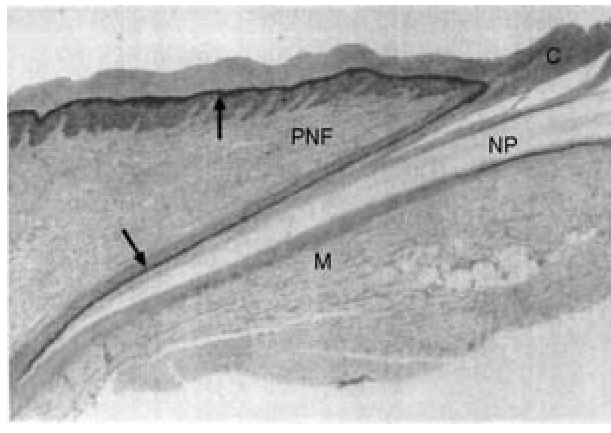

Figure 6 Longitudinal section of the proximal part of the nail apparatus. A stratum granulosum (*arrows*) is present in the dorsal and ventral part of the proximal nail fold epithelium but is absent in the matrix epithelium. *Abbreviations*: PNF, proximal nail fold; C, cuticle; NP, nail plate; M, matrix.

This technique, called "capillaroscopy," is useful in the diagnosis of Raynaud's phenomenon and connective tissue diseases. The ventral part of the PNF is a flat and rather thin epithelium that keratinizes with a stratum granulosum (SG). The latter can disappear in the most proximal part of the PNF that is the proximal matrix. The cuticle corresponds to the modified stratum corneum (SC) of the distal part of the PNF, at the angle of the dorsal and the ventral part.

- The nail matrix is a multilayered epithelium. Its keratinization process is characterized by an onychogenous zone devoid of keratohyaline granules (Fig. 6). It gives birth to the nail plate: the proximal part of the matrix gives birth to its dorsal part and the distal part of the matrix to its ventral part. Immunohistochemical studies have shown that the nail matrix epithelium is the sole site of hard keratin synthesis (10).

The epithelium of the matrix also contains melanocytes and Langerhans cells. Melanocytes are about 200/mm^2 in number (about 1150/mm^2 in the epidermis). Most of

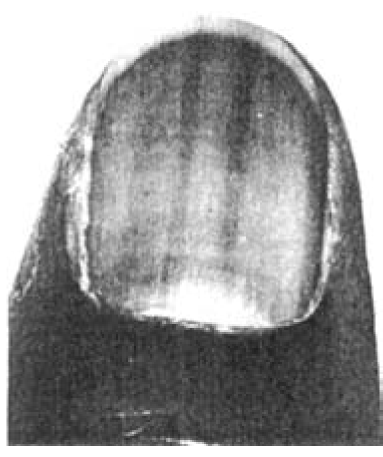

Figure 7 Multiple longitudinal melanonychia in an adult black patient.

them are dormant (11) and do not produce pigment. However, in dark-skinned individuals, longitudinal pigmented bands can be observed in nails. This racial physiological pigmentation is attributable to the activation of the matrix melanocytes and to the melanin incorporation in the nail plate (longitudinal melanonychia). It usually affects several nails and tends to become more frequent with aging; this can only be observed in 2.5% of newborn to 3-year-old black children, but in 96% of blacks older than 50 years (Fig. 7) (12).

- The nail bed epithelium is thin, reduced to few cellular layers. It keratinizes without any granular layer. The SG reappears only at the hyponychium, which represents the distal thickened part of the nail bed and is bordered by the distal groove and the digital pulp (Fig. 3).

Melanocytes are rare (47/mm^2) or may be absent in the nail bed (11).

From an immunohistological point of view, the nail bed is distinguished by the expression of basal keratin markers throughout the epithelium thickness and absence of markers of epidermis or mucosal differentiation. It has been suggested that the nail plate could act as a suprabasal layer for the nail bed. Additionally, expression of keratin 17, which is usually found with myoepithelial differentiation and epithelial mobility, could play a role in the sliding of the nail plate over the nail bed (10).

Finally, strong expression of a carcinoembryonic family antigen has also been described in the upper epithelial cell layers of the major central portions of the nail bed. It may play a part in the adhesion of the nail plate to the nail bed (13).

- The basal membrane of the nail apparatus is almost identical to that of the skin (14).
- The nail matrix and nail bed mesenchyme (dermis) does not contain pilosebaceous units. In the distal matrix, the connective tissue is loose and edematous. In the proximal matrix and the nail bed, it is characterized by dense collagen bundles, vertically orientated linking the nail apparatus to the periosteum. Elastic fibers are rare; eccrine sweat glands are usually absent. Glomus bodies, which are specialized arteriovenous anastomosis involved in the regulation of temperature, can also be observed in the dermis. In pathology, they give rise to glomus tumors, characteristically associated with paroxysmal pain. They represent one of the most frequent benign tumors of the nail apparatus.
- No genuine hypodermis is present in the nail but some adipose islets can be observed (7).

PHYSICOCHEMISTRY

The nail is highly rich in keratins, especially in hard keratins, which are close to those of hair and have a high content of disulfide linkage (cystine) (1,3). The high sulfur-containing keratins play an important role in the nail toughness and presumably in its good barrier property as well.

Sulfur represents 10% of the nail's dry weight; calcium represents 0.1% to 0.2%. The latter, contrary to conventional wisdom, does not intervene in the nail toughness.

Lipid content (particularly cholesterol) is low in nails: from 0.1% to 1% compared with 10% in the SC of the skin.

Water concentration varies from 7% to 12% (15–25% in the SC), but the nail is highly permeable to water: when its hydration level increases, it becomes soft and opaque, and when its hydration level drops, it becomes dry and brittle.

Studies carried on nail permeability are important for the development of cosmetic and pharmaceutical products specifically devoted to nails (15). As a permeation barrier, it has been shown that the nail plate reacts like a hydrogel membrane, unlike the epidermis that reacts like a lipophilic membrane (16).

The normal nail is hard, flexible, and elastic, which gives good resistance to the microtraumatisms it undergoes daily. Those properties are attributable to the following factors: the regular arrangement and important adhesion of onychocytes, the anchoring knots, the high sulfur-containing keratins and their regular orientation, and the hydration level of the nail.

PHYSIOLOGY

The nail growth is continuous. In a month, fingernails grow about 3 mm and toenails grow about 1 mm. A complete renewal, therefore, takes four to six months for normal fingernails, whereas 12 to 18 months are needed for toenails (1,3).

The origin of the nail plate production is still a debatable point. However, most studies agree and show that at least 80% of the nail plate is produced by the matrix. Indeed, studies based on cell kinetics, realized on squirrel monkey (17) or on human nails, (18) showed a cell proliferation largely limited to the matrix. This was further supported by later immunohistochemical markers of epidermal proliferation (19).

Finally, the use of keratin antibodies showing the production of hard keratin restricted to the matrix, matches the notion that the bulk of the nail plate derives from the matrix (10). It should be added that the main source of nail plate production is the proximal part of the matrix, 80% of nail plate cells being generated within the proximal 50% of the matrix. This probably explains why distal matrix surgery or nail bed surgery have a low potential for scarring compared with proximal matrix surgery (19).

Some studies suggest that the nail bed produces 20% of the nail plate, whereas others suggest that the nail bed hardly participates in the making of the nail plate (19,20).

It is not totally excluded that the ventral part of the PNF on the one hand, and the nail bed on the other, could contribute to the dorsal and ventral part of the nail plate, respectively, where soft keratin is observed.

ESTHETICS

For centuries, the nail has played an important esthetic role. Having clean nails is essential to looking well groomed and refined, and among women nails also need to be long and painted.

A "good-looking nail" has a smooth and shiny surface. It is transparent and adheres to its bed. Regarding the proximal groove, the cuticle has to be intact and thin. The distal and the lateral grooves have to be clean and the periungual tissues must be without hangnails and sores. The free border has to be smooth; its shape can be round, pointed, oval, or square. Women often wear long fingernails cut oval, which makes fingers look longer and thinner. Yet, square nails are in fashion. Too long nails can look unpleasant and can even be a nuisance.

Men wear short fingernails cut square. Both women and men have short toenails cut square. A normal nail structure and appropriate cosmetic care are necessary to obtain such "good-looking" nails.

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70 Nail Cosmetics: Handle of Skin Care

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THE MANICURE

The art of manicure is very ancient as can be testified by the Egyptian tomb of Niankhkhnum and Khnumhotep, dated approximately 2400 BC. It was discovered in the necropolis of Saqqarah in 1964. It is the tomb of two manicurist men who seem to have played an important role. Indeed, they were "Manicurist and Overseer of the Manicurists in the Palace, King's Acquaintance and Royal Confidant" (King Niouserrê, 5th dynasty, ancient empire) (1).

Nail Polish Remover

Currently, a professional manicure is made up of several steps (2):

The first one consists of removing any nail enamel remaining on the nail plate from a previous application. To do so, a cotton ball soaked with nail polish remover is used (Fig. 1). Nail enamel removers dissolve nitrocellulose and remove lipids from the nail plate. They mainly contain a mixture of organic solvents, with small amounts of oils added to counteract the drying effect of the solvents.

Typical formula (3):

- *Solvents (ca. 98%)—Example: acetone, butyl or ethyl acetate, ethoxyethanol.*
- *Lipids (ca. 2%)—Example: castor oil, lanolin oil.*

Other additives can also be found such as dyes, fragrances, preservatives, vitamins, and UV absorbers.

Nail polish removers should not be used more than once a week and should not be left too long in contact with the nails and the skin. Correctly used, they most probably do not cause nail brittleness (4).

Marketing usually insists on the fast action of the product, which is usually true. Nail polish removers specifically for brittle nails are also marketed. These are mainly nail polish removers without acetone. Actually, they are not better but are odorless.

Some varnish removers for brittle nails have a different composition, for example, on the basis of an ethoxy-diglycol solution, containing a mercapto-silanol complex (Si-nails remover®). This latter is purported to penetrate into the nail and to reinforce the disulfide linkage of the keratin. There is a lack in scientific studies, and the necessary time to remove the varnish is longer.

Cutting, Filing, Pumicing

Next, the nail is given the desired length, shape, and surface.

Therefore, the nail is cut and filed (Fig. 2). Filing is known to be less traumatic. This is probably only true when bad quality nail clippers are used.

If the nail surface is irregular, it can be trimmed, but not too much, to avoid nail plate thinning. This would be a source of nail brittleness.

Afterwards, the periungual grooves and folds are taken care of.

The nails are bathed in lukewarm, slightly soapy water. This cleans and softens the periungual tissue.

Cuticle Removers (5)

The cuticle is then covered with cuticle removers, which can be a liquid, gel, or cream. They usually contain sodium hydroxide and potassium hydroxide, in a 2% to 5% concentration.

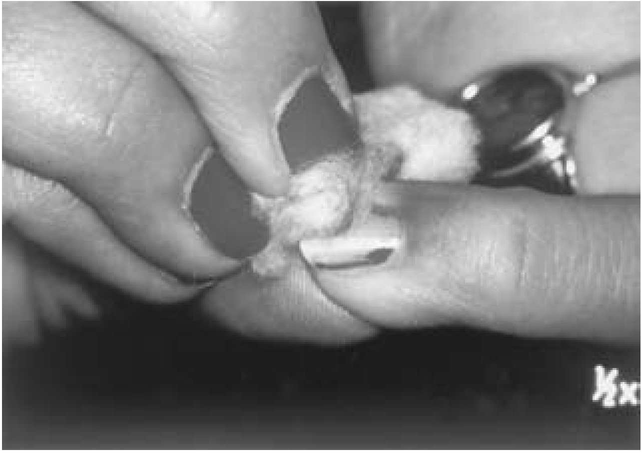

Figure 1 Nail polish removal.

Figure 2 Nail plate filing.

α-Hydroxyacids (1–5% lactic acid, pH 3–3.7) are also used. They attack keratin by disruption of the disulfide bonds of cystine. Cuticle removers contain various additives: emollients (lanolin) or humectants (glycerin, propylene glycol) whose purpose is to decrease evaporation, increase viscosity, and reduce irritation. Preservatives, perfumes, and thickening agents can also be added.

Typical formula (3):

- *Water (ca. 90%)*
- *Softening agent (1–5 %)—Example: potassium hydroxide*
- *Thickener (0.5–1 %)—Example: sorbitol, magnesium aluminum silicate*
- *Perfume (0.1%)*

The cuticle remover increases the softening of the cuticle and of the cuticle remnants, which adhere to the nail plate surface. These are then gently pushed back with an orangewood stick covered with cotton or a rubber-ended stick (Fig. 3). Of course, the goal of this step is not to destroy the cuticle, which is a very important seal between the proximal nail fold (PNF) and the nail plate. Its destruction can lead to paronychia (periungual inflammation). Inexperienced rough handling of the cuticle may also injure the matrix below.

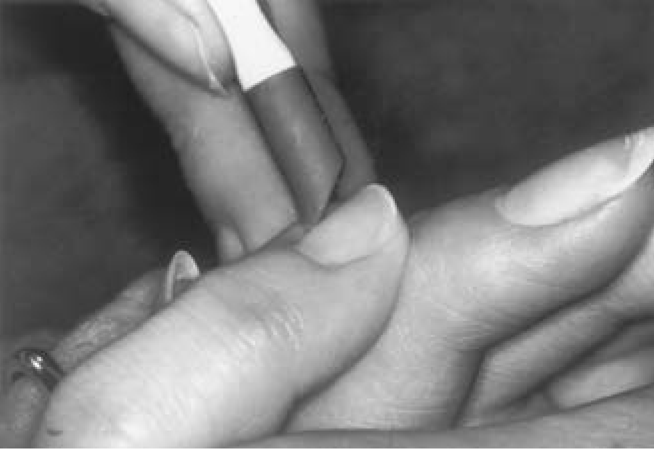

Figure 3 Rubber-ended stick, to push back the cuticle remnants.

Figure 4 Hangnails are cut with a special nipper.

The distal and lateral nail grooves are cleaned. It is most important to avoid overaggressive cleaning beneath the free edge of the nail, which can lead to onycholysis (detachment of the nail plate from the nail bed).

Finally, hang nails are cut with a special nipper (Fig. 4).

The manicure ends with a moisturising cream massage of the nails and the hands.

Buffing and Whitening

Other treatments are more rarely used: an abrasive powder, paste, or cream (stannic oxide, talc, silica, chalk, kaolin) (2) can be applied on the nail surface, which is then buffed with chamois leather. This produces a smooth and shiny nail surface, appreciated by women who do not wish to wear nail polish.

The undersurface of the nail can be painted with a whitening pencil, of which the core is kaolin, to reinforce the whiteness of the free edge.

Daily Care

Products are also marketed for daily use, which are to be used between manicures.

Cuticle softeners are not to be confused with cuticle removers. They are simply emollients to which quaternary ammonium compounds or urea are sometimes added to promote softening of the cuticles (2). These latter can then be gently reversed with the fingers.

Creams for brittle nails contain phospholipids. They are best applied after moistening the nail plate. Indeed, when a nail has been hydrated by immersion, phospholipids prevent dehydration, maintaining and increasing nail flexibility (6). Creams containing mandelic acid

are also marketed; in contrast to most α-hydroxyacid, mandelic acid would cause thickening in keratin.

Unwanted Effects of Manicure (7,8)

Unwanted effects in the nail area can mainly be attributed to technical errors. An overzealous manicure is likely an important source of microtraumatisms and can be the cause of multiple nail alterations: excessive pumicing or buffing can cause thinning of the nail plate and redness of the nail bed; repeated traumatisms on the nail matrix area can cause striate leukonychia (transverse white streaks) (Fig. 5) or Beau's lines (superficial transverse grooves).

The destruction of the cuticle leads to chronic paronychia.

The caring of the distal nail groove can cause onycholysis with proximal irregular indentation (Fig. 6), and caring of the periungual fold can cause small sores.

The damage to the cuticle, the onycholysis and the periungual sores favor bacterial and mycotic infections. The risk of transmitting infections in nail salons should not be neglected. A woman was awarded $3.1 million after contracting herpes, which spread to all 10 fingers. Precise standards of disinfection should be observed (7).

Figure 5 Leuconychia striata caused by overzealous manicure.

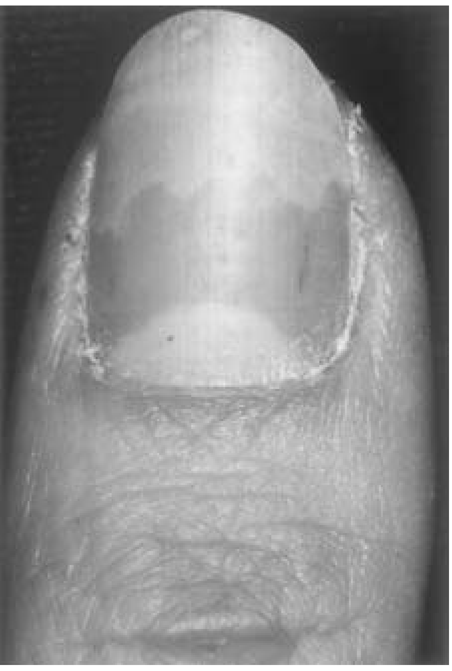

Figure 6 Onycholysis caused by excessive caring of the distal nail groove.

After contact with polish removers, the skin may have a white scaly appearance caused by stratum corneum dehydration; repeated application leads to irritant dermatitis. A single prolonged contact may even cause superficial blistering (9).

Cuticle removers should not be left too long in contact with the nail either, because they can cause irritation and should not be used on people who are susceptible to paronychia (10). In 1982, manufacturers reported receiving more complaints from cuticle removers than for nail polish removers: 2.3 and 0.33 complaints per million units sold, respectively (2).

Side effects of moisturizing creams (i.e., allergic contact dermatitis) are similar to those observed on other parts of the body.

Systemic Side Effects are Exceptional

Ingestion of nail polish remover may result in acetone intoxication, with central nervous system/respiratory depression, hyperglycemia, and ketosis (11). Inhaling nail polish removers containing toluene and aliphatic acetates (e.g., ethyl acetate) can cause central nervous system depression and a tight smothering feeling in the chest. As a consequence, excessive sniffing of polish removers could also produce toxic symptoms (9).

Classical nail polish removers should not be confused with artificial nail removers, which are much more dangerous systemically (see artificial nails/systemic side effects). In addition, nail polish removers are flammable and represent a fire hazard.

NAIL POLISH (SYNONYMS: NAIL VARNISH, NAIL ENAMEL, NAIL LACQUERS)

The concept of nail painting is not new. Numerous civilizations have used different products to enhance the beauty of nails. Henna is one of the most common product; it was probably already used in ancient Egypt and is still used today. Red balsam leaves mixed with alum were used in China, on the eve of the Mongol invasion (1250–1276) (12).

However, present polishes are quite recent; they appeared in the early 1920s. Their existence is linked to the discovery of nitrocellulose properties and to the progress made at that time in the automobile paint industry. Nitrocellulose was created by reacting natural cellulose fibers with concentrated nitric acid (HNO_3). Originally, it was used in high explosives, particularly during World War I. Later, another property of nitrocellulose was especially used: boiled in water, it decomposes enough to become soluble in organic solvents. After evaporation of the solvents, it produces a shiny and hard film. This quick-drying nitrocellulose lacquer rapidly achieved a great success in the developing car industry. Without the impetus provided by the automobile industry, it is doubtful that lacquer technology and the supply of good lacquer-grade nitrocellulose would have developed and been available for the manufacture of nail polishes (13).

For historic reasons, nail varnishes are also called "nail polishes," "nail lacquers," and "nail enamels" (13).

The perfect nail polish has to be easy to apply, and it should dry quickly. In addition, it should leave a shiny, smooth, even, hard, and flexible film that is able to last five days. Moreover, this polish should be removed without leaving any trace and should not have any side effects. Finally, it should be stable in the bottle and should offer a wide range of colors, enabling one to get the expected aesthetic effect.

The manufacture of nail polish is complex and potentially dangerous. It is only done in a few big factories. Nail varnishes are then contracted out by cosmetic companies, packaged, and labeled (14).

Composition (15)

The main constituent of the film that remains on the nail after drying (evaporation) is *nitrocellulose*. The film former has a lot of qualities. In particular, it is hard, tough, stable, and waterproof, but it is neither sufficiently adherent nor glossy or flexible.

To improve adherence and gloss, *film modifiers* are added. The most common one is Santolite® or toluene sulfonamide/formaldehyde resin (TSFR). TSFR is best designed under its INCI name: tosylamide/formaldehyde resin. It is the heart of the polish. As it is a potent sensitizer and contains formaldehyde, it tends to be replaced by other film modifiers that are said to be hypoallergenic: glycerophthalic polyester resin, 4-methylbenzene sulfonamide-epoxy

resin, phthalic polyester resin, polyester-saturated hydroxylated resin (16). However, in 1997, 42 nail polishes sold in Finland and belonging to 20 different brands were studied (17). They all contained TSFR, a factor of contact dermatitis, with concentrations from 0.02% to 11% in the bottle and from 0.1% to 25% in the dry polish.

With the nitrocellulose and the film modifier, the resulting film is hard, tough, adherent, and glossy. Unfortunately, it is still not flexible enough and cracks.

Plasticizers are added to increase flexibility. These are molecules with a high boiling point, which remain in the film after drying. They reportedly increase separation between the cellulose links as well as increasing the rate of solvent evaporation. Dibutyl phthalate and camphor enter this category. However, the former has been banned (65 Californian proposition in the United States). Other examples are castor oil, glyceryl tribenzoate, acetyl tributyl citrate, PPG-2 dibenzoate, glycerol, citrate esters, triacetin, and a polymer plasticizer called "NEPLAST" (a polyether-urethane) (15). While the film modifiers counterbalance the negative aspects of nitrocellulose, plasticizers modify the properties of the entire film. The glyceryl tribenzoate would even replace the film modifier in some nail polishes (15).

In the nail polish bottle, there are not only nitrocellulose, thermoplastic resin, and plasticizers but also solvents and thinners, pigments and dyes, thixotropic agents, as well as other additives.

The most commonly used *solvents* are alkyl esters (ethyl, *N*-butyl acetate) and glycol ethers (propylene glycol monomethyl ether). These molecules, which have different boiling points and evaporation rates, allow the regulation of drying time. They also allow lower viscosity, which improves brushability.

Thinners or diluents are not real nitrocellulose solvents but are miscible with them. This allows reduction of the nail varnish price. Thinners also help regulate the evaporation rates and stabilize viscosity. Indeed, a polish low in viscosity is easy to apply and leaves a homogeneous film. If viscosity is too low, the coverage of the film will be insufficient. If viscosity is too high, the film will be thick and streaky. Thinners essentially are aliphatic alcohols such as ethanol, isopropanol and butanol and aromatic hydrocarbures such as toluene. This latter, which is now considered to be cancerogenous and teratogenous, is no longer used in new nail lacquer formulations (15).

Pigments and colorants also need to draw the attention. Some are soluble in nitrocellulose and originate transparent polishes, very slightly colored. Most of them are not soluble and originate the most-used polishes: the nail enamels (13).

Example:

- *Mineral pigments: ferric ferrocyanide (Prussian blue), titanium dioxide (TiO2).*
- *Organic pigments: D&C red 6,7,34/FD&C yellow 5*

Prussian blue is used in small amounts to enhance blues and alter other shades. TiO_2 allows the attainment of pastel shades. D&C red 6 is a barium lake, D&C red 7 and 34 are calcium lakes, and D&C yellow 5 a zirconium lake, a lake being formed by precipitating a particular pigment with aluminum hydroxide to form a salt complex (15).

In pearlescent nail varnishes, there are guanine crystals, derived from scales of Atlantic herring and other fish, bismuth oxychloride, or mica coated with TiO_2.

The use of thermochrome pigments, of which the color changes according to the temperature, and photochrome pigments, of which the shade varies according to the light, offer new possibilities (18).

As a lot of pigments are insoluble, manufacturers had to cope with major problems of precipitation until new *thixotropic agents* were discovered in the 1960s (13). Thixotropic agents increase nail enamel viscosity at rest, thus preventing pigment precipitation. They, however, become fluid as soon as a mechanical constraint is exerted, either by shaking the brush or the bottle itself. This can be further improved by adding small metallic nickel (Ni) beads, often covered with plastic, in the bottle; Ni, indeed, is a common source of allergic eczema in women. Stearalkonium hectorite is the most frequently used suspending agent presenting settling out the pigments.

Theoretical composition of a nail varnish with 15% nitrocellulose, 7% resin, and 7% plasticizers is shown in Table 1.

Table 1 Nail Polish: Typical Formula. Comparison with Base Coat and Top Coat

Ingredients (approximate concentration in %)	Nail polish	Base coat	Top coat
Nitrocellulose	ca 15	↘	↗
TSFR	ca 7	↗	↘
Plasticizer	ca 7	↘	↗
Solvents–diluants	ca 70		
Butyl acetate	1.5–52		
Ethyl acetate	4–42	#	#
Isopropanol	2.7–7.7		
Toluène	20		
Suspending agent	ca 1		
Color pigments mixture	0.1		

TSFR: toluene sulfonamide formaldehyde resin; ↘: lower concentration; ↗: higher concentration; #: different concentrations
Source: From Refs. 3, 17.

Base Coats, Top Coats, Hardeners, Hypoallergenic Varnishes

Nail varnishes represent an important market estimated at $430 million a year, in the United States. (14). Besides classic nail polishes, base coats, top coats, hardeners, varnishes for brittle nails, varnishes for ridged nails, and hypoallergenic nail polishes are also marketed. What are they? All are nail varnishes with a slightly modified formulation.

The *base coats* contain more resin because they must increase the adherence of the varnish to the nail (Table 1). They contain no colorant to act as a protective antistain barrier between the nail plate and the shaded varnish (15).

On the contrary, the *top coats* contain more nitrocellulose and plasticizers because they must be tough and flexible to improve nail varnish resistance (Table 1). Often, they also contain special UV-absorbing materials, such as benzophenone 1 and 3, to help protect the underlying colored coats. Base coats and top coats also contain different proportions of thinners and solvents to ease application and to speed up drying.

Hardeners, varnishes for brittle nails, varnishes for ridged nails, etc. are in fashion. These are generally base coats to which are added nylon fibers, acrylic resins, or formaldehyde.

In hardeners, formaldehyde is in its free state, which is not to be confused with formaldehyde in the form of the TSFR in nail polishes (19). Formaldehyde is believed to crosslink protein in the nail plate, which increases surface hardness but decreases flexibility. In the long term, it may eventually be the cause of nail rigidity and cracks. Severe nail damage such as painful hemorrhages, paronychia, and nail bed involvement with subungual hyperkeratosis or onycholysis have been attributed to formaldehyde-based hardeners (Fig. 7). Lips hemorrhages are also possible in nail biters (19,20). Moreover, the presence of formaldehyde, which acts as an irritative agent, would favor TSFR sensitization (21). The presence of formaldehyde is now limited to less than 5%, and a shield for the skin should be used. Formaldehyde-containing hardeners are meant to be applied only to the free edge of the nail (19). In fact formaldehyde should be avoided.

New hardeners containing mandelic acid or silicium are marketed. In contrast to most α-hydroxyacid (AHA), acetyl mandelic acid in ISO propyl alcohol and butylenes glycol has been shown to cause thickenings in keratins. Silicium is purported to penetrate into the nail and to reinforce the disulfide linkage of the keratin. However, in both cases, biometric studies in nails are not available.

Calcium, vitamins, sulfured amino acid, and collagen can be added to the treating nail varnishes. These have probably no value. Two percent dimethyl urea is used in "Nail Intensity" by creative nail design (22). Chitosan has shown an increase in the linear nail growth in a double-blind study (23).

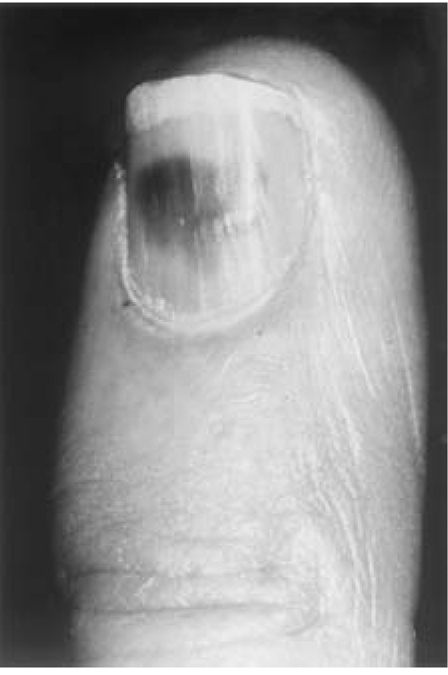

Figure 7 Subungual hemorrhage caused by a formaldehyde-based hardener.

Hypoallergenic nail polishes are polishes from which the most common substances causing allergic contact dermatitis, specially TSFR, have been removed. For example, the Pure Vernis® contains neither TSFR, nor phthalate or nickel balls. The resin is a polyester glycerophthalic resin. The plastifier is tributyl citrate acetyl. Nevertheless, these polishes do not guarantee the absence of reactions. Allergies to constituents of hypoallergenic varnishes have been described: polyester resin (24), epoxy resin (25), methyl acrylate (23), as well as rare cases of nitrocellulose allergy itself (26).

Recent Developments in Nail Polishes (14)

There is an increasing interest for quick-drying and long-lasting nail polishes, but these qualities are almost mutually exclusive. Nail polishes can be either quick drying or long lasting, not both. Quick-drying nail polishes contain low quantities of film former and are thus brittle.

Fragrances can now be added in nail polishes as well as metal or plastic glitters to give special effects.

Nail varnishes that are removed by peeling off also exist.

Water-based nail polishes have appeared. They are popular in Japan and with teenagers. As they contain water, they are prone to microbial contamination, and they have to be preserved, for example, with quaternium 15. Until now, water-based nail polishes are very brittle and crack but, according to Schoon (15), they probably constitute the future of nail polishes because a wider variety of additives could be added to them, which may eventually lead to truly preventing or help in treating common nail pathologies.

Use

After the manicure, the nail is degreased, dried, and covered with one layer of base coat, two layers of the colored nail enamel, and one layer of top coat (Fig. 8). The layers have to be thin and as uniform as possible. To do this, the quality of the nail polish and of the brush are both very important.

The varnish laying can be completed by spraying on or applying with a brush a film-drying accelerant that will protect the varnish while it is drying. (Silicone oil blends and silicone/water oil in water emulsions) (7).

The wearing of varnish is recommended for five of seven days.

Figure 8 Varnish laying.

Nail Polish Advantages

They are numerous. Firstly, nail polishes have an aesthetic advantage: they offer more refinement and elegance and allow for a wide range of personal preferences.

Secondly, the nail varnish plays a protective role: it forms a film on the nail surface, which is both tough and flexible, increasing the resistance of the nail to microtraumatisms. It also maintains a more constant degree of nail hydration.

In pathology, nail lacquers allow chromonychia and onycholysis to be hidden. Unfortunately, they can also hide a subungual melanoma and therefore delay its diagnosis (27).

Unwanted Effects of Nail Polishes (3,7,8)

They do exist but are relatively rare: in 1982, manufacturers reported receiving 0.28 complaints per million units distributed (2).

The nail polishes can cause an *orange staining of the nail plate*, prominent at the distal part. It is more frequently observed with the deeper shades of red and brown enamels and can be prevented by the former application of a base coat. But it has become rare, since soluble pigments are mainly used.

Nail lacquers can cause *keratin granulation* (Fig. 9), presenting as superficial friability. This happens when individuals apply fresh coats of enamels on top of old ones, for several weeks. Therefore, it is advisable to wear a varnish five in seven days only.

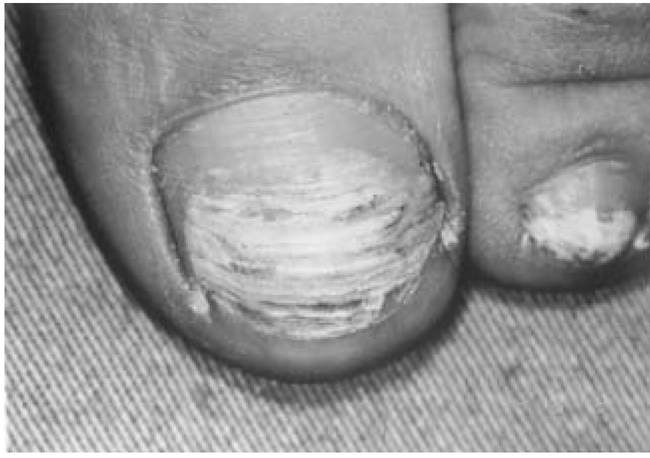

Figure 9 Keratin granulations *Source*: Courtesy of B. Richert, Belgium.

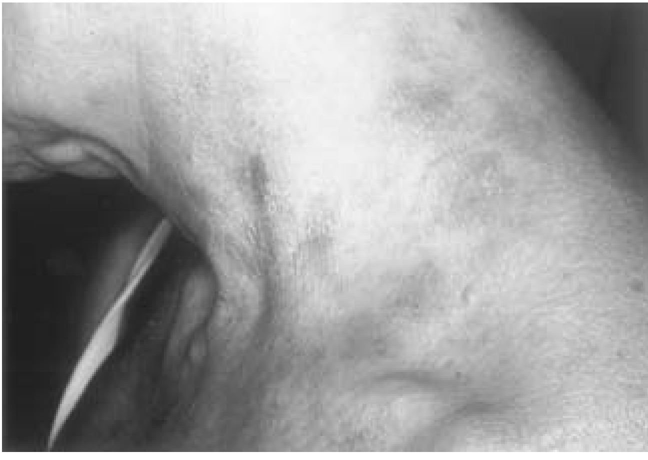

Figure 10 Eczema on the sides of the neck due to nail varnish allergy.

Nail varnishes can also cause *allergic contact dermatitis*, more rarely, contact urticaria.

Eczema breaks out especially on the eyelids, on the lower half of the face, on the sides of the neck (Fig. 10), and on the upper chest. In addition to distant (ectopic) contact dermatitis, allergic airborne contact dermatitis should be suspected when lesions on the face, neck, and ears are symmetrical. However, periungual dermatitis (28) is rare, but exists (29). The allergic contact dermatitis due to nail varnishes can have severe sociomedical consequences such as sick leave and hospitalization. Their diagnosis is easily missed (28).

The true incidence of reactions is not high: cosmetics cause 5.4% of contact dermatitis cases (30,31). Of the cosmetic reactions, nail products ranked fourth in the North American Contact Dermatitis Group study, producing 8% of the reactions (30). They ranked second, producing 13.4% of the reactions in a European study conducted by de Groot (31), in Holland. In a review of published cases, from 1925 to 1993, the most frequent allergen was by far TSFR, which was responsible for more than 347 cases (32). The guanine and the phthalates were respectively responsible for only four and three cases.

Among the most frequent allergens in cosmetics, TSFR comes in second place, after Kathon CG or paraphenylenediamine, in the studies of de Groot (31) and of Tosti (29) and in sixth place in the study of Adams and Maibach (30).

One case of both big toenail onycholysis, due to contact sensitivity to benzalkonium in a nail lacquer, has been reported (33).

Nail Polishes and Hospital

Nail polish should be removed routinely before anesthesia. Indeed, nail polishes, specially blue and green ones, significantly interfere with the measurement of oxygen saturation by pulse oximetry (34).

In health care workers, nail polish is allowed as long as it is worn on short nails and is fresh, not chipped (7). At equal length, the germ contamination rate does not seem higher for fresh painted nails than for natural ones.

THE ARTIFICIAL NAILS

Bringing very long nails into fashion may have originated from China (35). In the popular imagery, Chinese mandarins are often represented with very long nails. See, for example, the Chinese mandarin of Hergé in *Tintin and The Blue Lotus* (36).

The first artificial nails appeared in the United States, around 1935. Since then, thanks to their improving quality, they have become more and more popular. Annual business in nail salons in the United States is estimated at \$6.3 billion. (14). Artificial nails were almost

exclusively applied by professionally trained manicurists in nail salons. However, professional nail applications are expensive, and artificial nail kits designed for home use are now available over the counter (37).

Composition and Techniques

To make things easier, two different kinds of artificial nails can be considered: the artificial sculptured nails and the artificial preformed nails.

Sculptured Nail Technique

In the *sculptured nail technique*, artificial nail is made of an acrylic resin obtained by blending a methyl, ethyl, or isobutyl methacrylate monomer, which comes in a liquid form, and a polymethyl or ethyl methacrylate polymer, which is a powder. The methyl methacrylate monomer, banned in certain American states because of its side effects, may still be used, especially in discount price salons. Indeed, this monomer, which smells terrible, is cheap and allows to work quickly (14).

The monomer also contains a stabilizer such as hydroquinone and *N*,*N*-dimethyl-*p*-toluidine as an accelerator. The polymer also contains benzoylperoxide as a polymerization initiator.

Typical formula (3):

- *Liquid: Acryl-type monomer (ca 99%). Example: methylmethacrylate monomer*
  Stabilizer (ca 1%). Example: hydroquinone
- *Powder: Acryl-type polymer (ca 97%). Example: polymethylmethacrylate*
  Polymerization initiator (ca 3%). Example: benzoylperoxide

Other components such as plasticizers, solvents, accelerators, and pigments may be included (38).

The nail plate surface is pumiced. After disinfection, a metallized paperboard template is placed to frame the new nail, and a primer is painted on the nail (Fig. 11). The latter is a highly acidic solution, most commonly methacrylic acid with a pH as low as 2. It acts as a double-sided sticky tape.

Next, several layers of the acrylic paste that has just been blended are applied. For bigger refinement, a white paste is first applied on the template and shaped (Fig. 12); then a pinker one is applied on the natural nail and the template, and again shaped (Fig. 13).

After hardening at room temperature, which occurs rapidly, the template is removed and the nail is pumiced (Fig. 14), filed (Fig. 15), and buffed, producing a long, smooth, and attractive nail.

Figure 11 A metallized paperboard template is placed. Then a primer is painted on the nail.

Figure 12 A white paste is first applied on the template.

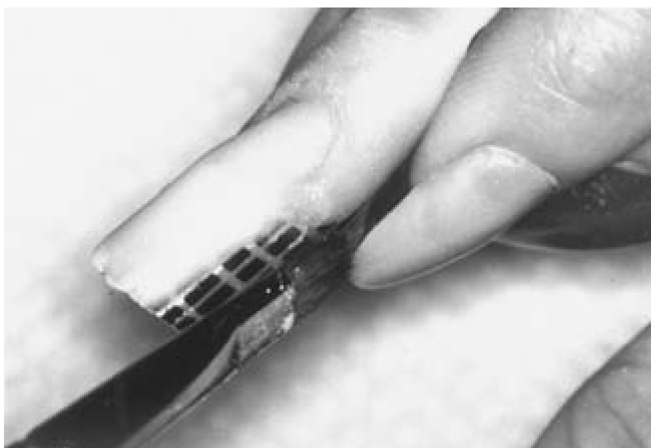

Figure 13 A pinker paste is applied on the natural nail and the template.

Figure 14 The sculptured nail is pumiced.

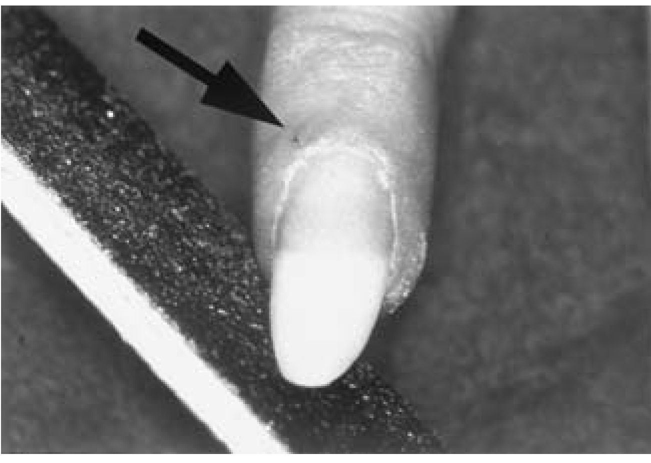

Figure 15 The sculptured nail is filed. Note the small sore in the proximal nail fold (*arrow*). The latter appeared while pumicing with the electric device shown.

This sculptured nail technique is rather difficult to perform. Its apprenticeship is long and requires good skills from the nail sculptor. However, this technique can be used on seriously damaged nail surface. Clients need maintenance filing.

Sculptured nails can be dissolved in acetone.

Artificial Preformed Nails

The second technique uses *preformed plastic tips*, which are packaged in several shapes and sizes, adapted to the different fingernails (Fig. 16).

The nail plate surface is buffed. After disinfection, the preformed plastic tip is simply fixed with cyanoacrylate glue, on the distal half of the nail (Fig. 17).

Typical formula of cyanoacrylate glue (38):

- *Ethyl cyanoacrylate 90.6%*
- *Polymethyl methacrylate 9%*
- *Hydroquinone 0.4%*
- *Stabilizer (organic sulfonic acid) Trace*
- *Plasticizers and thickenings agents may be added.*

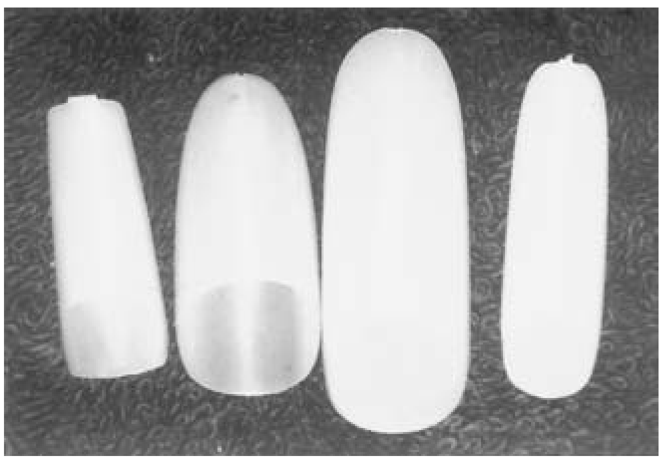

Figure 16 Preformed plastic tips.

Figure 17 Preformed plastic tips glued on the distal part of the nail.

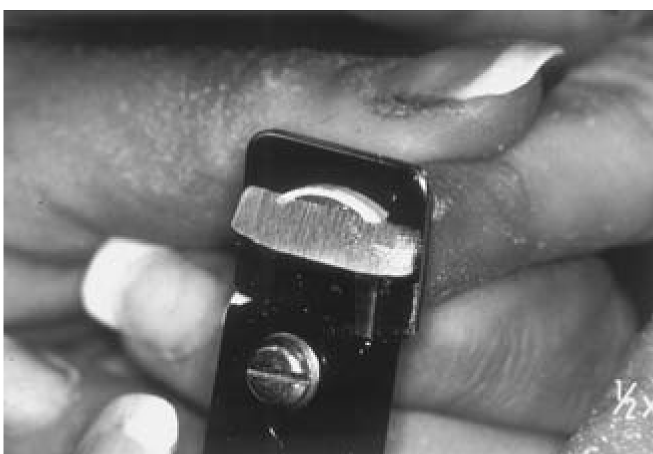

Figure 18 The artificial nail is cut with a special "guillotine clipper."

Then, the artificial nail, which is too long, is cut with a special "guillotine clipper" (Fig. 18). It is also filed and buffed to give it the desired shape and length.

At this stage, the nail surface is not smooth; it presents a bump between the proximal natural nail and the proximal border of distal artificial nail (Fig. 19). An acrylic gel is therefore painted on the nail like a nail polish (Fig. 20). It will harden, in other words polymerize, after UV exposure.

This technique is much easier to learn than the sculptured nail technique. It does not require any special skills, but there is a normal nail to be present for attachment. If not, the plastic nail will never be sufficiently glued. They should not remain for more than one or two days in time.

Light-Cured Gels

Gels are a premixed variant of sculptured nails. They are made of a mixture of acrylic monomers and polymers directly provided by the manufacturer.

They are more and more popular because they are odorless, give a more natural aspect to the nails, and do not require irritant (meth)acrylic acid as a primer.

Figure 19 Bump (*arrow*) between the proximal natural nail and the distal artificial nail.

Figure 20 An acrylic gel is painted on the nail.

New gel formulations are regularly marketed, but there are two main types of gels:

1. Acrylic light-cured gels in which the polymerization or hardening is obtained by exposure to light (most often to UV). These gels may contain (meth)acrylated urethanes, triethyleneglycol dimethacrylate, methacrylated epoxy resin or hydroxyfunctional methacrylates (39), and a photoinitiator. The bonding is similar to "restorative dental bonding" commonly used by many dentists worldwide (40).

   It should be pointed out that acetone will have no effect on UV-gels in contrast to artificial sculptured nail. If a patient becomes allergic, the artificial nails must be grinded off with heavy abrasive (20).
2. Cyanoacrylate gels in which the polymerization is obtained by spraying or brushing an activator. For example: ethyl-cyanoacrylate gel; activator, *N,N* di-methyl paratoluidine.

There are gels with different consistencies, designed for different uses, the consistency being determined by the resin to monomer ratio. There are also colored gels.

According to their composition, the gels can be used for different purposes (20):

- For the sculptured nail technique. However, gels do not allow production of as long and resistant nails than the classical liquid-powder technique.

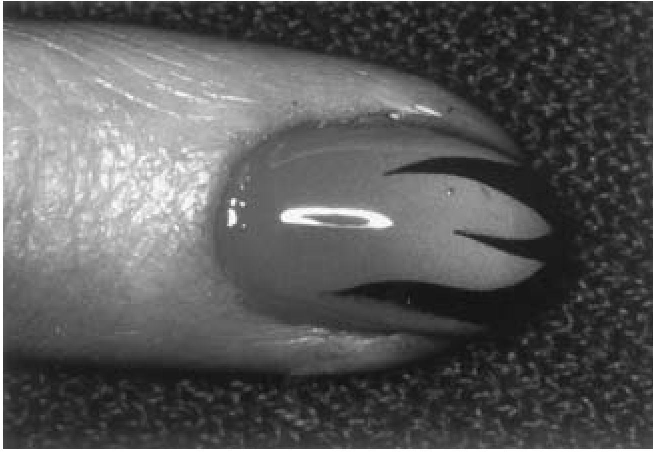

Figure 21 Air brushes made "nail art."

- Over plastic tips as we have just seen.
- To protect a natural or polished nail. This procedure is known as "nail capping."
- Capping with fabric (silk, linen, and fiberglass) adds strength and is known as "nail wrapping."
- Sprinkle resins based on cyanoacrylate resins, moisture cure, and trace quinone-type polymerization are present. Amine-based spray or brush-on polymerization activator may be used.

Advantages of Artificial Nails

Artificial nails are much more resistant than natural ones; nail polishes easily remain on them for three weeks.

They allow refinement and fantasy, which are much superior to those obtained with nail enamels. They can be decorated with jewels. Genuine work of art can be realized with special airbrushes (nail art) (37) (Fig. 21).

Artificial nails are mainly used for brittle or broken nails and for onychophagia. However, artificial nails should not be recommended in onychophagia associated with small wounds. Indeed, periungual sores that are so frequent in nail biters could favor acrylic sensitisation.

In pathology, they allow to hide more serious dystrophies than the nail polishes, such as posttraumatic permanent nail dystrophy (Figs. 22 and 23) or racket nails. After big toenail shedding in a tennis player or a skier, an artificial nail can prevent the hypertrophy of the periungual soft tissues, allowing a painless nail regrowth.

Artificial nails should not be recommended in case of nail psoriasis or lichen planus because they can worsen the condition by Koebner phenomenon (20).

Unwanted Effects of Artificial Nails (5,37)

Artificial nails are expensive and time consuming. It takes about one hour to put a set of 10 artificial nails in place, and moreover, they must be taken care of every two to three weeks. As the natural nail continues to grow, the proximal part of the artificial nail must be refilled. The adhesion between the natural and the artificial nail must also be checked; it must remain watertight.

Side effects of artificial nails can be classified in two main groups:

- Nonallergic reactions because of technical errors, bad care, or wearing of too long nails, which are most frequent
- Allergic or toxic reactions

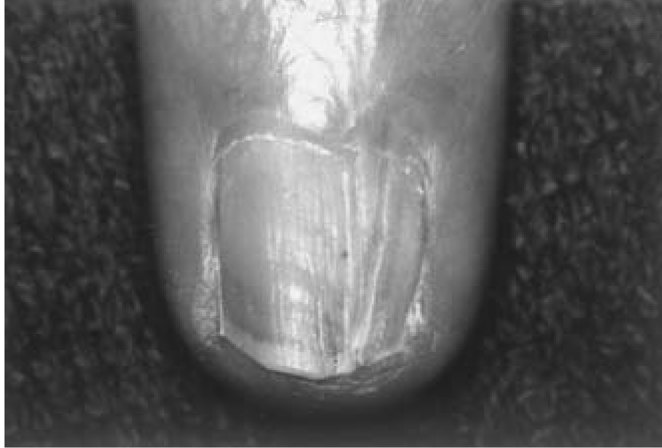

Figure 22 Posttraumatic permanent nail dystrophy.

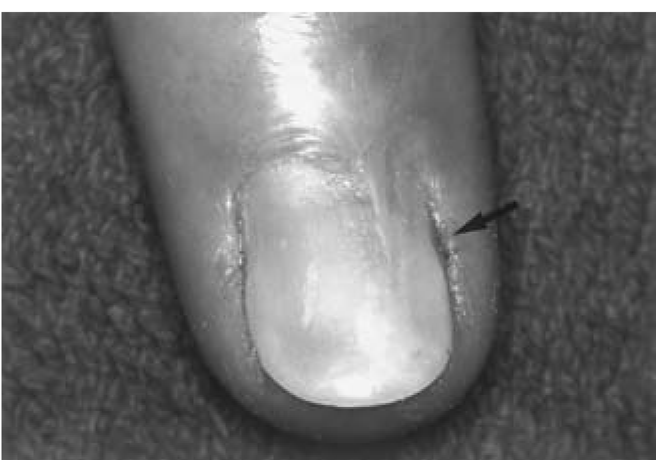

Figure 23 Posttraumatic permanent nail dystrophy as seen in Fig. 22, after application of an artificial nail. Note the small sore in the lateral nail groove (*arrow*).

NONALLERGIC REACTIONS

Technical errors or bad care cause brittleness of the natural nail by excessive filing or pumicing and inadequate use of the primer. After two or four months of wear, it is not unusual for a sculptured nail to damage the underlying natural nail. If it becomes yellow or crumbly, this means that the product was applied and maintained incorrectly. Instead of wearing prosthetic nails for no more than three consecutive months, with reapplication after one-month interval, it should be more appropriate to find a better-qualified nail technician. The problem may well not be the acrylic nail materials but rather the thinning of the nail because of over-filing with heavy abrasives. Small periungual sores (Figs. 15 and 24) are frequently observed. They could favor acrylic sensitization.

The penetration of water between the natural and the artificial nail is a frequent complication in artificial nails, which shows up as superficial nail plate discoloration (Fig. 25). Individuals in frequent contact with water, such as nurses, bartenders, and so on, have difficulty keeping on acrylic nails. This side effect is well known by nail technicians who remove the artificial nail, buff the nail surface to make the discoloration disappear, and sculpture a new, tightly adherent, artificial nail.

The wearing of too long nails favors onycholysis as well as nail fracture. It impairs finger and hand performance (41).

Figure 24 Severe alteration of the nail plates with distal onycholysis due to artificial nail allergy.

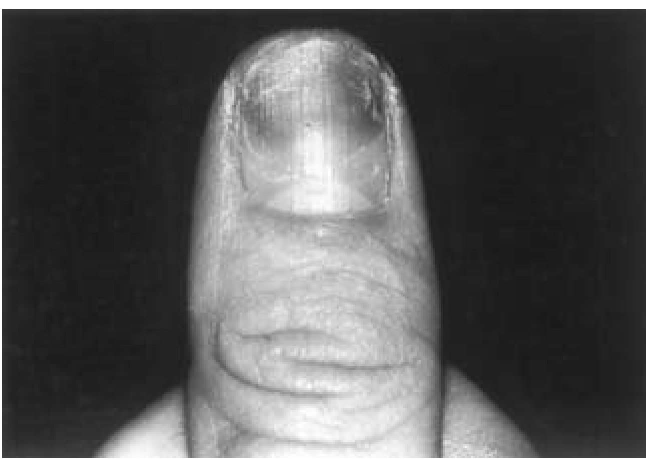

Figure 25 Superficial nail plate discoloration due to bad adherence between the normal and the artificial nail.

The wearing of too long nails, the onycholysis, the periungual sores, the penetration of water favor mycotic and microbial infections. These can be severe, especially in diabetics and immunocompromised people (42). Three cases of *Pseudomonas* corneal ulcers after artificial fingernail injuries have been described (43). There is a recent concern for microbial contamination of artificial nails in health care workers (see below).

Inappropriate Use

A case of "pseudo-onycho-palatitis" has recently been described. A mother had offered her right index finger in place of a pacifier to her baby. The mother's artificial nail became detached and was stuck on the palate. Several hours later, correct diagnosis was made when the infant was brought to hospital (44).

ALLERGIC OR TOXIC REACTIONS

Contact dermatitis to artificial nails can affect the client, less frequently the manicurist (40,45). Here, unlike nail-lacquer dermatitis, paronychia accompanied by onycholysis or subungual dermatitis are more likely to be present. Eyelid dermatitis is frequently associated. Women are usually not aware that artificial nails are a possible cause of allergy (46,47), and diagnosis is often delayed. When the use of artificial nail is discontinued, it is worth noting that it takes several months for the nails to return to normal.

Sculptured Nails

The most frequent allergen is the (meth)acrylate monomer, whereas the polymer is considered to be a weak sensitizer. Sensitization seems to be primarily caused by the monomer, which remains unpolymerized, in the final sculptured nail and in the filing dust, produced when the sculptured nail is trimmed (40). This is specially observed with self-curing resins, but even in photobonded acrylic nails monomer persists. The nail technicians should apply thin successive gel layers and expose each layer to UV, which is rarely done.

Allergic contact-type reactions were first described with methyl methacrylate monomer, but other monomers (e.g., ethyl and butyl methacrylates) can induce sensitisation, and cross-reactions also occur (39,48). They may be even stronger sensitizers than methyl methacrylate (49).

The allergic reaction usually starts two to four months, and even as long as 16 months, after the first application (37). The first symptom is an itch in the nail bed, followed by painful paronychia, which can be associated with paresthesia. Nail bed hyperkeratosis or onycholysis is frequently observed. Distant allergic contact dermatitis may affect the eyelids and the face, but more widespread lesions are also possible (50). Six cases of occupational asthma due to ethyl methacrylate have been reported in cosmetologists working with artificial nails (37).

Exceptional cases of severe paresthesia evolving for several years were described. These were accompanied by Raynaud's-like syndrome and permanent nail loss (48). They could result from a direct, toxic effect on the wounded Ranvier's cutaneous nerves in nail biters for instance. Patch tests may remain negative (51).

Prolonged paresthesia of the fingertips were also observed with photobonded acrylic-sculptured nails (39), and two natural nails had to be surgically removed because of resistant superinfections (40).

In contrast to the manufacturers' declarations, many "hypoallergenic" products continue to include acrylate functional monomers and therefore cause allergic sensitization (40).

Cyanoacrylate Nail Preparations

Initially, it was believed that the cyanoacrylates were not sensitizers. We now know that such cyanoacrylates can produce allergic reactions (39). Cyanoacrylates do not usually crossreact with the (meth)acrylate monomers used in nail preparations (38,45,48), although in a study performed by Koppula (52) ethyl α-cyanoacrylate did crossreact with several acrylates.

The cyanoacrylate glue, either used for nail wraps composed of silk, linen, fiberglass, or with plastic tips that should never cover more than distal half the nail, can be responsible for an eczema of the fingertips, with nail involvement [onycholysis, subungual hyperkeratosis (46), rough, split, deformed (53), or discolored (39) nail plates] (Figs. 25 and 26). An eyelid dermatitis as well as a nummular eczema can be present, particularly over the dorsal hand (54). More widespread eruption were also described (46); one case mimicked a small plaque parapsoriasis eruption (55).

Figure 26 Same patient as seen in Figure 25: positive patch tests with ethyl-cyanoacrylate glue and one of the patient's own product.

Persistence of the dermatitis until the nails grow out is frequently observed and is probably caused by retained adhesive. A positive patch test to nail clippings was indeed obtained in one patient, some two months after stopping the use of cyanoacrylate (46).

Patch testing is very easy, using a few drops of the cyanoacrylate adhesive, placed on the gauze portion of an adhesive plaster and allowed to dry before application (46).

Paresthesia has not yet been reported from cyanoacrylate glue.

Methacrylic Acid–Containing Primers

Nail-care products are a common cause of accidental poisoning in children. Such products accounted for 198,084 exposures (16% of exposures to cosmetics and personal-care household products) reported to the American Association of Poison Control Centers, in 1997 (56). Most nail product exposures involved either polish or nail polish removers, both of which are generally of low-order toxicity. However, products used in the application and removal of artificial nails are potentially hazardous and their packaging and labeling information are inadequate.

Methacrylic acid–containing primers are particularly involved: more than 759 exposures to methacrylic acid–containing artificial nail preparations were gathered by the American Association of Poison Control Centers. Seventy-five percent involved preschoolers younger than six years and almost 10% resulted in either moderate or severe injuries. Dermal burns were mainly reported, but burns of the airway and gastrointestinal tract with residual esophageal dysfunction were also described (57). Out of the 759 exposures, 84.9% had occurred at home, expressing the recent trend toward domestic use of artificial nail products, previously restricted to professional cosmeticians in nail salons. This home use has been done without a concomitant review of packaging safety. It is now recommended that the primers should be dispensed in child-safety containers (56).

Systemic Side Effects

Acetonitrile-containing artificial nail glue removers were removed from the market, after causing several childhood cyanide-poisoning deaths (58).

Six cases of profound methemoglobinemia were reported in children aged 13 to 27 months (59). They appeared following ingestion of small quantities of *artificial nail removers containing nitroethane*. The authors concluded that these products should be packaged in child-safety containers and properly labeled. Their availability for home use should be questioned.

N,N-dimethyl-p-toluidine can also cause methemoglobinemia when ingested (60,61).

ARTIFICIAL NAILS AND HOSPITAL

Unpolished acrylic nails do not affect pulse oximetry measurements of oxygen saturation. Theoretically, patients may not need to remove them before surgery (62); however, thick ornately painted gel false nails, which may be difficult to remove, present a real challenge to pulse oximetry (37).

There is a Real Concern for Microbial Contamination of Artificial Nails (35)

In health care workers with artificial nails, it has been shown that there is not only an increased amount of carriage of pathogen such as gram-negative bacteria (63–64) but also *Staphylococcus aureus* and yeasts, and this is observed both before and after washing the nails (65). In one study, *Serratia, Acinetobacter, and Pseudomonas* were cultured only from the fingertips of nurses wearing artificial nails (64). In a recent epidemiologic and molecular investigation of endemic *Pseudomonas aeruginosa* infection in a neonatal intensive care unit, it was shown that the use of artificial nails or nail wraps was a risk factor for colonization of the health care worker's hands (66).

Two nosocomial infectious outbreaks have been described where nurses wearing artificial nails could have played an important role in the transmission of potentially lethal infections. The first report dealt with *Serratia marcescens* infections in a cardiovascular surgery unit (67). The other report dealt with *P. aeruginosa* infection in a neonatal intensive care unit (68).

It should be added that individuals with artificial nails tend to wear their nails longer (65); they are more careful about their nails when washing their hands, and sanitary conditions for application of artificial nails, whether at the nail salon or at home, are paramount in preventing nail infections (68).

In the United States, the association of operating room nurses has guidelines concerning the nails. It is recommended that the nails should be kept short and clean. Nail polish can be used only fresh (but not chipped) and artificial nails should not be worn.

These guidelines should probably be extended to all health care workers, especially when they are dealing with immunocompromised patients. In addition, the 3-mm rule for end-of-fingernail length should be emphasized (69).

NAIL PROSTHESIS

For permanent nail degloving, following accidental or surgical origin and in congenital nail or missing digit, thimble-shaped digital fixation, for example, brings aesthetic and functional comfort to the patient (70).

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71 Surfactants: Classification

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INTRODUCTION

Surfactants are chemical materials used by humans for centuries; a very remote use is reported as a kind of soap already prepared with ash and vegetal oils by Sumerian people (ca. 10,000 BC). Surfactants are used today in many practical applications in industry, home, and institutions. Cosmetics is an important application area among others (detergents, foods, fabric softeners, biocides, textiles industries, paints, inks, adhesives, dyes, herbicides, insecticides, fire extinguishers, etc.).

The term "surfactant" applies to a group of molecules having both a hydrophilic part and a hydrophobic (or lipophilic) part. Surfactants modify the interfacial properties of the liquids in which they are incorporated; this property stems from their tendency to concentrate at the interfaces separating immiscible phases.

This peculiar property favors the formation of micelles and structured liquid phases, which are involved in numerous facets of the cosmetic world: cleansing action, direct and inverse emulsions, gels, foam production, etc.

Depending on the nature of the hydrophilic moiety ensuring water affinity of the molecule, surfactants are distributed in anionic, cationic, amphoteric, and nonionic classes.

Regarding the hydrophobic moiety of the molecule, it is a hydrocarbon chain in most common surfactants; however, in some more specialized surfactants, this hydrophobic part can be a non-hydrocarbon chain such as a polydimethylsiloxane or a perfluorocarbon.

The selection of surfactants in the frame of cosmetic products development is a delicate task in which numerous factors have to be taken into account. Among others, one should consider those directly related to functions to be fulfilled (detergency, emulsification, foam quality, rinsability, mildness for skin, skin feel, etc.), and also those related to cost, toxicity, and biodegradability.

Although few surfactants are naturally produced such as saponins or lecithins, there is a trend to produce more and more surfactants issued from natural materials.

The aim of this chapter is to provide a classification of various commercially available surfactants.

IONIC SURFACTANTS

Anionic Surfactants

In aqueous solution, anionic surfactant molecules carry negative charges if the composition pH is not too low (slightly acidic, neutral, or alkaline). The ionized moiety can be a carboxylate, sulfate, sulfonate, or phosphate. Among the most frequently used surfactants in skin care products, the alkyl sulfates and alkyl ethoxylated sulfates can be mentioned for their high-foaming capacity. Anionics are generally used in association with other surfactants (nonionics or amphoterics), which bring improvements in the skin tolerance, foam quality, or product viscosity.

Other anionics are also used in personal products, however, as secondary surfactants, often for their milder profile and their low-foaming properties (isethionates, sulfosuccinates, taurates, sarcosinates, phosphoric acid esters, acylglutamates, etc.).

Carboxylates

Carboxylates salts. Surfactants belonging to this class generally derive from oleochemistry. Carboxylate salts (or soaps) are directly produced by the alkaline hydrolysis (or saponification) of animal and vegetable glycerides and result from the neutralization of fatty acids.

Saturated sodium soaps are extremely soluble in water up to C_8; those with chain lengths approaching C_{18} become less soluble, and they are insoluble above C_{20}.

Starting from C_{16} chain lengths, the fatty acids can be either saturated or unsaturated.

Unsaturated fatty acids are prone to undergo oxidation and form oxides and peroxides, which cause rancidity and yellowing.

Potassium soaps and salts of alkanolamines are more fluid and also more soluble than sodium salts.

The extremely low solubility of alkaline earth and heavy metals' fatty acid salts make this class of surfactants less appropriate for use in hard water.

$$R{-}C(=O){-}O^{\ominus}\ M^{\oplus} \qquad M = Na, K, NH_4, etc. \qquad R = CH_3{-}(CH_2)_x{-}$$

Alkyl carboxylate

The main application of fatty carboxylates is found in the soap bars widely used in the world for fabric hand wash (generally based on tallow/coconut oil mixtures).

Water-soluble soaps are mainly used in skin cleansers (soap bars or liquids), shaving products (sticks, foams, or creams), and deodorant sticks.

Mixtures of fatty acids and their salts are used in "acid soaps."

Water-insoluble soaps form gels in nonaqueous systems and, because of their hydrophobicity, they can be appropriate surfactants for w/o emulsions.

Ester carboxylates. This class of surfactants is a subcategory of the previously discussed surfactant group based on carboxylic acids; they are monoesters of di- and tricarboxylic acids.

These esters are produced by condensation reactions involving different types of molecules, either an alcohol with a polycarboxylic acid (e.g., tartric or citric acid) or a hydroxyacid (e.g., lactic acid) with a carboxylic acid.

The reacting alcohol may have been previously ethoxylated.

$$HO{-}C(COONa)\left[CH_2C(=O){-}O(CH_2CH_2O)_7{-}C_{12}H_{25}\right]_2$$

Sodium dilaureth-7 citrate

Because of their good foaming properties and substantivity on the hair, ester carboxylates are especially suitable in shampoos; in combination with alcohol ethoxy sulfates (AEOS), they provide reduced skin irritation.

Short-chain lactylates (i.e., issued from lactyllactic acid) are substantive on the skin and show humectant properties.

Ether carboxylates. Alkyl polyglycol ether carboxylates are the best-known surfactants in this category.

These surfactants are formed by the reaction of sodium chloracetate with ethoxylated alcohols.

Because of the addition of ethoxylated groups, ether carboxylates are more soluble in water and less sensitive to water hardness compared with conventional soaps. Also, keeping the best properties of nonionic surfactants, they do not exhibit any cloud point and show good wetting and foam stability.

Ether carboxylates do not undergo hydrolysis in presence of alkali or acids.

$$RO{-}(CH_2CH_2O)_n{-}CH_2{-}COONa$$

Alkyl polyglycol ether carboxylate, sodium salt

Ether carboxylates are used as general emulsifier and emulsion stabilizers.

In personal care, they impart mildness, creamy foaming, skin feel, and hair-conditioning benefits. Therefore, they are especially suitable in shampoos in combination with alcohol ether sulfates and possibly with cationics.

More recently, a new generation of alkyl glucose carboxylates is emerging. These surfactants exhibit both the high mildness of alkyl polyglucoside (APG) surfactants and additional attributes such as foaming and sensory benefits. A typical surfactant of this class is the sodium lauryl glucose carboxylate.

Sulfates

Alkyl sulfates. Alkyl sulfates are organic esters of sulfuric acid; they vary by the length of the hydrocarbon chain and by the selected counterion.

Alkyl sulfates are produced by sulfation of the corresponding fatty alcohols.

The properties of alkyl sulfates depend mainly on the chain length and on the degree of branching of the hydrocarbon chain as well as, to a smaller extent, on the nature of the counterions.

They are generally good foamers, more especially in hard water; best foam characteristics are obtained in the C_{12} to C_{14} chain length range.

Sodium lauryl sulfate (SLS) has a 12-carbon chain length and is one of the most common surfactants. It is not well tolerated by the skin. When the chain length increases, i.e., in the C_{14} to C_{18} range, surfactant penetrability through the stratum corneum decreases along with its irritation potential; but the foaming capacity is accordingly depressed. Chains with carbon number lower than 12 are better tolerated by the skin than SLS but exhibit more pronounced smell. Combination with other surfactants allows considerable improvement of the lauryl sulfate compatibility with skin while keeping a good foam. SLS is, however, less frequently used than its ethoxylated counterpart. Lauryl sulfate is available under the form of various salts: SLS, ammonium lauryl sulfate (ALS), magnesium lauryl sulfate [$Mg(LS)_2$] and triethanolamine lauryl sulfate (TEALS). Skin tolerance of lauryl sulfates is as follows: $Mg(LS)_2$ > TEALS > SLS > ALS

$$R-CH_2-O-S(=O)_2-O^-\,Na^+$$

Sodium alkyl sulfate

Alkyl sulfates are used in cosmetics and personal care areas [e.g., diethanolamine (DEA) lauryl sulfate in shampoos]; they are associated with other surfactants and improve foaming characteristics of detergent systems.

Pure SLS is also used in oral care and incorporated in dental creams, essentially as a foaming agent.

Alkyl ether sulfates. Alkyl ether sulfates (AESs), which are also identified as AEOS, result from the sulfation of an ethoxylated alcohol.

Compared with alkyl sulfates, the ether sulfates show higher water solubility, improved foam stability in hard water, and better skin tolerance. The viscosity of surfactant solutions of ether sulfates is much more sensitive to the presence of electrolytes than alkyl sulfates; formulators often take advantage of this opportunity to bring liquid formulations to the desired viscosity by simply adjusting the salt level (e.g., NaCl).

The higher the number of ethoxy groups (EOs) in the molecule, the lower the surfactant's ability to penetrate the stratum corneum, and the less irritant for skin it will be. Similar ranking is true for eye irritation. Also, the foaming capacity decreases as ethoxylation degree increases.

$$R-CH_2-O-(CH_2-CH_2-O)_n-SO_3Na$$

Sodium alkyl ether sulfate

AESs are extensively used in personal products such as liquid soaps, shower gels, foam baths, and, more especially, shampoos. Sodium lauryl ether sulfate (SLES) is today the most currently used primary tensioactive, and more especially, under the forms SLES-2 EO and SLES-3 EO, which combine good foaming and skin compatibility properties.

Amide ether sulfates. The amide ether sulfates are obtained by sulfation of the corresponding ethoxylated amide.

The magnesium salts foam well, and their skin compatibility is excellent.

$$\left[R-\underset{\underset{O}{\|}}{C}-NH(CH_2CH_2O)_3-SO_3 \right]_2 Mg^{2+}$$

Magnesium PEG-3 cocamide sulfate

Owing to their weak lipid removal effect, amide ether sulfates are used in very mild personal cleaners.

Alkyl glyceride sulfates. The best-known surfactant of this class is the cocomonoglyceride sulfate (CMGS). It is obtained by transesterification of coconut oil with glycerol followed by a sulfation with sulfur trioxide and a neutralization with sodium hydroxide.

$$\begin{array}{l} H_2C-O-\overset{\overset{O}{\|}}{C}-R \\ \;\; | \\ HC-OH \\ \;\; | \\ H_2C-O-SO_4Na \end{array}$$

Sodium alkyl monoglyceride sulfate

This surfactant is very well designed for cosmetic and personal care products. Compared with the corresponding AES, it shows similar foaming power. Because this surfactant acts as a foam booster, it can be advantageously combined with APG. Such mixtures also show a thickening ability induced by salt addition. CMGS is said to present a better skin compatibility profile than ether sulfate or other anionic surfactants.

Sulfonates

On a chemical standpoint, there is an important difference between the previously discussed alkyl sulfates and the alkyl sulfonates: in the former, the sulfur atom is linked to the carbon chain via an oxygen atom, and in the latter, the sulfur atom is directly linked to the carbon atom.

Alkyl sulfonates. Three major types of alkyl sulfonates must be considered: the primary and secondary paraffin sulfonates [PS and secondary alkyl sulfonate (SAS)] and the α-olefin sulfonates (AOSs).

The paraffin sulfonates are water-soluble surfactants, good foamers, and good o/w emulsifiers. Their solutions do not thicken easily upon salt addition. Therefore, they are particularly appropriate to formulate fluid liquids or highly concentrated products.

The AOS have general properties fully comparable to LAS (see sect. "Alkyl-Aryl Sulfonates"); they are good o/w emulsifiers, wetting, and foaming agents.

$$CH_3-(CH_2)_m-\underset{\underset{H}{|}}{\overset{\overset{H}{|}}{C}}-SO_3Na \qquad CH_3-(CH_2)_m-\underset{\underset{CH_3(CH_2)_n}{|}}{\overset{\overset{H}{|}}{C}}-SO_3Na$$

Primary sodium alkyl sulfonate — Secondary sodium alkyl sulfonate

±35% { $R{-}CH_2{-}CH_2{-}CH{=}CH{-}CH_2{-}SO_3Na$
$R{-}CH_2{-}CH{=}CH{-}CH_2{-}CH_2{-}SO_3Na$

±65% $R{-}CH_2{-}CH_2{-}CH(OH){-}CH_2{-}CH_2{-}SO_3Na$

Constituants of α-olefin sulfonate: sodium alkene sulfonates and sodium hydroxy alkane sulfonate

Alkane sulfonates (PS and SAS) are mainly used in Europe in detergent products.
AOSs have been mainly used in Asia as surfactants for heavy- and light-duty laundry detergents, synthetic soap bars, and household products. Because they are less irritating than alkyl-aryl sulfonates, they have also been used in the United States in several personal products (liquid soaps, bubble baths, and shampoos) as alternatives to alcohol ether sulfates. They are also marginally used in oral care formulations.

Alkyl-aryl sulfonates. Today, LAS (linear alkylbenzene sulfonate) is the most important surfactant on a volume basis, but its use in personal care is very limited.

It is worth mentioning that some methyl or methyl-ethyl-substituted aryl sulfonates, i.e., sodium xylene, toluene, or cumene sulfonates (SXS, STS, or SCS, respectively), although not showing typical surfactant properties are used as hydrotropes (i.e., solubilizing agents, which decrease hydrophobic effects in aqueous systems).

$CH_3{-}(CH_2)_x{-}CH{-}(CH_2)_y{-}CH_3$ (CH bearing a benzene ring substituted with SO_3Na)

Sodium linear alkylbenzene sulfonate (LAS)

LAS is a very cost-effective surfactant that is extensively used in a broad variety of detergents for household, fabric care, institutional, and industrial products.

Because of its too high detersive action, LAS has a relatively low compatibility with skin and is only scarcely used in cosmetics except in some anti-seborrheic preparations.

Sulfosuccinates. Sulfosuccinates are the sodium salts of alkyl esters of sulfosuccinic acid; they generally result from the condensation of maleic anhydride with a fatty alcohol, followed by a sulfonation with sodium bisulfite $NaHSO_3$. Some variants of sulfosuccinates are derived from other substituted fatty molecules such as fatty alcohol ethoxylates, fatty amines (yielding sulfosuccinamates), or fatty alkanolamides.

$NaO_3S{-}CH(C(=O){-}OR){-}CH_2{-}C(=O){-}OR$

Sodium dialkyl sulfosuccinate

Monoesters disodium salts are the most common sulfosuccinates used in cosmetic applications. Monoesters of alkanolamines (sulfosuccinamates) are milder than monoesters of fatty alcohols (sulfosuccinates). Monoesters derived from ethoxylated alcohols or alkanolamides are extensively used in personal products and especially in shampoos; they are known for their mildness and skin irritation reduction when used in association with other anionic surfactants.

Sulfo fatty acid esters. These surfactants are sometimes known under their abbreviated names: FES, MES, and ASME, for fatty ester sulfonate, methyl ester sulfonate, and alpha sulfo (or α-sulfo) methyl ester, respectively. Most of α-sulfo fatty acid esters derive from fatty acid methyl esters.

$R{-}CH(SO_3Na){-}C(=O){-}O{-}CH_3$

Methyl ester of α-sulfc fatty acid, sodium salt.

$R{-}CH(SO_3Na){-}C(=O){-}O{-}R'$

Alkyl ester of α-sulfo fatty acid, sodium salt

α-Sulfo methyl ester surfactants deriving from C_{16} to C_{18} fatty acid (e.g., ASMT, the tallowate) are appropriate for use in laundry detergents. ASME is also used in the formulation of syndet bars (laundry bars based on synthetic surfactants).

To our knowledge, these surfactants are not used in personal care.

Fatty acid isethionates and taurides. Fatty acid isethionates are usually prepared by reaction of a fatty acid chloride with sodium isethionate ($HO{-}CH_2{-}CH_2{-}SO_3{-}Na$), itself resulting from the addition of sodium bisulfite to ethylene oxide (EO). These surfactants are insensitive to water hardness and show good wetting, foaming, and emulsifying properties. In addition, they are very mild and have excellent compatibility with the skin. Taurides (or taurates) are acyl–amino alkane sulfonates that have chemical structures close to isethionates. They can be used in association with other surfactants to increase the viscosity.

$R{-}C(=O){-}OCH_2CH_2{-}SO_3Na$

Fatty acid isethionate

$R{-}C(=O){-}N(CH_3){-}CH_2CH_2{-}SO_3Na$

Sodium methyl acyl tauride

Acyl isethionates have been used in shampoos and personal cleansers. They are also incorporated in syndet bars together with various soaps. The most currently used isethionate is the cocoyl isethionate.

Taurides (or taurates), which have properties similar to soaps (except the sensitivity to water hardness), have been extensively used in shampoos but are now replaced by AEOS. Today they are limitedly used in cosmetics mainly in foam baths and toilet bars.

Taurides are also used in soap bars especially designed for laundering with seawater, in agriculture, and in textile dying.

Phosphates Esters

This class of surfactants includes alkyl phosphates and alkyl ether phosphates.

$RO{-}P(=O)(OH){-}OH$

Alkyl phosphoric ester

$RO{-}P(=O)(OR){-}OH$

Dialkyl phosphoric ester

Phosphate esters as surfactants are especially useful in applications for which a particular tolerance to pH, heat, or electrolytes is required. They are also used in acidic cleaning products for household as well as industrial applications. Mild for the skin, alkyl phosphates sometimes enter the composition of facial and cleansing products.

Acyl–Amino Acids and Salts

Acyl glutamates. These surfactants are formed by acylation of a natural amino acid, the glutamic acid $HOOC{-}CH_2{-}CH_2{-}CH(NH_2){-}COOH$ (or α-aminoglutaric acid).

These surfactants are mild for the skin and the eyes, deliver improved skin feel, but are poor foamers.

$NaOOC{-}CH_2CH_2CH(NH{-}C(=O){-}R){-}COONa$

Sodium acyl glutamate

Acyl glutamates are mainly used in personal products such as shampoos.

Acyl peptides. These surfactants are formed from hydrolyzed proteins (e.g., animal collagen).

Depending on the protein hydrolysis process (chemical or enzymatic), the average polypeptide molecular weight can vary from about 350 to 2000, and some free amino acids may be present in the hydrolysate. An acylation reaction occurs on the amine terminal functions and, possibly, on some side groups (e.g., the hydroxyls) and thus leaves the carboxyl groups free, which must be neutralized.

Products containing such surfactants are prone to be contaminated by various germs and have to be properly preserved.

$R{-}C(=O){-}[NHCH(X)C(=O)]_x{-}NHCH(X)C(=O){-}ONa$

Sodium acyl polypeptide (X = amino acids side groups)

Acyl peptides are mild surfactants designed for the personal care area; they are especially used in shampoos owing to their substantivity on the keratin of hair and, therefore, they effectively deliver the expected benefits of conditioning agents.

Acyl sarcosides. Sarcosinates (or salts of acyl–amino acids) are the condensation products of fatty acids with *N*-methylglycine $CH_3{-}NH{-}CH_2{-}COOH$ (or sarcosine).

$R{-}C(=O){-}N(CH_3){-}CH_2COONa$

Sodium acyl sarcosinate

Sarcosinates are good surfactants for cosmetic use because of their mildness to skin, substantivity on skin and hairs when incorporated in formulations around neutral pH, conditioning action, and foaming resistance in the presence of soaps or sebum. Incorporated in shampoos with alkyl sulfates, they boost the lather.

Sarcosinates are also used as corrosion inhibitors.

Cationic Surfactants

From a very general standpoint, cationic surfactants differ from anionic and nonionic ones by the fact that they carry a positive charge. Their major interest in cosmetic industry resides in hair care; in this frame, they are used as hair conditioners and antistatic agents.

Cationics are also found in the personal care area as emulsifiers in some cosmetic preparations and as bactericidal agents.

Alkylamines

Primary, secondary, and tertiary alkyl amines, and more especially their salts, are included in this surfactant class.

$R-NH_2$ — Alkyl amine

$R-N(CH_3)_2$ — Dimethyl alkyl amine

$R-C(=O)-NH-(CH_2)_3-N(CH_3)_2$ — Alkylamido dimethy propylamine

Amines and their salts are mainly used in textile treatment and occasionally in rinse fabric softeners. Salts of amines are used in cosmetics together with other surfactants. Their usage is restricted to specialties; they exhibit conditioning and antistatic properties in hair care applications. Amidoamines are also used in cosmetic products.

Alkylimidazolines

Reaction of a fatty acid with a substituted ethylene diamine forms imidazoline. Heating the resulting amido-ethylamine yields the imidazoline with a five-member substituted ring.

The tertiary nitrogen atom can be quaternized.

R' = $CH_2CH_2NH_2$ ⇒ alkyl aminoethyl imidazoline

R' = CH_2CH_2OH ⇒ alkyl hydroxyethyl imidazoline

Imidazolines are cationic o/w emulsifiers.

Considered to be irritating, they are scarcely used in cosmetics as substantive hair-conditioning agents.

Quaternary Ammonium Compounds

Quaternary ammonium compounds form a class of surfactants that contain a positively charged nitrogen atom linked to four alkyl or aryl substituents.

The positive charge is permanent, regardless of pH.

Tetra alkyl(-aryl) ammonium salts. Tetra alkyl ammonium salts have the structure $[R_1R_2R_3R_4N^+]$ X^- where R_1, R_2, R_3, and R_4 are alkyl or aryl groups and X^- represents an anion. The water solubility of quaternaries mainly depends on the nature of R substituents.

Low-solubility quaternaries can adsorb on various substrates and impart various useful conditioning effects (softening, antistat, corrosion inhibition, etc.).

With the exception of *N*-alkyltrimethyl ammonium salts, quaternary surfactants usually show poor detergency, wetting, and emulsifying capacities. Quaternaries are generally not compatible with anionics because of the formation of water-insoluble complexes.

$$\left[R-N^+(CH_3)_2-R' \right] X^-$$

Quaternary compound

The major usage of quarternaries is related to their ability to adsorb on natural or synthetic substrates and fibers. They are widely used as softening agents in rinse fabric softeners.

Their softening and antistatic properties are similarly exploited in hair-conditioning shampoos or after-shampooing rinses.

It is worth to note that, in cosmetic applications, quaternaries may cause ocular and local irritation. Among quaternaries, some are used as germicides and disinfectants (e.g., didecyl dimethyl ammonium chloride and benzalkonium chloride).

Heterocyclic ammonium salts. Heterocyclic quaternaries are derived from heterocyclic aliphatic or aromatic compounds in which a nitrogen atom constitutive of the cycle is quaternized.

$CH_2CH_2NHCOC_{17}H_{35}$; $C_{17}H_{35}$; $N-CH_3$; N ; $CH_3OSO_3^-$

Imidazolinium quaternary compound

The quaternaries derived from imidazoline and morpholine are used as hair conditioners and antistatic agents. Those derived from aromatic heterocycles are used as germicides.

Alkyl betaines. Alkyl betaines, which are *N*-trialkyl derivatives of amino acids ($[R_1R_2R_3]$ N^+CH_2COOH), are classified as cationics because they exhibit a permanent positive charge. Because they also have a functional group able to carry a negative charge in neutral and alkaline pH conditions, they are often regarded, although this position is questionable, as amphoterics.

The positive charge is always carried by a quaternized nitrogen, while the anionic site can be a carboxylate (betaine), a sulfate (sulfobetaine or sultaine), or a phosphate (phosphobetaine or phostaine).

Betaines are good foaming, wetting, and emulsifying surfactants, especially in the presence of anionics. Alkylamido betaines deliver more stable foam and are better viscosifiers than alkyl dimethyl betaines. Betaines are compatible with other surfactants, and they frequently form mixed micelles; these mixtures often deliver unique properties that are not found in the individual constitutive surfactants.

Betaines have low eye irritation and skin irritation; moreover, the presence of betaines is known to decrease the irritation effect of anionics.

$$R-N^+(CH_3)_2-CH_2COO^-$$

Alkyl dimethyl betaine

$$R-C(=O)-NH-(CH_2)_3-N^+(CH_3)_2-CH_2CH(OH)CH_2SO_3^-$$

Alkylamidopropyl hydroxysultaine

Because of their ability to improve the skin's tolerance against irritating anionic surfactants, and also, because of their high price, betaines are usually used in association with other surfactants.

Betaines are especially suitable in personal care applications (e.g., shampoos, foam baths, liquid soaps, shower gels), fabric hand wash products, and dishwashing products.

Quaternized APG. These surfactants, derived from natural sources, are recently available. They are made of alkyl polyglycoside with a cationic backbone. These very mild quaternary compounds are substantive to the skin and hair and provide a soft after feel. Thanks to the sugar moiety, they show low irritation potential, and therefore, they are particularly suitable

for personal care formulations. A major benefit is a longer-lasting effect associated with a reduction of irritation compared with traditional quaternary surfactants.

Some potential applications reside in hair care products (e.g., in wet combing benefit, flyaway hair control), w/o cationic emulsions, formulations in the presence of anionics, foam building in shampoo formulations, or surfactants for baby wipes.

Ethoxylated Alkylamines

These surfactants can be considered as cationic or nonionic depending on the degree of ethoxylation and the pH at which they are used. Polyethoxylated amines are formed by ethoxylation of primary or secondary fatty amines.

$$C_{12}H_{25}N[(CH_2CH_2O)_3H]_2$$

Laurylamine + 6 EO(or POE 6)

$$R{-}N(CH_2CH_2O)_xH{-}CH_2CH_2CH_2N[(CH_2CH_2O)_yH][(CH_2CH_2O)_zH]$$

Alkyl propanediamine ethoxylate

The ethoxylated alkylamines have various application fields; they are generally exploited for their capacity of adsorbing on surfaces.

In personal care, ethoxylated alkylamines are used as emulsifiers and hair-conditioning agents.

Ethoxylated amidoamines find applications in rinse fabric softeners.

Esterified Quaternaries

Esterified quaternaries (or esterquats) are produced by the esterification of the hydroxyl group (s) of secondary or tertiary amino-alcohols with selected fatty acids.

$$\left[H_3C{-}N^{\oplus}(HO{-}H_2C{-}H_2C)(CH_2{-}CH_2{-}O{-}C(=O){-}R)_2 \right] MeSO_4^{\ominus}$$

Esterquat: *N*-Methyl-*N*,*N*-bis[$C_{16/18}$-acyloxy)ethyl]-*N*-(2-hydroxyethyl)ammonium-methosulfatesalt

The esterquats are suitable substitutes for straight quaternaries; they present improved environmental profile and comparable softening properties compared with straight quaternaries.

Amphoteric Surfactants

Amphoteric surfactants are characterized by the fact that these surfactants can carry both a positive charge on a cationic site and a negative charge on an anionic site. The use of amphoteric terminology is still more restrictive: the charge of the molecule must change with pH, showing a zwitterionic form at intermediate pH (i.e., around the isoelectric point).

The surfactant properties are accordingly influenced by pH: around the isoelectric point, the zwitterionic form takes place, exhibiting the lowest solubility; in alkaline conditions, the anionic form is predominant, delivering foam and detergency; and in acidic conditions, the cationic form prevails, providing surfactant substantivity.

Although betaines are commonly classified among amphoterics, this classification is improper because these surfactants never exhibit in single anionic form.

Amphoteric surfactants are generally used as secondary tensioactives for their foam-stabilizing effect, their thickening capacity, and their skin irritation reduction capacity on alkyl sulfates and alkyl ethoxy sulfates.

Acyl Ethylenediamines and Derivatives

These surfactants are made by the reaction of an alkyl imidazoline with chloroacetic acid (yielding amphoglycinates) or with acrylic acid (yielding amphopropionates).

$$R-C(=O)-NH-CH_2CH_2-N(CH_2COONa)-CH_2CH_2OH$$

Acylamphoacetate

$$R-C(=O)-NH-CH_2CH_2-N(CH_2CH_2COONa)-CH_2CH_2OCH_2CH_2COONa$$

Acylamphodipropionate

Amphoterics of this class are mainly used in personal products (e.g., coco amphocarboxy glycinate). Incorporated in baby shampoos, they reduce eye irritation.

Other applications are fabric softeners, industrial cleaners, and car cleaners.

N-Alkyl Amino Acids or Imino Diacids

These molecules are chemical derivatives of amino acids that can be produced by the reaction of chloroacetic acid or acrylic acid with an alkyl amine.

Their compatibility with other surfactants is excellent.

These surfactants are good emulsifiers and show optimal wetting and detergency under alkaline pH. They are good foamers at neutral and alkaline pH but lose their foaming properties under acidic conditions.

They are substantive to surfaces and provide antistatic effects.

They provide skin and eye irritancy reduction in combination with anionics.

$$R-NH-CH_2CH_2-COOH$$

Alkyl aminopropionic acid

$$C_{12}H_{25}-NH-CH_2-COONa$$

Sodium coco glycinate

$$R-N(CH_2CH_2CH_2-NH_2)-CH(COOH)CH_2CH_2-C(=O)-NH_2$$

Aminopropyl alkylglutamide

$$R-N(CH_2CH_2-COONa)(CH_2CH_2-COONa)$$

Sodium alkyliminodipropionate

Amphoterics of this class are mainly used in personal products.

Polycarboxylates deliver reduced eye irritation and provide hair-conditioning benefits. Their zwitterionic forms are substantive on the hairs.

NONIONIC SURFACTANTS

Nonionic surfactants do not dissociate into ions in aqueous medium. They generally deliver a weak to moderate foam. They are appreciated for their good skin and eye compatibility as well as for their anti-irritant potential when they are combined with anionics in appropriate concentration ratio. Therefore numerous products for sensitive skin, babies, or the face incorporate nonionics as major surfactant.

Fatty Alcohols

Fatty alcohols are primarily used as a chemical precursor for the production of several other surfactants.

$$R-CH_2-OH$$

Fatty alcohol

Because they are not water soluble, the use of fatty alcohols is very limited in liquid products. They are mainly used as opacifiers, thickening agents, and foam depressors (e.g., lauric alcohol).

Ethers

Alkoxylated Alcohols

This class of surfactants mainly covers ethoxylated or propoxylated alcohols.

Ethoxylated alcohols (also called "polyethyleneglycol ethers" or "PEG ethers") are produced from the reaction of fatty alcohols with EO.

Similarly, propoxylated alcohols (also called "polypropyleneglycol ethers" or "PPG ethers") are obtained with propylene oxide (PO).

The hydrophilic-lipophilic balance (HLB) of ethoxylated alcohols can be adjusted by properly balancing the hydrophilic ethoxylated chain and the hydrophobic fatty chain.

Ethoxylate nonionics are compatible with all surfactants. Some beneficial associations with ionic surfactants are often shown.

In the frame of personal care applications, ethoxylated alcohols often result from the transformation of natural lipids. The nomenclature specific to cosmetic chemicals [i.e., international nomenclature of cosmetic ingredients (INCI) names[a]] is applied to these nonionics: they are denominated by using the root of the fatty acid name terminated by the suffix "-eth" (contraction of "ethoxylated"), directly followed by the ethoxylation degree (e.g., laureth-4, oleth-5, and myristeth-7).

As some raw materials yield on hydrolysis various fatty chain lengths, the names of the derived nonionics are either drawn from the natural source (e.g., laneth-16 for a lanolin-derived nonionic) or from the combined abbreviations of the constitutive fatty chains (e.g., ceteareth-20 for a combination of cetyl and stearyl).

$$CH_3(CH_2)_x—O—(CH_2CH_2O)_nH$$

Alkyl polyethyleneglycol ether or alcohol ethoxylate

(e.g., laureth 20 for $x = 11$ and $n = 20$)

$$CH_3-\left[CH_2\right]_x\left[O-\underset{\displaystyle CH_3}{\underset{|}{C}}HCH_2\right]_y\left[OCH_2CH_2\right]_z OH$$

EO/PO alkyl ether

(e.g., propyleneglycol capreth-4 for $x = 9$, $y = 1$, and $z = 4$)

Applications of ethoxylated alcohols are numerous in industrial as well as in household products.

When properly selected, alkoxylated alcohols are also useful for personal products as good emulsifiers and solubilizers. The cosmetic applications remain, however, limited because of their rather weak foaming capacity.

Because they are prone to undergo degradation by oxidation, the following precautions can greatly improve the stability of ethoxylate nonionics: storage in the dark, minimal air contact, low-temperature storage, avoiding storage of diluted products, and the addition of an antioxidant.

EO/PO Block Polymers

These polymeric surfactants have some similarity with the previously discussed alkoxylated alcohols. They consist in the combination of the assembly of PPG (hydrophobic part) and PEG chains (hydrophilic part). Such surfactants are known under the denomination "poloxamers" (INCI name) and are called EO/PO block copolymer nonionics.

A major property of EO/PO nonionics is their low-foaming profile.

As straight EO nonionics, EO/PO copolymers exhibit the cloud point phenomena.

[a]The International Cosmetic Ingredient Dictionary provides a nomenclature of conventional names for cosmetic ingredients that are defined by the CTFA (The Cosmetic, Toiletry, and Fragrance Association).

EO/PO nonionics are also mild surfactants.

$$HO(CH_2CH_2O)_x\left[\begin{array}{c}CHCH_2O\\ |\\ CH_3\end{array}\right]_y(CH_2CH_2O)_zH$$

Ethoxylated PPG ether

These surfactants are especially useful for applications in which foaming must be significantly depressed, such as automatic dishwashing detergents, laundry detergents, and rinse aids.

Owing to their mildness, EO/PO block polymers also find applications in cosmetic products. They are generally used as emulsifying, solubilizing, or fluidizing agents.

APGs

Alkyl polyglycosides are most often known by the simple abbreviation APGs.

APGs are produced by the alkylation of short-chain glucosides resulting from acidic alcoholysis of polysaccharides such as starch. Commercial products consist of mixtures of mono-, di-, and triglucosides. Accordingly the glucosidic average chain length varies between 1.2 and 3 glucose units, depending on the production conditions.

Surfactants of this class are good emulsifiers and provide good wetting and foam profile.

APGs are compatible with all other surfactants. They show good chemical stability at neutral and alkaline pH, and are impaired under acidic conditions (pH < 5).

Alkylpolyglucoside

APGs are used in detergents and personal care cleansers (e.g., shampoos). They are claimed to be very mild for skin as well as to reduce the skin irritation potential of anionics. Additionally, they impart an excellent skin feel.

Their thickening effect in the presence of anionics and their foam stabilization capacity are also exploited in personal care applications.

APGs made from fully natural and renewable sources are now available, the glycosidic chain being made from corn, potato, or wheat starch and the alkyl chain from coconut and/or palm oil. Such surfactants are readily biodegradable and approved for eco labels (e.g., EU Flower, Nordic Swan, and Bra Miljöval).

Ethoxylated Oils and Fats

This class of surfactants essentially covers ethoxylated derivatives of lanolin (i.e., aliphatic alcohols and sterols and fractionation products of wool fat) and of castor oil (i.e., fatty acids with a high ricinoleic acid fraction extracted from ricinus seeds).

$$CH_3(CH_2)_5\underset{\displaystyle O-(CH_2CH_2O)_x-H}{\underset{|}{C}}HCH_2CH{=}CH(CH_2)_7-\overset{\displaystyle O}{\overset{\|}{C}}-O-(CH_2CH_2O)_y-H$$

PEG castor oil derivative

Ethoxylated products of lanolin and castor oil are good and excellent emulsifiers, respectively. These surfactants are mainly used in the cosmetic industry; their major interest is to allow claims based on the natural origin of the constitutive surfactant systems.

Alkanolamides

Straight Alkanolamides

Alkanolamides are *N*-acyl derivatives of monoethanolamine and diethanolamine.

$RCONHCH_2CH_2OH$ $\quad$ $RCON(CH_2CH_2OH)_2$ $\quad$ $RCON(CH_2CH_2OCOR)(CH_2CH_2OH)$

Monoalkanolamide dialkanol amide ester-amide

Alkanolamides have been largely used in household detergent products; their consumption has now significantly declined because of the extensive use of alkyl ethoxylated detergent products.

Because of their foam boosting and viscosity-enhancing capacity in the presence of anionics, alkanolamides are also usefully incorporated in personal care, especially in shampoos.

Ethoxylated Alkanolamides

Reaction of an alkanolamide with EO leads to an ethoxylated amide.

$RCONH(CH_2CH_2O)_nH$

Polyethoxylated monoalkanolamide

It is more expensive than its corresponding ethoxylated alcohol and has therefore restricted usage. The benefits of thickening, foam stabilization, and dispersibility are exploited in personal care cleansers.

Esters

In this surfactant class, there are five major subcategories to be considered:

1. Ethoxylated fatty acids
2. Glycol esters, glycerol esters, and ethoxylated derivatives
3. Sorbitan esters and ethoxylated derivatives
4. Alkyl carbohydrates esters
5. Triesters of phosphoric acid

Ethoxylated Fatty Acids

This class of surfactants comprises mono- and diester that result from the reaction of fatty acids with either EO or polyethylene glycol.

$$R-C(=O)-O-(CH_2CH_2O)_n-H$$

PEG fatty acid ester

$$R-C(=O)-O-(CH_2CH_2O)_n-C(=O)-R$$

PEG fatty acid diester

Given their outstanding emulsifying properties, ethoxylated fatty acids are useful in domestic and industrial detergents, more especially in degreasing compositions.

If properly balanced, combinations of esters with low and high ethoxylation provide excellent emulsifiers for creams and lotions. They are also used as mild cleaners or viscosifying agents (e.g., PEG-150-distearate).

In cosmetics (shampoos), less water-soluble grade (i.e., ethylene glycol monostearate) is used as a pearlescent agent.

Glycol Esters, Glycerol Esters, and Ethoxylated Derivatives

A common point among the surfactants grouped in this class and the following two classes (sorbitan esters and alkyl carbohydrates esters) is that they all derive from the condensation reaction of a polyhydroxyl compound (e.g., glycol, glycerol, sorbitol, sucrose) with a fatty acid. Some of them can be directly extracted from natural sources.

The resulting esters can be additionally ethoxylated to increase their HLB value and, thereby, their solubility in water.

These surfactants show poorer wetting and foaming properties in comparison with alcohol-derived nonionics. Emulsifying properties are excellent.

In general, esters and lower ethoxylates are appropriate for w/o dispersions, whereas higher ethoxylates are more suitable emulsifiers for o/w dispersions.

$R-C(=O)-OCH_2CH_2OH$ Ethylene glycol ester

$R-C(=O)-OCH_2CH(CH_3)-OH$ Propylene glycol ester

Monoglyceride

1.3-diglyceride

Polyethoxylated 1.3-diglyceride

$HOCH_2CH(OH)CH_2O(CH_2CH(OH)CH_2O)_nCH_2CH(OH)CH_2O-C(=O)-R$ Polyglyceryl monoester

Because of their high compatibility, these surfactants are widely used in the cosmetic and food industry.

Glycol and glycerol esters are used in the pharmaceutical and cosmetic industries either as emulsifying agents or as oily compounds, refatting agents, emollients and skin conditioners in various products such as creams, lotions, ointments, and gels.

Stearate derivatives also deliver thickening and opacifying properties (e.g., the glyceryl stearate). Some of them are also used as pearlescent agents (i.e., glycol stearate and distearate).

Ethoxylated derivatives are used as solubilizing agents, emulsifiers, and even as emollients. Some show effective thickening effect when combined with other surfactants (e.g., PEG-200 glyceryl stearate)

Sorbitan and Sorbitol Esters and Ethoxylated Derivatives

Sorbitan molecule is generated from the dehydration of the sorbitol molecule, which results in an internal ether bond.

Sorbitol $\xrightarrow{-H_2O}$ 1,4-Sorbitan

Sorbitol and sorbitan esters are obtained by acylation of hydroxyl groups, using most frequently natural fatty acids such as lauric, palmitic, stearic, or oleic. These surfactants can be optionally ethoxylated. Acylation (or ethoxylation) can occur on almost all hydroxyl groups present in the original polyol molecule.

1,4-Sorbitan monoester   1,4-Sorbitan triester   Polyethoxylated Sorbitan monoester

The field of application of sorbitan esters and their ethoxylated derivatives is identical to the one of glycol and glycerol esters (see the sect. "Glycol Esters, Glycerol Esters, and Ethoxylated Derivatives").

The sorbitol esters with a higher degree of ethoxylation (e.g., sorbitol septaoleate 40 EO) are also used as spreading aids in emollient bath oils.

Alkyl Carbohydrates Esters

Surfactants of this class are better known as "sugar esters" or "sucrose esters."

The sucrose esters are obtained by transesterification of sucrose with fatty acid methyl esters or triglycerides.

Surfactants of this class are good emulsifiers.

Of great interest about such surfactants is their natural origin and good biodegradability.

It is worth noting that some glucosides surfactants, e.g., the so-called *saponins*, are already present in nature and directly available from vegetal sources.

Saccharose fatty acid monoester

Sucrose esters are food grade ingredients and have similar uses as the previously described glycol, glycerol, and sorbitan esters in the food and cosmetic industries.

They are very mild surfactants and can be used as emulsifiers or as cleansing agents with emollient properties.

Amine Oxides

Amine oxides are produced by the oxidation of tertiary amines using a 35% hydrogen peroxide solution as the oxidizing agent.

Amine oxides remain mainly nonionic in neutral and alkaline conditions (pH >7) but can become weakly cationic under acidic conditions.

In current amine oxides, the initial reactive are alkyl dimethyl amines with chain lengths ranging from C_{12} to C_{18}.

Amine oxides are compatible with all other surfactants.

Amine oxides are also known to increase the skin compatibility of detergent products.

A small amount of amine oxide increases the cloud point of nonionics.

$$R{-}N(CH_3)_2 + H_2O_2 \xrightarrow{60{-}80^{\circ}C} R{-}N(CH_3)_2{\rightarrow}O + H_2O_2$$

Incorporated in shampoos, amine oxides contribute to impart viscosity, reduce eye and skin irritancy, and enhance foam properties (more creamy). They are especially suitable in slightly acidic or neutral formulas.

NON-HYDROCARBON SPECIALTY SURFACTANTS

Alkoxylated Polysiloxanes

Surfactants, which can be classified in the chemical group of organosilicones, are structurally derived from polydimethylsiloxanes in which some methyl are replaced by hydrophilic groups that can be of anionic, cationic, or nonionic nature.

The nonionic derivatives are mostly represented by the polyether-polydimethylsiloxane copolymers.

The general structure of these surfactants is illustrated below. The hydrophilic chain(s) generally contains EO/PO block copolymers.

$$(CH_3)_3Si{-}O{-}\left[Si(CH_3)_2{-}O\right]_m{-}\left[Si(CH_3)\left((CH_2)_p{-}O{-}(C_2H_4O)_x{-}(C_3H_6O)_y{-}H\right){-}O\right]_n{-}Si(CH_3)_3$$

Polysiloxan-polyether copolymer (*p* generally equals 0 or 3)

These surfactants are specialty ingredients and are used in very different fields (e.g., painting, foam control, phytosanitary products).

They are also used in cosmetics and hair care:

- In cosmetic or personal care products as emulsifiers (e.g., in protective creams, hydrating body milks, liquid soaps, and shave creams) and
- In hair care products (e.g., shampoos, conditioners, gels, lotions, and foams) to act as combing out auxiliaries, reduce the irritancy of surfactant system, provide improved skin feel, or control the foam. The CTFA-adopted name of these surfactants is dimethicone copolyol.

Fluorosurfactants

Fluorosurfactants form a distinct group of surfactants besides the conventional surfactants based on hydrocarbon chains.

Fluorosurfactants differ from hydrocarbon surfactants by the hydrophobic moiety of the molecule, which is made of perfluoroalkyls chains $F{-}(CF_2{-}CF_2)_n{-}$, in which n ranges from about 3 to 8.

As for conventional surfactants, a broad variety of hydrophilic functional groups (e.g., ethoxylated chains, sulfonates, quaternaries, and betaines) can be grafted on fluorosurfactants.

Depending on their nature, these surfactants show variable emulsifying and foaming characteristics.

Although fluorosurfactants have some potential prospects in personal care (e.g., improved hair conditioning), we are not aware of any significant application in this field. We can, however, report their use in barrier creams that require good spreading and stable o/w emulsions.

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72 Encapsulation to Deliver Topical Actives

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INTRODUCTION

The insertion of active ingredients into protective boundaries that can isolate, disperse, mobilize, enhance activity, and transport these ingredients in different barriers has been and is a very important tool for the cosmetics industry.

The effects of encapsulation on the performance of ingredients can be rated by the following criteria:

- Transport them across biological media,
- Increase their active lifetime,
- Improve ingredients' solubility in desired media,
- Protect the body from possible poisonous side effects of ingredients, and
- Concentrate active ingredients in the desired biological target area.

Various encapsulation systems have been studied successfully in the past 30 years, with the aim of controlling the drug release and improving the efficacy and selectivity of the formulations. Among the techniques, nanotechnology is one of the most prominent factors of the scientific revolution we are witnessing. Good reviews, including topical applications, are made in literature (1–6). As for biotechnology, nanotechnology is the outcome of an interdisciplinary, new approach to old technological issues ranging from device manufacturing to drug design.

Nanotechnology is considered by many as the logical step in science, integrating engineering with biology, chemistry, and physics (7). Nanometric systems have a great surface area, which renders them highly satisfactory for application on the skin (8). In the last couple of years, the term "nanotechnology" has been inflated and has almost become synonymous for things that are innovative and highly promising. On the other hand, it is also the subject of considerable debate regarding the open question on toxicological and environmental impact (9,10).

The various nano formulations currently being used in cosmetics are

- nanoemulsions (submicron-sized emulsions)
- nanosuspensions (submicron-sized suspensions)
- nanospheres (drug nanoparticles in polymer matrix)
- nanotubes (sequence of C60 atoms arranged in a long thin cylindrical structure)
- nanopowders
- nanocapsules (encapsulated drug nanoparticles)
- lipid nanoparticles (lipid monolayer enclosing a solid lipid core)
- dendrimers (nanoscale three-dimensional macromolecules of polymer)

In this chapter, an overview of various methods of encapsulation including microparticles, nanotechnology, vesicles and special emulsions are given, as well as their advantages and limitations for topical applications.

MICROPARTICLES

Microencapsulation is a process in which very thin coatings of inert natural or synthetic polymeric materials are deposited around micro-sized particles of solids or droplets of liquids.

Commercial microparticles typically have a diameter between 1 and 1000 μm and contain 10 to 90 wt.% core. Most capsule shell materials are organic polymers, but fat and wax are also used. Various types of physical structure of the product of microencapsulation such as mononuclear spheres, multinuclear spheres, multinuclear irregular particles, etc., can be obtained depending on the manufacturing process.

A thorough description of the formation of microparticles is given by several reviews (11–14).

The efficacy of microparticles into the human skin depends on the size and the type of the formulation with which they are topically applied. Microparticles with a diameter of >1 μm do not penetrate the human skin. They are located on the skin surface and form a film, which can be used for protection against ultraviolet (UV) radiation in sunscreens, among other examples. While the penetration of the microparticles in the lipid layers of the stratum corneum is limited, they penetrate efficiently into the hair follicles up to a depth of >2 μm (15,16).

Concerning microsponge systems, each microsphere is composed of thousands of small beads wrapped together to form a microscopic sphere capable of binding, suspending, or entrapping a range of substances.

Skin absorption of benzoyl peroxide from a topical lotion containing freely dispersed drug was compared with that of the same lotion in which the drug was entrapped in a controlled-release styrene-divinylbenzene polymer system (Microsponge®). The studies done by Wester et al. (17) showed (*i*) in vivo, less benzoyl peroxide was absorbed through rhesus monkey skin from the polymeric system, (*ii*) reduced skin irritation in cumulative irritancy studies in rabbits and human, and (*iii*) when the experimental formulations were evaluated for antimicrobial activity in vivo, their efficacy was in line with that of conventional products.

Jelvehgari et al. (18) showed that the drug-polymer ratio influenced the particle size and drug release behavior of microsponges. An increase in the ratio resulted in a reduction of the release rate of benzoyl peroxide attributed to the microsponge's decreased internal porosity.

The cidofovir distribution in porcine skin, after topical application of microparticles and drug solution, was determined by horizontal slicing of the skin. The amount of cidofovir found in the epidermis was higher with microparticles than with the control solution, and the quantity retained decreased with depth (19).

Scalia et al. (20) loaded lipid microparticles with the sunscreen agent 4-methylbenzylidene camphor (4-MBC). The influence of the microparticle's carrier system on percutaneous penetration was evaluated after its introduction in a topical formulation (emulsion). The amount of sunscreen penetrating the stratum corneum was greater for the emulsion containing nonencapsulated 4-MBC compared with the formulation with the sunscreen-loaded microparticles.

Lipid microparticles loaded with butyl methoxydibenzoylmethane (BMDBM) were prepared and evaluated for skin permeation in vivo and in vitro. Following in vivo human skin application of an oil-in-water (O/W) emulsion containing 2% of BMDBM loaded in lipospheres, 15% of the applied sunscreen was found accumulated in the uppermost layers of the stratum corneum (21).

On the basis of this literature, it can be concluded that the general release of active substances from microparticulate systems is directly or indirectly related to some factors:

- The presence of pores in the microparticulate system; a good example is provided by microsponges.
- Particle size: particles with >1 μm are retained in the skin surface or deposited on the surface of the hair follicles.
- Intrinsic characteristics of delivery systems being directly related to the process and materials used.

NANOPARTICLES

Nanoparticles can be defined as submicron (<1 μm) colloidal systems, generally, but not necessarily made of polymers (biodegradable or not). According to the preparation process used, nanocapsules or nanospheres can be obtained. Nanocapsules are vesicular systems in

which the drug is confined to a cavity surrounded by a unique polymeric membrane. Nanospheres are matrix systems in which the drug is dispersed throughout the particles.

There are many methods for preparing nanoparticles, whereas the most common methods are the required polymerization reaction or from a preformed polymer.

Good reviews with methods of preparation for nanoparticles can be found in the literature (6,22–25). Other reviews (26,27) reports that the system of solid lipid nanoparticles (SLNs) is one of the most attractive systems for encapsulating cosmetic ingredients.

Drug release from colloidal carriers is dependent on both the type of carrier and the loading mechanisms involved.

Nanospheres

Release from nanospheres may be different according to the drug entrapment mechanism involved. When the drug is superficially adsorbed, the release mechanism can be described as a partitioning process (rapid and total release if sink conditions are met). When the drug is entrapped within the matrix, diffusion plus bioerosion will be involved with a biodegradable carrier, whereas diffusion will be the only mechanism if the carrier is not biodegradable. The entrapment rate within the matrix of nanospheres may lead to a sustained release, which may be related to the polymer's biodegradation rate.

Nanocapsules

Release from nanocapsules is related to partitioning processes within immiscible phases. The equilibrium between the carrier (loaded drug) and the dispersing aqueous medium (free drug) is dependent both on the partition coefficient of the molecule between the oily and aqueous phases, and on the volume ratio of these two phases. This means that the amount released is directly related to the dilution of the carrier and that release is practically instantaneous when sink conditions exist.

Because of the increasing use of nanotechnology, the number of papers on topical nanoparticles has increased proportionally. Following is a short summary of some of these works.

Shim et al. (28) evaluated the effect of size of self-assembled nanoparticles on skin penetration of minoxidil in vitro and in vivo. Self-assembled 40 and 130 nm nanoparticles were prepared with poly (caprolactone)-block-poly(ethyleneglycol) and applied onto the skin of both hairy and hairless guinea pigs in the Franz diffusion cell. In hairy guinea pig skin, the permeation of the minoxidil incorporated in 40 nm nanoparticles was 1.5-fold higher in the epidermal layer and 1.7-fold higher in the receptor solution than that of 130 nm nanoparticles.

Lombardi et al. (29) describe techniques to characterize drug loading to carrier systems and skin penetration profiles by using the lipophilic dye nile red as a model agent. Nile red was incorporated into the lipid matrix of SLNs and nanostructured lipid. Nile red concentrations were followed by image analysis of vertical sections of pigskin treated with dye-loaded nanoparticular dispersions and an O/W cream for four and eight hour in vitro. Following the SLNs dispersions, dye penetration increased about over fourfold. Nanostructured lipid carriers turned out to be less potent (<3-fold increase), and penetration appeared even more reduced when applying nanoemulsions.

Trimethylpsoralen (TMP) permeates moderately the skin barrier. Three formulations were performed. Each form (liposomes, nanospheres, and EtOH solution) contained 0.05% of TMP. The results indicated that the controlled release of TMP by incorporation into PLG nanospheres [poly (DL-lactide-co-glycolide)] may increase drug content in the skin, while maintaining a minimal percutaneous absorption (30).

The nature of the vehicle used can enhance or block the percutaneous absorption of UV filters. Luppi et al. (31) suggested that polymeric nanoparticles hydrophobically modified (polyvinyl alcohol with fatty acids) were able to prevent benzophenone-3 movement toward the skin. Nanoparticles prepared with a high degree of substitution (fatty acids) prevented benzophenone-3 percutaneous absorption.

Santos Maia et al. (32) incorporated prednicarbate [(PC), 0.25%] into SLNs of various compositions. Conventional PC cream of 0.25% and ointment served as reference. Local tolerability as well as drug penetration and metabolism were studied in excised human skin and reconstructed epidermis. The drug recovery from the acceptor medium was about 2% of the applied amount following PC cream and ointment, but 6.65% following nanoparticle dispersion.

The influence of cross-linked pullulan nanoparticles on human dermal fibroblasts in vitro has been assessed in terms of cell adhesion, cytotoxicity, and light microscopy. Results from cell adhesion/viability assay suggest that the pullulan nanoparticles are nontoxic to cells and do not cause any distinct harm to cells. Fibroblasts were healthy and maintained their morphology and adhesion capacity (33).

Wissing (34) showed that the crystalline cetylpalmitate in SLNs has the ability of reflecting and scattering UV radiation on their own, thus leading to photoprotection without the need for molecular sunscreens. The photoprotective effect after the incorporation of the molecular sunscreen 2-hydroxy-4-methoxybenzophenone (Eusolex® 4360) into the SLN dispersion was increased threefold compared with a reference emulsion. Further, film formation on the skin was investigated by scanning electron microscopy, showing particle fusion due to water evaporation and formation of a dense film.

In another study (35), the same team showed the influence of the carrier on the release rate in vitro. It could be decreased by up to 50% with the SLN formulation. In vivo, penetration of oxybenzone into stratum corneum on the forearm was investigated by the tape-stripping method. It showed that the rate of release is strongly dependent upon formulation and could be decreased by 30% to 60% in SLN formulations.

SLN dispersions with different crystallinity indices of the lipid matrix were produced. Their characterization and occlusion factor were determined after 6, 24, and 48 hours. It was shown that the occlusion factor depends strongly on the degree of the crystallinity of the lipid matrix (36).

The actual sunscreens contain nanoparticles of titanium dioxide (TiO_2) or zinc oxide (ZnO), which are colorless and reflect UV. Most available data suggest that insoluble nanoparticles do not penetrate human skin. In vitro cytotoxicity studies on TiO_2 report uptake by cells, oxidative cell damage, or genotoxicity that should be interpreted with caution, since such toxicities may be secondary to phagocytosis of the cells exposed to high concentrations of insoluble particles.

There is little evidence that smaller particles have greater effects on the skin. Overall, the current knowledge suggests that nanomaterials such as nano-sized vesicles or TiO_2 and ZnO nanoparticles currently used in cosmetic preparations or sunscreens pose no risk to human skin or health, although other nanoparticles may have properties that warrant safety evaluation on a case-by-case basis before human use (37).

Other nanoscale materials such as carbon *fullerene* have been used in some cosmetic products because of their antioxidative properties. Bianco et al. (38) showed a good review on functionalized carbon nanotubes that are emerging as new tools in the field of nanobiotechnology and nanomedicine. They can be easily manipulated and modified by encapsulation with biopolymers or by covalent linking of solubilizing groups to the external walls and tips.

Certain chemical forms of fullerenes have been reported to elicit oxidative damage to cell in culture experiments (39). Adverse effects have not been reported following the application of fullerenes in formulations such as topically applied lotions or creams. Two studies are often cited about fullerenes, Oberdorster (40) and Zhu et al. (41) reported toxicity in many aquatic species treated with fullerenes in water. It is important to mention that the toxicological potential of nanoscale materials such as fullerenes can be evaluated through current safety evaluation processes, and these materials should be only used in products once their safety is confirmed.

Dendrimers are artificial macromolecules, which have the structure of a tree. They are hyperbranched and monodispersed three-dimensional molecules with defined molecular weights, large numbers of functional groups on the surface, and well-established host-guest entrapment properties. Dendrimers may be engineered to meet the specific needs of biologically active agents, which can either be encapsulated within dendrimers or chemically attached to these units. Recently, dendrimers have successfully proved themselves as promising nanocarriers for drug delivery because they can render drug molecules a greater water solubility, bioavailability, and biocompatibility (42,43).

Finally, *DNA vaccines* have been shown to elicit both broad humoral and cellular immune responses. Recent human clinical studies with needle-free injection devices and the gene gun have validated the direct targeting concept of dendritic cells (Langerhan's cells) in the viable epidermis of the skin. Cui and Mumper (44) showed that several different chitosan-based nanoparticles containing plasmide DNA (pDNA) resulted in both quantifiable levels of luciferase expression in mouse skin 24 hours after topical application as well as significant

antigen-specific Immunoglobulin G (IgG) titers at 28 days. In another study (45), the same authors showed that pDNA-coated nanoparticles, especially the mannan-coated pDNA nanoparticles with DOPE (dioleoyl phosphatidyl ethanolamine), resulted in significant enhancement in both antigen-specific IgG titers (16 fold) and splenocyte proliferation over "naked" pDNA alone.

Regarding the action mode of nanoparticles, we can say that they are associated with the skin surface, facilitating drug transport by changing the vehicle/stratum corneum partition coefficient.

NANOEMULSIONS

The main characteristic of nanoemulsions is the droplet size that must be inferior to 1 μm. Usually, the average droplet size is between 100 and 500 nm. The particles can exist as water-in-oil (W/O) and O/W forms where the core of the particle is either water or oil, respectively. The terms "submicron emulsion" (SME) and "miniemulsion" are used as synonyms. Usually, nanoemulsions contain 10% to 20% oil stabilized with 0.5% to 2% of an emulsifyng agent.

Emulsions prepared by use of conventional apparatus, e.g., electric mixers and mechanical stirrers, show large droplet sizes and wide particle distribution. The techniques usually employed to prepare nanoemulsions involve the utilization of ultrasound, evaporation of solvents (46), PIT (phase-inversed temperature) method (47), two-stage homogenizer (48,49), and the microfluidizer (50,51).

In recent years, nanoemulsions have been gaining more and more attention in the cosmetic industry, which has rapidly grasped its benefits. The patents assert that these systems penetrate through the skin to a greater extent compared with usual topical compositions.

Nanoemulsions are so strongly compressed that they become ultralight and, like vesicular systems, constitute a new form that could prove extremely fruitful for the release of substances.

Nanoemulsions have a much higher surface area and free energy than macroemulsions, which make them an effective transport system. They can be formulated in a variety of formulations such as foams, creams, liquids, and sprays.

Many studies have shown that nanoemulsion formulation processes improved both transdermal and dermal delivery properties in vitro (52–55) as well as in vivo (56,57).

The determination of silicones and hydrogenated didecenes deposited on human hair from shampoo applications is described by Haake et al. (58). A transparent shampoo containing 1.8% of hydrogenated didecenes delivered via a nanoemulsion showed a good performance profile.

The penetration of octyl methoxycinnamate formulated in nanocapsules was compared with one obtained from a nanoemulsion and one from a conventional O/W emulsion. The percutaneous penetration, assessed by the tape-stripping technique, demonstrated that nanoemulsion increased the extent of octyl methoxycinnamate penetration. The accumulation in the skin was significantly greater with nanoemulsion than with emulsion or nanocapsules (59).

Calderilla-Fajardo et al. (60) evaluated the influence of sucrose laureate and sucrose oleate on in vivo percutaneous penetration of octyl methoxycinnamate. The sunscreen agent was formulated in nanoemulsions, nanocapsules, and conventional O/W emulsions. The results showed that nanoemulsions of sucrose laureate exhibited the highest stratum corneum penetration compared with the other formulations.

The skin transport of inulin incorporated in O/W nanoemulsions was found to be significantly higher (5–15-fold) than that obtained with micellar dispersions or aqueous controls. The results suggest that W/O nanoemulsions are more compatible with the lipophilic sebum environment of the hair follicle (61,62).

VESICLES

In the 1960s, Bangham et al. (63) clearly demonstrated that the dispersion of natural phospholipids in aqueous solutions leads to the formation of "closed vesicles structures," which morphologically resemble cells. Since 1975 (64), vesicles have been prepared from

surfactants. Mezei and Gulasekharam (65) published the first paper to report the effectiveness of liposomes in the skin.

In 1986, the first commercial product incorporating liposomes appeared on the market (Capture®). At the same time, a synthetic one made by nonionic surfactants (66) was also launched (Niosomes®).

These microscopic vesicles contain from one to several concentric lipid bilayers with intercalated aqueous compartments. Transepidermal penetration of the content of vesicles is proportional to the "fluidity" of their lipids and negative charge. Several drugs and cosmetics in this form are already commercially available and successfully used, presenting a better dose-effect ratio and provoking less side effects.

In the 1990s, transfersomes, i.e., lipid vesicles containing large fractions of fatty acids, were introduced. Transfersomes (67–69) consist of a mixture of a lipidic agent with a surfactant. In consequence, their bilayers are much more elastic than those of most liposomes.

Niosomes can be prepared from various classes of nonionic surfactants, e.g., polyglycerol alkyl ethers (66,70), glucosyl dialkyl ethers (71), crown ethers, and polyoxyethylene alkyl ethers and esters (72).

The ethosomes are soft phospholipid vesicles, whose size can be modulated from tens of nanometers to microns. These vesicular systems have been found to be very efficient for enhanced delivery of molecules with different physicochemical characteristics to/through the skin. They can be modulated to permit enhancement into the skin strata as far as the deep dermis or to facilitate transdermal delivery of lipophilic and hydrophilic molecules (73).

Transfersomes have been shown to be versatile carriers for the local and systemic delivery of various steroids, proteins, and hydrophilic macromolecules (69). The mechanism proposed by the author for transfersomes is that they are highly deformable, thus facilitating their rapid penetration through the intercellular lipids of the stratum corneum. The osmotic gradient, caused by the difference in water concentrations between the skin surface and skin interior, has been proposed as the major driving force for transfersome penetration (68).

The effectiveness of vesicles has been investigated by several research groups. Liposomes, in particular, have received a great deal of attention (74–77).

In several studies, the diffusion of a drug was facilitated or achieved certain selectivity into human and nonhuman skin by vesicle encapsulation (85% of the papers). Other studies show that the influence of vesicles on drug transport is negligible (10%). Only a few papers claimed that the vesicles have no effect on the skin (5%).

OTHER ENCAPSULATION SYSTEMS

Cyclodextrins (CDs) are cyclic oligosaccharides containing at least 6 D–(+) glucopyranose units attached by α-(1, 4) glucosidic bonds. The three natural CDs, α-, β-, and γ-CDs (with 6, 7, or 8 glucose units, respectively) differ in their ring size and solubility.

CDs have been used to optimize local and systemic dermal drug delivery. Applications of CDs in transdermal drug delivery include enhancement of drug release and/or permeation, drug stabilization in formulation or at absorptive site, alleviation of drug-induced local irritation, sustaining of drug release from vehicle, and alteration of drug bioconversion in the viable skin (78).

CDs increase topical availability of drugs by solubilizing lipophilic drugs and thus increasing availability of the drug at the skin surface. The drugs are therefore solubilized without modifying their intrinsic property of lipophilicity. Polymers, such as hydroxypropyl methylcellulose (HPMC), polyvinylpyrrolidone (PVP), and carboxymethylcellulose sodium (CMC), may be added to increase permeability. The type and amount of CDs are two important parameters for maximizing the permeability of the drug in formulation. Finally, use of other permeation enhancers has been seen to aid the CD-mediated delivery of drugs, such as prostaglandin E1 through the skin (79).

Microemulsions are stable dispersions in the form of spherical droplets, whose diameter is in the range of 10 to 100 nm. They are composed of oil, water, and usually surfactant and cosurfactant.

These systems show structural similarity to micelles and inverse micelles, resulting in O/W or W/O microemulsions, respectively. They are highly dynamic systems showing fluctuating surfaces caused by forming and deforming processes.

The main characteristics of microemulsions are the low viscosity associated with a Newtonian-type flow, a transparent or translucid appearance, isotropic, and thermodynamic stability within a specific temperature setting. Certain microemulsions may thus be obtained without heating, simply by mixing the components as long as they are in a liquid state.

One of the conditions for microemulsion formation is a very small, rather than a transient negative, interfacial tension. This is rarely achieved by the use of a single surfactant, almost always necessitating the addition of a cosurfactant. The presence of a short chain alcohol, e.g., can reduce the interfacial tension from about 10 mN/m to a value less than 10^{-2} mN/m. Exceptions to this rule are provided by nonionic surfactants which, at their phase inversion temperature, also exhibit very low interfacial tensions.

Since microemulsions were discovered approximately six decades ago, their applications in several fields, including cosmetics, have been increased because of their good appearance, thermodynamic stability, high solubilization power, and ease of preparation (80).

Liquid crystals are defined as the intermediary state between solid and liquid and also called mesomorphous or crystalline phase, presenting characteristics of the mentioned physical states. That intermediary phase, in simple emulsions, can act as forms of encapsulation of drugs providing its controlled liberation and can increase cutaneous hydration. These characteristics evidence the differentiation of the developed formulations and the use of the same ones in the release of new cosmetic vehicles (81).

Multiple emulsions are emulsions in which the dispersion phase contains another dispersion phase. Thus, water-in-oil-in-water (W/O/W) emulsion is a system in which the globules of water are dispersed in globules of oil, and the oil globules are themselves dispersed in an aqueous environment. A parallel arrangement exists in oil-in-water-in-oil (O/W/O) type.

The first commercial use of a W/O/W-type multiple emulsion is unique moisturizing by Lancaster, which was introduced on the market in 1991.

Cosmetic applications of multiple emulsions have been protected in the patents issued for their composition. The patents show that multiple emulsions are highly recommended for all kinds of cosmetic applications: sunscreens, makeup removers, cleansers, and nutritive, hydrating, and cooling products.

Ferreira (82,83) showed three emulsions types (W/O/W, O/W, and W/O) containing a water-soluble molecule (glucose). The release of glucose from the O/W emulsion was the fastest. From the W/O emulsion, it was the slowest, while the one from the W/O/W emulsion was intermediate. The W/O/W emulsion showed some tendency toward steady state during the first 3 to 12 hours, and the flux was found to be 1.7 times greater than that from the W/O emulsion.

CONCLUSION

Encapsulation is now part of our everyday life and should be used to improve our life. However, much care needs to be taken to decipher completely and exhaustively the effects of nanomaterials before they may be commercialized.

Molecular biology has provided us with tools to identify and build genetic materials that can be used for treatment of hereditary diseases. Developing a carrier for gene therapy is one of the main challenges that the encapsulation field faces today.

What will the future bring us? We have already indicated where, on the basis of our present knowledge, encapsulation in many different vectors offer a rational advantage as active carrier systems to the skin. Therefore, efforts should be made to obtain a better understanding concerning the mechanisms of these systems at the molecular and supramolecular level. This could lead to new formulation processes and open new prospects in the area of active delivery by means of encapsulated systems.

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73 Elastic Vesicles as Topical/Transdermal Drug Delivery Systems

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INTRODUCTION

The topical/transdermal (TT) delivery route for drug administration has many advantages over other pathways, including avoiding the hepatic first-pass effect, continuous drug delivery, fewer side effects, and improved patient compliance (1). A major obstacle to TT drug delivery is the low penetration of drugs through skin. The stratum corneum (SC) provides a principal barrier to TT delivery of applied drugs and consists of corneocytes that are embedded in an intercellular lipid matrix composed of ceramides, free fatty acids, and cholesterol (CHOL) (2). Several approaches have been used to weaken this skin barrier and to improve TT drug delivery (3–8).

One possibility for increasing the penetration of drugs is the use of vesicular systems such as liposomes. Because of their biocompatibility and capability of incorporating both hydrophilic and lipophilic drugs, liposomes have been investigated as parenteral drug and antigen-carrier systems and more recently as TT drug delivery systems (9–18). Despite improvements in TT delivery, conventional liposomes were not efficient at delivering transdermally across the skin because they do not deeply penetrate the skin, but rather remain confined to the upper layer of the SC. Thus, several investigators developed novel elastic vesicles to deeply and easily penetrate across the skin (19–21).

In the early 1990s, novel series of liquid-state vesicles have been developed, and these vesicles could better facilitate drug transport across the skin as compared with conventional vesicles. Novel types of vesicular systems have been recorded to penetrate intact skin if applied nonocclusively in vivo by virtue of their very high and self-optimizing deformability. Elastic vesicles are classified with phospholipid and detergent-based types (Table 1). Because of high flexibility, elastic vesicles squeeze through small pores in SC less than the vesicle sizes. Elastic vesicles were more efficient at delivering a low- and high-molecular-weight drug to the skin in terms of quantity and depth (24–26,33–38). The precise quantity and depth of elastic vesicles' penetration through skin depends on the carrier type, the total mass applied, the entrapment efficiency, and the detailed application conditions such as occlusion, pretreatment of vesicles, duration, and application volume. Elastic vesicles prolonged the release and showed better biological activity in comparison with conventional liposomes and ointments.

This review focuses on the effect of elastic vesicles for enhancing the penetration chemicals, and it defines action mechanism and optimal condition of elastic vesicles.

ELASTIC VESICLES–SKIN INTERACTION

Vesicle–skin interactions can occur either at the skin surface or in the deeper layers of the SC. Hofland et al. (39) and Abraham and Downing (40) have shown fusion and adsorption of vesicles onto the SC surface, forming stacks of lamellae and irregular structures on top of the skin. Vesicle–skin interactions are strongly influenced by the composition of the vesicles, resulting in differences in their phase, state, and elasticity. Liquid-state vesicles have been shown to have superior to gel-state vesicles (41). When comparing the interactions of elastic and rigid vesicles with hairless mouse skin in vivo, only the elastic liquid-state vesicles affected the ultrastructure of the viable tissue. No changes in the ultrastructure were observed with any of the conventional liposomes (42).

Table 1 Drug Skin Penetration from Phospholipid- and Detergent-Based Elastic Vesicles

Drug (comments)	Animal	Composition	Enhancing factor	Reference
Dipotassium	Pig	PC:KG (4:1)	5.9	22
Glycyrrhizinate (KG)		HPC:KG (4:1)	5.5	
Methotrexate	Pig	PC:KG (2:1)	5.2	23
		HPC:KG (2:1)	5.9	
Dexamethasone	Rat	PC:CHOL (7:3)	1.0	24
		PC:deoxycholate (85:15)	2.2	
		PC:Tween-80 (85:15)	1.9	
		PC:Span-80 (85:15)	2.3	
Diclofenac	Rat	Commercial form	1.0	25
		Lotion-like transfersomes	30–100	
Gap junction protein	Mouse	Soybean PC	1.0	26
(Antibody production)		PC/sodium cholate/SDS	4.7	27
Insulin (decrease of	Mouse	PC liposomes or micelle	No change	
blood glucose)		PC/cholate (8.7:1.3)	20–30%	
Cyclosporin A	Mouse	PC/cholate (10:2.8)	16.2	28
Estradiol	Human	PC/cholate (84:16)	18	29.30
		PC/Span 80 (84:16)	16	
		PC/Tween 80 (84:16)	15	
		PC/oleic acid (84:16)	13	
5-Fluorouracil	Human	PC/cholate (84:16)	6.9–13.2	31
Rotigotine	Human	L-595/PEG-8-L (50:50)	30.6	32
Pergolide	Human	L-595/PEG-8-L (50:50)	2.7	33

Abbreviations: PC, phosphatidylcholine; KG, dipotassium glycyrrhizinate; HPC, hydrogenated lecithin; CHOL, cholesterol; SDS, sodium dodecyl sulfate.
Source: From Refs. 22–33.

Cevc and Blume (19) suggested that elastic vesicles (Transfersomes) were able to penetrate through intact SC under the influence of a transepidermal osmotic gradient. Although the transport of most compounds is increased during occlusive application, Cevc and Blume (19) have suggested that elastic vesicles (Transfersomes) are most efficient under nonocclusive conditions. Nonocclusive conditions are necessary to create a transepidermal osmotic gradient, which is believed to be the driving force for elastic transport into the skin. The osmotic gradient is caused by the difference in water concentrations between the skin surface and skin interior. Transfersomes are highly deformable, and this property facilitates their rapid penetration through the intercellular lipid pathway of the SC. Schatzlein and Cevc (43) reported the existence of irregularities within the intercellular lipid packing of murine SC that can act as virtual channels through which Transfersomes could penetrate.

In the case of detergent-based elastic vesicles, Van den Bergh et al. (42,44) investigated the interaction of elastic and rigid vesicles with murine skin in vivo and with human skin in vitro. Unlike Transfersomes, these studies did not show any evidence that elastic vesicles could penetrate through the SC. However, Honeywell-Nguyen et al. (45) demonstrated a fast penetration of intact elastic vesicles into human SC, and these vesicles were localized within channel-like regions. They also investigated the in vivo interaction of elastic vesicles with human skin, using the tape-stripping technique in combination with freeze–fracture electron microscopy, and demonstrated a fast penetration of intact elastic vesicles into human SC (46). They proposed that the channel-like regions represent imperfections within the intercellular lipid lamellae in the areas with highly undulating cornified envelopes. Taken together, intact elastic vesicles may penetrate into human SC via channel-like regions.

Ethosomes are also phospholipid elastic vesicles having a high content of ethanol. Ethanol interacts with lipid molecules in the polar head group region, resulting in a reduction in the melting point of SC lipid, increasing their fluidity. The interaction of ethanol into the polar head group environment can result in an increase in membrane permeability. This is followed by a fusion of ethosomes with cell membranes. In addition to the effects of ethanol on SC structure, ethosome itself interacts with the SC barrier and then can penetrate the disturbed SC bilayer (47,48).

TRANSFERSOMES

Skin has small virtual pores (20–40 nm), and this limits passing through intercellular passages in the outer skin layers (20,49). To overcome this problem, Cevc developed a new liposomal system with more deformable aggregates called Transfersomes (20). Transfersomes differ from more conventional liposomes in several respects. Transfersomes resemble liposomes in morphology, but not in function (50). The most important is the extremely high and stress-dependent adaptability of such mixed lipid aggregates. Because of high deformability, Transfersomes squeeze through pores in SC that are less than one-tenth in the liposomes's diameter. Thus, sizes up to 200 to 300 nm can penetrate intact skin (27). Transfersomes contain phosphatidylcholine (PC) and a surfactant (edge activator) and also consist of at least one inner aqueous compartment surrounded by a lipid bilayer. Sodium cholate, Span 80, Tween 80, oleic acid, and dipotassium glycyrrhizinate (KG) (20,22,29,30) were employed as edge activators. For transdermal DNA delivery, DOTAP (positive-charged molecule), as a component, was used in producing Transfersomes instead of PC (38). Subsequent studies have documented that Transfersomes were more effective than conventional liposomes or ointment in the enhancement of small and large drug molecules across mouse and human skin (24–26,28,31,35–38).

Several investigators reported that Transfersomes prolonged the release and improved the biological activity in vivo (24,25,35,51). The in vivo performance of Transfersomes was studied by a carrageenan– and arachidonic acid–induced edema model with dexamethasone and triamcinolone acetonide (TRMA). Jain et al. (24) showed that dexamethasone Transfersomes could provide a maximum of 82.32% inhibition of paw edema, whereas conventional liposomes and ointments prevented approximately 38.32% and 25.35% of paw edema, respectively. With TRMA, the drug dose of 0.2 mg cm^{-2} suppressed 75% inhibition of ear edema for 48 hours. In contrast, a conventional formulation of TRMA required a 10-fold higher drug dosage to achieve a similar effect (35). A similar result was obtained with hydrocortisone and diclofenac Transfersomes (25,35,51). Diclofenac association with Transfersomes prolonged the effect and reached 10-fold higher concentrations in the tissue under the skin in comparison with the drug from a commercial hydrogel (25).

In addition to chemical drugs, Transfersomes could deliver large molecules into the body through intact skin. Paul et al. (26) investigated the effect of Transfersomes on the transdermal immunization with protein antigen. They applied Transfersomes to the intact skin surface with gap junction proteins (GJP) and showed that the specific antibody titers were higher than those elicited by subcutaneous injection of GJP in Transfersomes, mixed micelles, or conventional liposomes. Hofer et al. (37) reported the formulation of IL-2 and IFN-α containing Transfersomes for transdermal application. They showed that IL-2 as well as IFN-α were trapped by Transfersomes (75–80%) in biologically active form and in sufficient concentration for immunotherapy. Cevc (36) also reported the transdermal delivery of insulin with Transfersomes. Transfersomes could deliver insulin through skin barrier with a reproducible drug effect that resembled closely to that of insulin injected under the skin. In addition, Kim et al. (38) investigated the effect of Transfersomes (DOTAP and cholate) on the transdermal application of DNA in mice-intact skin. They reported that the GFP expression was detected in some organs, such as liver and lungs, when green fluorescent protein (GFP) was complexed with Transfersomes, whereas the GFP mixed only with phosphate buffered saline (PBS) did not observe with GFP expression. Transfersomes were capable of penetrating DNA into intact skin of mice when topically applied. Thus, Transfersomes may be developed further as a noninvasive protein and gene delivery system.

Transfersomes have several advantages on the TT drug delivery. They can be entrapped and delivered with small and large molecules through TT delivery. When applied on the intact skin, Transfersomes are not detrimental to the skin. Phospholipid, as a component of Transfersomes, even seems to improve the hydration of the aged skin (25). The advantages include a faster onset of drug effect, longer times of action, a biological action that is unaffected by mechanical abrasion, and the ability to reduce the necessary dosage needed to achieve therapeutic effects. Thus, the use of Transfersomes on the skin offers unprecedented opportunities for well-controlled and modern topical medication, not only just for low-molecular weight, but also for a variety of macromolecular therapeutics.

EFFECT OF TYPE SURFACTANTS AND CONCENTRATION

To prepare Transfersomes vesicles, edge activators (surfactants) were incorporated into the vesicular membranes; sodium cholate or sodium deoxycholate, Span 80, and Tween 80 have been used for this purpose. El Mghraby et al. (29,30) investigated the effect of surfactants on the formation of Transfersomes and permeation into the skin using cholate, Span 80, Tween 80, and oleic acid. Also, they investigated the effect of surfactant concentration on the skin permeation.

Transfersomes significantly improved the epidermal delivery of estradiol compared with the aqueous solution. The maximum flux increased by 18-, 16-, 15-, and 13-fold for Transfersomes containing sodium cholate, Span 80, Tween 80, and oleic acid compared with control. The skin deposition also increased by eight-, seven-, and eight-fold for cholate, Span 80, and Tween 80, respectively, compared with control. The efficiencies of Span 80 and Tween 80 were comparable with that of sodium cholate, but efficiency of oleic acid was less than that of sodium cholate. With dexamethasone Transfersomes, Span 80 was more effective as compared with sodium deoxycholate and Tween 80 as edge activators on penetration and edema inhibition assay.

With respect to drug delivery from vesicles, J_{max} first increased with increasing surfactant concentration, then decreased. These results suggested that too low or too high concentrations of surfactants are not beneficial in vesicular delivery of estradiol through skin and also indicated that the possible penetration enhancing effect of surfactants is not mainly responsible for improved estradiol skin delivery from deformable vesicles. The surfactant concentrations in the refined formulation were assessed to be 14.0%, 13.3%, and 15.5% w/w for sodium cholate, Span 80, and Tween 80, respectively (29). A possible explanation for lower drug delivery at high surfactant concentrations may be that surfactant at high concentrations decreased the entrapment efficiency and disrupted the lipid membrane so that it became more permeable to the entrapped drug. This will in turn reduce the delivery. The overall results suggested that there may be an optimum concentration of surfactant in lipid vesicles for maximum skin delivery of drug using Transfersome vesicles.

In addition, KG is used in preparing elastic vesicles. KG has emulsifying properties and good solubility. Thus, it is widely used in cosmetics. Trotta et al. (22) evaluated the ability of KG to produce elastic vesicles with soya lecithin (PC) or hydrogenated lecithin (HPC). They compared KG permeation with elastic vesicles and aqueous solution in the pigskin. All systems showed a negligible flux, but differed in the residual amount of KG in the skin. PC and HPC elastic vesicles promote the transfer of KG into the pigskin (60 ± 8 to 71 ± 10 mg cm^{-2}), while the KG solution failed to achieve transport (12 ± 3 mg cm^{-2}). There were no significant differences between elastic vesicles containing PC or HPC. The skin deposition increased by fivefold compared with 0.25% KG control solution.

Trotta et al. (23) also reported the effect of KG elastic vesicles on the dermal penetration of methotrexate (MTX) in pigskin. In an estimation of the cumulative amount of MTX permeated after 24 hours through pigskin, aqueous solution and conventional liposomes are quite similar in terms of MTX delivery through skin, whereas elastic vesicles show an increase in the amount of MTX permeated. In a skin deposition after 24-hour application, PC and HPC elastic vesicles promoted the transfer of MTX into pigskin. Skin deposition increased by a factor of 3 compared with either aqueous solution or conventional liposomes. Thus, KG acts as a good edge activator to produce elastic vesicles for TT drug delivery.

Which detergents are the best choices in Transfersomes formulation? The effect of detergents was different from other formulation conditions. The use of cholate seems to be better than other detergents in the case of skin delivery of large molecules. In addition to cholate, KG is a good candidate. KG has chemical stability, good solubility, emulsifying property, and anti-inflammatory activity. Thus, the use of elastic vesicles containing KG creates new opportunities for the well-controlled and modern topical medication. However, a more extensive study about compositions should be undertaken to define the optimal formulation regardless of drug types.

NONPHOSPHOLIPID-BASED ELASTIC VESICLES

In addition to phospholipid-based elastic vesicles (Transfersomes and ethosomes), Van den Bergh et al. (21) developed a series of elastic vesicles, consisting of the bilayer-forming surfactant L595 (sucrose laurate ester) and the micelle-forming surfactant PEG-8-L

(octaoxyethylene laurate ester). L595 consisted of 100% sucrose laurate ester (30% monoester, 40% diester, and 30% triester). Surfactant-based elastic vesicles consisted of L595, PEG-8-L, and sulfosuccinate as stabilizers in the molar ratio 50:50:5. Several investigators reported that L595–PEG-8-L elastic vesicles were effective in enhancing the skin permeation of various drugs (32,33,52,53). However, drug transport was influenced by fluidity of elastic vesicles. The most rigid vesicles consisting of L595/PEG-8-L (100:0) significantly reduced the flux by 50% compared with the buffer control ($p < 0.05$). However, increasing the PEG-8-L content—and thereby increasing the vesicle elasticity—resulted in a significant higher flux for the L595/PEG-8-L (70/30 or 50/50) elastic vesicles ($p < 0.01$). This was a threefold increase to the buffer treatment and a sixfold increase to the most rigid vesicle treatment (33). Similar results were obtained from rotigotine elastic vesicles (32).

Elastic vesicle transport and the appearance of vesicle material in human SC can be affected by several factors, including pH, entrapping efficiency, pretreatment of vesicles, occlusive volume, and duration of application (32,45,46). The optimal pH differs depending on the drugs. The optimal pH was found to be 5.0 in case of pergolide, giving the highest drug incorporation as well as the highest drug transport. There was more than a fourfold difference between the highest flux at pH 5.0 (371.0 ± 51.7 ng cm^{-2}) and the lowest flux at pH 7.0 (89.3 ± 9.1 ng cm^{-2}) (33). Unlike pergolide, optimal pH was found to be 9.0, giving the highest drug incorporation ($99.8\% \pm 0.02\%$), in case of rotigotine elastic vesicles. At pH 5.0, the entrapment efficiency is very low ($22.1\% \pm 9.6\%$). The flux and cumulative amount of vesicle solution at pH 9.0 with high drug entrapment efficiency was 2.7-fold higher than those resulting from the vesicle solution at pH 5.0. Vesicles solution at pH 9.0 (3251 ± 902 ng cm^{-2}) gave rise to enhancement effect of factor 80 as compared with the corresponding buffer solution (42 ± 29 ng cm^{-2}). In contrast, vesicles solution at pH 5.0 (1072 ± 160 ng cm^{-2}) did not significantly enhance the drug transport as compared with its corresponding buffer control (1133 ± 241 ng cm^{-2}) (32). Table 2 summarizes the effect of elastic vesicles on pergolide and rotigotine delivery into the skin. As shown in Table 2, enhancement of rotigotine was much higher than that of pergolide. Entrapment efficiency and drug properties may result in this difference.

Nonocclusive co-treatment with elastic vesicles improved the skin delivery of pergolide compared with nonocclusive buffer control by more than twofold. Occlusion improved drug transport from both elastic vesicles as well as buffer solutions because of the fact that water is an excellent penetration enhancer for pergolide (45). In contrast to nonocclusive application, occlusive treatment with elastic vesicles showed a lower flux compared with occlusive treatment with the buffer control. A higher volume of application could increase the partitioning of vesicles into the skin, thereby increasing the enhancement effect. Pergolide transport from the 40- and 100-mL application were much higher within the first 20 hours as compared with that from the 20-mL application. However, there were no significant differences in the total cumulative amounts of drugs transported (306 ± 47.4, 462.0 ± 112.0, and 509.7 ± 141.9 in 20, 40, and 100 μL application, respectively) (45). They also investigated the effect of co-application and pretreatment on the rotigotine transport. Co-application (3483 ± 1067 ng cm^{-2}) significantly enhanced the drug transport by many folds, whereas pretreatment (126 ± 18 ng cm^{-2}) clearly had no effect on the drug transport as compared with buffer control (133 ± 27 ng cm^{-2}) (32). Similar result was obtained from pergolide elastic vesicles.

Table 2 Comparison of Pergolide and Rotigotine Skin Delivery with Elastic Vesicles

Factors	Rotigotine	Pergolide
Use	Dopamine agonist	Dopamine agonist
Compositions	L595/PEG-8-L-sulfosuccinate (50/50/5)	L595/PEG-8-L-sulfosuccinate (50/50/5)
Enhancement ratio	30.6-fold	2.7-fold
Occlusion		Increase penetration
Optimal pH	9.0	5.0
Entrapment	99.8% ± 0.02% at pH 9.0	2.5 mg/mL at pH 5.0 (Saturated)
Average size	117 ± 6 nm	100 ± 5 nm
Pka	7.9	5–6
Lipophilicity	Lipophilic at pH 9.0	Lipophilic and positive charge

From these results, detergent-based elastic vesicles were found to be powerful drug delivery systems across the skin. Co-application (co-treatment) and entrapment efficiency were essential factors for an optimal drug delivery by elastic vesicle formulations. The drug entrapment efficiency is strongly dependent on the pH of the drug–vesicular system. Thus, pH was also an important factor to deliver drugs with TT pathway.

ETHOSOMES

Ethosomes are phospholipid liposome carriers containing high content of ethanol (20–45%) (34). However, due to the interdigitation effect of ethanol on lipid bilayers, it was believed that high concentrations of ethanol are detrimental to liposomal formulation. Touitou developed ethosomes for transdermal drug delivery (54). Currently, ethanol can only be found in relatively low concentrations in liposome formulations: 7% to 10% for Transfersomes, 14% for Mibelle, and 16% for Natipide II. But, high content of ethanol was used in case of proniosomes (30–50%) (55,56). Ethosomes are soft, malleable vesicles tailored for enhanced delivery of various drugs to/through the skin and cellular membranes. Unlike conventional liposomes, which are known mainly to deliver drugs to the outer layers of the skin, ethosomes were shown to enhance permeation through the SC barrier. They penetrate skin and enhance drug delivery to deep skin SC (34,47,54,57). They are noninvasive delivery carriers that enable drugs to reach the deep skin layers and the systemic circulation.

Touitou et al. (34) investigated the effect of phospholipid and ethanol concentration on the size distribution of ethosome vesicles. In the ethanol concentration range of 20% to 45%, the size of the vesicles increased with decreasing ethanol concentration, with the largest sizes in preparation containing 20% ethanol (193 $\pm$ 8 nm) and the smallest in preparations containing 45% ethanol (103 $\pm$ 9 nm). The dependence of vesicle size on phospholipid content was determined for ethosomes containing 30% ethanol and PC concentration ranging from 0.5% to 4%. An eightfold increase in PC concentration (from 0.5% to 4%) resulted in a twofold increase in ethosome size (from 118 $\pm$ 2 to 249 $\pm$ 24 nm). Also, ethanol imparted a negative charge to the vesicles and improved the vesicle stability. Hydrophilic and hydrophobic chemicals can be entrapped into ethosomes, and entrapment efficiency of hydrophobic chemicals was higher than that of hydrophilic chemicals. Entrapment efficiencies of minoxidil, testosterone, trihexylphenidyl (THP), and bacitracin were 83% $\pm$ 6%, 90% $\pm$ 3.5%, 75% $\pm$ 0.8%, and 77.4% $\pm$ 2.9%, respectively (34,47,58).

Enhanced delivery of chemicals from the ethosomal carrier was observed in permeation experiments with fluorescent probes (for detection of penetration depth) and drugs to nude mouse skin. Hydrophilic calcein penetrated the skin to a depth of 160, 80, and 60 mm from ethosomes, hydroethanolic solution (30% ethanol in water), and liposomes, respectively. Lipophilic rhodamine red (RR) penetrated the nude mouse skin to a depth of 140 mm from both the ethosomal system and from hydroethanolic solution. The probe into the fluorescence intensity was significantly greater from the ethosomal system [150 arbitrary unit (AU) for ethosomes and 40 AU for hydroethanolic solution]. Fluorescence was still visible at the deepest skin layers (20 AU at 260-mm depth) in case of ethosomal system. Deep penetration from liposomes was almost negligible (20 AU at 40-mm depth) (59).

Touitou et al. (34) also investigated the ability of ethosomes to deliver minoxidil to the deep layers of the skin. When it permeated the skin, it was 45 and 35 times higher from the ethosomal system than 30% ethanolic solution and absolute ethanol, respectively; the amount of minoxidil in the skin was also seven and five times greater than control systems, respectively. Similar results were obtained using acyclovir®, testosterone, and ionic molecules such as propranolol and THP (34,47,60). Horwitz et al. (60) reported that acyclovir delivered from an ethosomal system performed significantly better than Zovirax® (GlaxoSmithKline, Middlesex, U.K.). The amount of testosterone permeated in the rabbit skin in 24 hours was 30 times greater from the ethosomal system than from Testoderm® (848.16 $\pm$ 158.38 mg vs. 27.79 $\pm$ 16.23 mg). The amount of testosterone in the skin was also almost seven times greater when the drug was delivered from ethosomal system (130.76 mg vs. 18.32 mg) (34). THP flux from ethosomes (0.21 mg/cm^2/hr) was 87, 51, and 4.5 times higher than that from liposomes, buffer, and hydroethanolic solution, respectively (47). These data indicate that the ethosomal system is a more effective permeation enhancer than ethanol and hydroethanolic solution. Table 3

Table 3 Drug Skin Permeation from Ethosomal Vs. Control System

Drugs	Animal model	System	Qr[a] (μg cm^{-2})	Qs[a] (μg cm^{-2})	References
Minoxidil®	Nude mouse	Ethosomes	673.0 ± 92.0	69.6 ± 11.0	34
		30% EtOH	13.1 ± 3.5	10.0 ± 2.3	
Trihexyphenidyl®	Nude mouse	Ethosomes	1750 ± 250	586 ± 77	47
		30% EtOH	500 ± 50	415 ± 21	
Cannabidiol®	Nude mouse	Ethosomes	110.07 ± 24.15	–	61
Bacitracin®	Human	Ethosomes	12.0 ± 1.0	–	58
Testosterone®	Rabbit	Ethosome patch	848.16 ± 158.38	130.76 ± 20.23	34
		Testoderm®	27.79 ± 16.23	18.32 ± 8.34	

[a]The quantity of drugs that permeated the skin (Qr) and the quantity of drugs in the skin (Qs) in 24 or 18 hours (trihexyphenidyl) were measured in diffusion cells from systems, each containing 0.5% minoxidil, 0.1% bacitracin, 1% trihexyphenidyl, 0.25 mg cm^{-2} testosterone.
Source: From Refs. 34, 47, 58, and 61.

summarizes the ethosomal drug delivery to skin and cell membranes. Lodzki et al. (61) also reported the cannabidiol® transdermal delivery in a murine model. The flux of cannabidiol differs depending on skin sites. After a 24-hour application, it permeated 37.43 ± 13.58 and 110.07 ± 24.15 mg cm^2 into the hip skin and abdominal skin, respectively. Cannabidiol was also detected in the muscle, liver, pancreas, and blood. Godin and Touitou (58) evaluated the skin-depth penetration from bacitracin® ethosomes in vivo in rats. After an eight-hour topical application to rat abdomen, bacitracin penetrated more deeply into the skin from ethosomes than 30% ethanolic solution and liposomes. Taken together, the ethosomal system is effective in delivering drugs deeply into and through the skin.

Ethosomal systems are easy to prepare, nonirritant, and composed mainly of phospholipids and ethanol; compounds commonly found in pharmaceutical preparations. Ethosomes, because of their unique structure, are able to entrap and deliver through the skin highly lipophilic molecules such as cannabinoids, testosterone, and minoxidil as well as cationic drugs such as propanolol and trihexyphenidil. Enhanced delivery of bioactive molecules through the skin and cellular membranes by means of an ethosomal carrier opens numerous challenges and opportunities for the research and future development of novel improved therapies.

ACTION MECHANISM OF ELASTIC VESICLES ON PENETRATION

How do phospholipid- and detergent-based elastic vesicles enhance drug penetration into the skin? Two mechanisms can be proposed. First, the elastic vesicles can act as penetration enhancers, whereby vesicle bilayers enter the SC and subsequently modify the intercellular lipid matrix. This will facilitate the penetration of free drug molecules into and across the SC (mechanism 1). Second, the elastic vesicles can act as drug-carrier systems, whereby intact vesicles can enter the SC-carrying vesicle-bound molecules into the skin (mechanism 2) (32,45). In order to assess whether a drug-carrier mechanism of action is involved or whether elastic vesicles simply act as penetration enhancers, two important questions should be answered: (*i*) Is pretreatment of the skin with empty vesicles sufficient (for mechanism 1), or is it essential to incorporate drugs into the vesicle solution (for mechanism 2)? (*ii*) What is the effect of the entrapment efficiency on the drug transport? Does higher entrapment efficiency result in a higher drug transport?

Cevc et al. (19) proposed that Transfersomes are drug-carrier systems that can deliver across the intact skin. It is believed that the successful passage of such carriers is based on two important factors: the high elasticity (deformability) of the vesicle bilayers and the existence of an osmotic gradient across the skin. Because of high deformability, Transfersomes could—under influence of the transepidermal osmotic gradient—squeeze themselves between the cells in the SC and carry large amounts of drugs across the intact skin. Fang et al. (18) investigated the mechanism of vesicular system across the skin with soybean PC liposomes containing enoxacin®. After a 12-hour pretreatment, drug permeation across PC-treated skin was higher

than that across nontreated skin. Also, Verma et al. (17) reported that PC liposomes carry not only the entrapped hydrophilic drug but also the nonentrapped drug into the SC and possibly into the deeper skin layers. These results indicated that PC liposomes could serve as permeation enhancers for drug delivery via the skin. With 5-fluorouracil Transfersomes, the percentage of drug penetrated (13.5%) was higher than the drug entrapment efficiency (8.8%) of Transfersomes (31). This strongly suggested that Transfersomes components may have altered the skin structure, as a penetration enhancer. Taken together, Transfersomes may have two functions to enhance drug transport across the skin, as a carrier system as well as a penetration enhancer.

Several investigators reported that pretreatment of detergent-based elastic vesicles did not improve the transport of pergolide and rotigotine, whereas higher entrapment efficiency resulted in higher drug transport (32,46). These data suggest that a penetration-enhancing process is not the main or the only mechanism of action, and detergent-based elastic vesicles may act as a carrier system. Honeywell-Nguyen and Bouwstra (45) proposed that detergent-based elastic vesicles facilitate drug transport by a fast partitioning in the SC, thereby carrying vesicle-bound drug molecules into SC. The vesicles remain in the SC and do not penetrate into the deeper skin layers. Hence, there are four major steps determining the effectiveness of the elastic vesicles system: (*i*) the drug association to the vesicle bilayers, (*ii*) the partitioning of vesicles into the SC, (*iii*) the drug release from the vesicles once in the SC, and (*iv*) the diffusion of free drugs in the SC and partitioning into the viable skin tissue, and subsequently into the systemic circulation (45). Taken together, they proposed that a penetration-enhancing effect of the individual surfactant component is not the main or the only mechanism of action for the elastic vesicles, and it is essential to apply drug molecules together with the vesicles.

Touitou et al. (34) proposed the action mechanism of ethosomal systems. First, ethanol disturbs the organization of the SC lipid bilayer and enhances its lipid fluidity. The flexible ethosome vesicles can then penetrate the disturbed SC bilayers. The release of the drug in the deep layers of the skin and its transdermal absorption could then be the result of a fusion of ethosomes with skin lipids, and drug release at various points along the penetration pathway. Unlike other elastic vesicles, occlusion slightly increased the skin penetration of ethosomes. This result indicated that the existence of an osmotic gradient across the skin was not an important factor (58). These data differ from that observed with elastic vesicles, where permeation enhancement occurred only in nonocclusive conditions, and point toward different mechanisms of action of the two carriers.

To further investigate mechanism of ethosomal skin permeation, Godin and Touitou (58) used double-staining methods; ethosomes co-loaded with two fluorescent probes, RR and FITC-bacitracin (FITC-Bac). Both probes were delivered from ethosomes to a maximal possible depth of 200 mm. When the two probes were observed separately at the skin depth of 90 mm, it was clearly seen that the delivery of FITC-Bac from ethosomes was followed by the delivery of ethosomal components in the same area. Skin penetration profile date indicated that penetration of ethosomal vesicles into the skin peaked at approximately 40 mm, while depth of maximum bacitracin penetration was approximately 90 mm, suggesting that the release of the drug in deep skin layers occurred. In a double-staining study, the bacitracin, delivered from ethosomes, entered the skin between the coreocytes through the intercellular lipid domain. High content of ethanol fluidizes the ethosomal membranes to produce highly deformable vesicles, and subsequently ethosomes squeeze drugs between the cells in the SC and carry large amounts of drugs across the intact skin (31,48). Additionally, we cannot exclude the possibility that ethosomes can be trapped in follicles and delivered to deep layers of the skin.

CONCLUSIONS

Highly deformable elastic vesicles (Transfersomes, ethosomes, detergent-based elastic vesilces) improve the transdermal delivery of low and high molecules in vitro and in vivo systems. The use of elastic vesicles as a vesicular drug carrier could overcome the limitation of low penetration ability of conventional liposomes or commercial ointment across the skin. Penetrating-enhancing effects of phosphlipid- and detergent-based elastic vesicles act as a drug carrier system as well as penetration enhancers. For optimal drug delivery, it is essential

that drug molecules are associated with vesicles and applies with co-application (co-treatment) conditions. In in vivo study, Transfersomes and ethosomes showed better biological activity in comparison with conventional liposomes or commercial ointment. Thus, many topical drugs may be developed using elastic vesicles. However, a more extensive study about vesicular type and compositions should be undertaken to fully establish the optimal condition, for which elastic vesicles are the most suitable vehicles.

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74 Polymers Effect on Chemical Partition Coefficient Between Powdered Human Stratum Corneum and Water

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INTRODUCTION

Macromolecules have gained interest as potential active entities and as modulators of percutaneous delivery systems. Some success in percutaneous delivery using enhancer is being reported, despite these compounds exceeding the “500 Dalton rule” (1). Another approach is to use macromolecules as transdermal transport facilitators, where the presence of macromolecules can interact at the skin surface that facilitates the passage of another chemical or drug (2). These macromolecules can interact at the skin surface, interphasing with cosmetic ingredients to alter the initial phase of percutaneous absorption, which is the initial partition into the skin.

Powdered human stratum corneum (PHSC), a product made from foot callus, can be cut into smaller pieces and ground with dry ice to form a powder. Uniformity in particle size is achieved with sieving. The callus is human stratum corneum (SC) derived and thus, should retain some physical and chemical characteristics of human SC (3,4). Two macromolecular polymers have been synthesized to hold cosmetic and drug compounds on the skin surface. The effect these two polymers have on the partition coefficient (PC) (SC/water) was investigated on the PHSC.

MATERIALS AND METHODS

Compounds

MacroDerm™ L (lipophilic MW 2081) is a block polymer of propylene oxide with hydrocarbon end groups that is completely insoluble in water. MacroDerm H (hydrophilic MW 2565) is a block polymer of ethylene oxide with hydrocarbon end groups. This latter material swells in water and exhibits amphipathic characteristics, but does not dissolve. Both materials are soluble in most organic solvents, including ethanol (5).

[^{14}C]-Estradiol—a model compound—(DuPont NEN) had specific activity of 54.1 mCi/mmol, and radiochemical purity was 99%, as determined by high-performance liquid chromatography (HPLC) and thin-layer chromatography (TLC).

Preparation of Powdered Human Stratum Corneum

Human foot callus was cut into fine pieces and ground in a Micro-mill Grinder (Bel-Air Products, Pequannode, New Jersey, U.S.) in the presence of dry ice to form powder. Particles of PHSC that pass through a 50-mesh sieve but not 80-mesh sieve were used. This particle size (180–300 μm) has previously been used in published studies (3,4).

Partition Coefficient Incubations

Into a specially designed glass centrifuge tube (6 cm length × 1 cm diameter; O.Z. Glass Company, Pinole, California, U.S.), with a glass fritte (40 μm) fitted in on the end, 10 mg of PHSC was mixed with 4 mL of [^{14}C]-estradiol in water. After incubating with estradiol for different lengths of time (0, 2, 6, 12, and 24 hours), the mixture was separated by centrifugation (15 minutes at 1500 × g). Water was passed through the glass fritte and collected into receiving

centrifuge tubes. PHSC was then washed twice with water, with vigorous mixing and further centrifugations. Each subsequent supernatant was collected separately. Water and remaining dry PHSC were mixed with the scintillation cocktail (Universol-ES, ICN, Costa Mesa, California, U.S.), and then assayed for radioactivity by liquid scintillation spectrophotometry. Five replicate tubes were prepared for each test sample. Three concentrations of estradiol were chosen (2.8, 0.28, and 0.028 μm/mL).

Delipidization of PHSC

PHSC was delipidized by incubation in chloroform:methanol (2:1) for 16 hours, then centrifuged and air-dried.

Polymer Incubation

Three concentrations of each polymer were used (1, 5, and 10% w/v in ethanol). There was a 30-minute preincubation period (with agitation) with the polymer solutions, and PHSC was washed twice with 50% ethanol and finally once with water. The resulting dry PHSC was then incubated for two hours with the same three concentrations of estradiol as previously described.

Calculation of PHSC/Water Partition Coefficient

The following equation was used to calculate PC:

$$PC_{PHSC/Water} = \frac{\text{dpm PHSC/mg PHSC}}{(\text{dpmi} - \text{dpm PHSC/mg water})}$$

where dpmi represents the initial concentration of estradiol in water (in desintegration per minute units). Statistical significance was determined using sigma stat.

RESULTS AND DISCUSSION

The PC values obtained after different incubation times, different estradiol concentrations, and in delipidized PHSC are shown in Table 1. There was no statistically significant effect ($p > 0.05$) on PC values when the concentration of estradiol was increased 100-fold (from 0.028 to 2.8 μm/mL), when the incubation period was increased from 0 to 24 hours, or when PHSC was delipidized prior to incubation with estradiol. In general, the log PC values obtained for estradiol ranged from 1.6 to 1.9, indicating an intermediate affinity of estradiol for PHSC compared with water. The data indicate a rapid partitioning of estradiol into human skin with no apparent lag time in partitioning for this chemical, which is probably because of the lipophilicity of the compound (log $P_{o/w} = 2.70$). Therefore, a two-hour incubation period was chosen for further studies with estradiol. The log PC values obtained (1.6–1.9) agreed with other similar work; for example, Surber et al. (6) reported a log $PC_{PHSC/water}$ value for estradiol of 2.1 and Hui et al. calculated the value to be 1.2. Therefore, the new glass vials developed for these assays appear consistent with older methods. Surber et al., (6) also showed the process of delipidization of the SC that had little or no effect on the PC of estradiol. This result is consistent with the data reported here.

Table 1 Effect of Time, Estradiol Concentration, and PHSC Delipidation on the PHSC/Water Partition Coefficient

	Log $PC_{PHSC/water}$ (mean ± SD, $n = 5$)			
Time (hr)	Delipidized PHSC [2.8 μm/mL][a]	Normal PHSC [2.8 μm/mL][a]	Normal PHSC [0.28 μm/mL][a]	Normal PHSC [0.028 μm/mL][a]
0	1.22 ± 0.17	1.57 ± 0.17	1.81 ± 0.30	1.20 ± 0.32
2	1.67 ± 0.22	1.62 ± 0.14	1.68 ± 0.11	1.71 ± 0.13
6	1.89 ± 0.09	1.68 ± 0.14	1.78 ± 0.07	1.79 ± 0.05
12	1.75 ± 0.15	1.71 ± 0.26	1.70 ± 0.33	1.70 ± 0.23
24	1.88 ± 0.05	2.16 ± 0.55	1.79 ± 0.04	1.79 ± 0.17

[a]Concentration of estradiol used in each incubation.

Table 2 Effect of Two Polymers (L and H) on the Estradiol PC Between PHSC and Water

	LogPC$_{PHSC/water}$ (mean ± SD, $n = 5$)		
	Estradiol concentration (μm/mL)		
Polymer concentration	2.8	0.28	0.028
Polymer H (hydrophilic polymer)			
10%	2.31 ± 0.22[a]	2.36 ± 0.14[a]	2.13 ± 0.07[a]
5%	1.93 ± 0.10[b]	2.06 ± 0.21[b]	1.94 ± 0.06[b]
1%	1.71 ± 0.10	1.61 ± 0.19	1.59 ± 0.26
Polymer L (lipophilic polymer)			
10%	1.74 ± 0.10	1.65 ± 0.07	1.61 ± 0.14
5%	1.70 ± 0.20	1.62 ± 0.17	1.65 ± 0.09
1%	1.59 ± 0.19	1.57 ± 0.15	1.71 ± 0.07
Control (no polymer)	1.62 ± 0.14	1.68 ± 0.11	1.71 ± 0.13

[a]Statistically significantly different from control ($p < 0.01$).
[b]Statistically significantly different from control ($p < 0.05$).

The results in Table 2 show that polymer L had no effect on the estradiol PC between PHSC and water. This result was consistent for all three concentrations of estradiol. Polymer H showed a significant increase ($p < 0.01$) in log PC for estradiol concentrations of 2.8 and 0.28 mg/mL; this increase was dependent on the polymer concentration. At 10% polymer H, PC was increased by a factor of 2, and at 1% polymer, there was no statistical difference from control, untreated values. At the lowest estradiol concentration (0.028 μm/mL), the increase in PC was not as prominent, although still significant ($p < 0.05$) at 10% and 5% polymer. These results suggest that no polymer adhered to the PHSC during the 30-minute preincubation, or was completely removed by washing. The hydrophilic polymer H had a statistically significant effect ($p < 0.05$) on the estradiol PHSC/water PC, with increases of up to sixfold observed. The binding also exhibited a dose concentration-dependent response. This dose dependency is indicative of polymer binding to the SC and estradiol preferentially "binding" to the polymer rather than water. In addition, the dose dependency may be due to hydrophilic nature of polymer H, which can "swell" in the presence of water, hence absorbing additional water containing estradiol.

The data show a promising effect for a polymer in increasing the partitioning of a chemical of intermediate lipophilicity (be it a fragrance or other cosmetic ingredient) into the outer layers of the skin. Thus, a macromolecular chemical has the potential to alter the first step in percutaneous absorption, the partitioning of a drug or fragrance from its formulation into the skin. The data also show that PHSC may be a simple and cost effective method to screen macromolecules for potential improvement in percutaneous delivery. However, the consistency of this effect with other chemicals, of differing physicochemical properties, must first be established before firm conclusions can be drawn. These data derived from a model compound—estradiol—possibly to likely will be relevant to cosmetic/cosmeceutical ingredients of related physical/chemical properties. In addition, the newly designed glass tubes have proven to be a quick and reliable method for the determination of PHSC/water PCs.

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75 General Concepts of Ethics in Human Testing

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COSMETICS MUST BE SAFE

According to the European Union (EU) Cosmetics Directive (Council Directive 93/35/EEC, article 2), cosmetic products must not cause damage to human health when used under normal and foreseeable conditions of use. This is in line with the expectations in society and among consumers. Cosmetic products must be safe.

Side Effects Do Occur

However, on the other hand, consumers also know that they may experience adverse reactions related to the use of cosmetic products. Usually, the reactions are so mild that the consumers do not seek medical advice, but just stop using the suspected product or change to alternative products. A 20-year old questionnaire survey, including about 1000 individuals from the normal population in United Kingdom, showed that 8.3% claimed to have experienced adverse reactions from the use of cosmetics (1). About 1% of the study group were later shown to have allergic patch test reactions to a cosmetic ingredient. A Danish study evaluated sensitization to cosmetic ingredients in an unselected population, comprising 567 subjects patch tested with ready-to-use patch test kits (TRUE test panel 1 and 2). It was found that 3.2% of males and 4.2% of females were sensitized to cosmetic allergens in this series of 24 common contact allergens (2). From dermatological clinics also it is well documented in eczema patients that cosmetic products are a common source of irritant- and allergic-contact dermatitis. Further, there are other types of skin side effects reported; such as photosensitivity, acnegenicity, contact urticaria, changes in pigmentation, and subjective symptoms as burning and stinging sensations in the skin.

Debate on Cosmetic Safety and Ethics of Testing is Needed

This is the background for the discussion on ethics and safety issues in relation to cosmetic testing. If we do not face the ethical problems, the impression may be that they do not exist! The key notions of the scientific community are therefore an open debate among peers, enforcement of law and regulations, training of authorities, industry and safety assessors, and information to the public and debate.

TESTING OF COSMETIC PRODUCTS IS NOT COMPULSORY, BUT MAY BE WARRANTED

The EU Council Directive 93/35/EEC regulates the manufacture and marketing of cosmetic products in the EU. In theory, cosmetic ingredients and products testing can be performed in vitro, in vivo in animals, and in humans.

Safety Tests

Testing of products is not required, because in general the toxicity profile of a cosmetic product can be derived from the knowledge of the toxicity of cosmetic ingredients. The directive contains positive and negative lists of cosmetic ingredients. Most ingredients are tested according to the methods required for industrial chemicals as given by the EU or OECD, and certain types of ingredients as preservatives, UV filters, and colorants need approval prior to their inclusion in cosmetic formulations. However, industry wants additional information on the finished products under certain circumstances; for example, to test for compound effects caused by interaction between ingredients, which may change the toxicological profile of the product.

Tests to Support Claims

On the other hand product testing may also be warranted by industry to document claimed efficacy and to support marketing and not just to provide assurance about the safety of the product.

No "Cookery Book" Testing

There is no "cookery book" description on how to test cosmetic products. In predictive testing, in general, it is important to recognize that a test method only gives answers related to the type of side effect it is developed for!

Possible adverse reactions include skin irritation, contact allergy, photo-mediated reactions, acne, contact urticaria, pigment changes, hair and nail changes, subjective symptoms, and various end points measured by noninvasive techniques (elasticity, skin thickness, wringling, roughness etc.). Therefore, it is not possible to make a complete list of current testing methods. The design of the test protocol depends on the specific question asked. In each case the reasoning and the scientific background of the test should be given.

Animal Tests

For more than 50 years, animal assays for skin irritation and skin sensitization have been routinely used with success (3). They have proven good predictivity for significant skin sensitizers and corrosive and moderately irritant substances. However, the animal assays have limited discriminative power, when it comes to mild effects, as the ones expected from cosmetic ingredients and products (4). When it concerns skin irritation, the animal skin with important structural and physiologic differences compared with human skin simply lacks the multitude of reaction patterns possible in human skin. Until recent years, laboratory animal-test methods have been used for testing of cosmetic ingredients and products in spite of the inherent difficulties related to extrapolation, from animal-test data to human–consumer exposure risk. However, the 7th amendment to the Cosmetics Directive [2003/15/EC] says that safety of cosmetic ingredients and products in the future must be assured without the use of animal tests. The consumer council in Brussels has managed to reach a qualified majority agreement on the proposal to ban:

- The placing on the market after 2013 of cosmetic products, of which the final formulation is tested on animals, as well as cosmetic products containing ingredients tested on animals for those cases where validated alternative test methods are available.
- Testing final products on animals.
- Testing of ingredients or combinations of ingredients on animals, when such tests can be replaced by one or many alternative methods figuring in Annex V of Directive 67/548/EEC or in Annex IX of the cosmetics directive.

Until 2007, Annex IX of the cosmetics directive contains no alternative methods that offer consumers a degree of protection similar to the animal tests that they aim to replace. The validation process for alternative methods is complicated and time consuming, so the definitive ban is postponed until further notice. New in vivo animal data for cosmetic ingredients and formulations will not be generated. The agreement tries to strike a balance between human health and animal welfare, while abiding by World Trade Organization rules. This change could result in animal testing moving into third countries instead of avoiding them. This is because cosmetic products tested on animals outside the EU could be sold in the EU without any restrictions. As a consequence, this measure could take the pressure from the authorities and the industry to further develop and adopt alternative methods.

HUMAN SAFETY TESTS

Ethical Considerations

The risk to a volunteer participating in cosmetic testing cannot be weighed against a benefit, as is the case when testing of pharmaceuticals. Finished cosmetic products must be shown to be essentially safe prior to human exposure. Transient effects may be ethically acceptable, such as

slight irritation, whereas adverse and permanent health effects, such as sensitization or scarring, are not. A *minimal risk* is acceptable (5). This is equivalent to daily life situations where there is either a chance of a trivial reaction, such as slight eczema or sunburn, or where there is a minimal risk of a serious disability or death, comparable with that of being a passenger in a scheduled airline flight. The Food and Drug Administration has also defined minimal risk, as the probability and magnitude of harm or discomfort not greater than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests.

On the other hand, it is not sufficient to satisfy requirement for minimal risk, recruitment, and informed consent of volunteers. Clinical studies should aim to answer an experimental question that cannot be answered in any other way. The test results should add value to the understanding of the product toxicity and benefit the consumers in terms of additional safety. Studies with ill-defined aims and poor experimental design will be of no value to the product risk assessment and may just generate a false feel-good factor, and the results may be used as an unethical basis for marketing purposes. There is a gray zone to efficacy testing that can only be performed when there is evidence that the product does not cause local or systemic adverse reactions. Market research trials may give additional confidence in the degree of tolerance in the consumer population. Any adverse reaction data from such human volunteer market research trials may be useful in the evaluation of product compatibility, but this should not be the primary purpose of the trial design. The rationale for human volunteer use in cosmetics testing must be continually challenged to ensure good clinical practice and ethical justification.

Notes of Guidance

In the "Notes of Guidance for Testing of Cosmetic Ingredients for their Safety Evaluation" (6), published by the Scientific Committee on Consumer Products (SCCP), human data are mentioned as a possibility when evaluating the safety of cosmetics. However, the document does not specify these in detail. Regarding skin irritation, the SCCP considers that at present human testing of cosmetic ingredients should not be preferred to animal testing.

In this context, the scientific and ethical considerations for testing cosmetic ingredients on human subjects need to be defined more clearly. The skin irritancy reaction in humans is not an absolute measure and must be related to appropriate controls defining the range of response.

Prerequisites for Human Testing

At the European Community level, there is virtually no formal legislation addressing human experimentation.

A number of points shall be considered in relation to human tests (7):

1. Tests in animals or validated alternative methods are of limited value regarding their predictive value for exposure of a human population. Therefore, confirmatory safety tests in humans may be necessary scientifically and ethically provided that the toxicological profile of a compound is available on the basis of animal or alternative methods.
2. Confirmatory testing of compounds in humans must only be undertaken when there is adequate information to suggest that a high degree of safety is to be expected.
3. Confirmatory tests of ingredients/products in humans must be limited to situations where no irreversible damaging effects are to be expected for the volunteers, and where the study goal is reasonably achievable with a study population of limited size.
4. Human volunteers should not be employed in investigation for eye irritation and sensitization or other toxicological trials where the outcome may be irreversible.
5. The recruitment of human volunteers should be in line with the "World Medical Association Declaration of Helsinki" in its current revision: Human testing is to be conducted and monitored under the direction of relevantly trained personnel to ensure the health and well being of volunteer subjects involved in the testing. The health and welfare of the subject has first priority and is highly protected.
6. "*The Good Clinical Practice for trials on Medicinal Products in the European Community*" is a valuable guide.
7. National regulations regarding human studies should be followed.
8. Test protocols should be submitted to the responsible ethical committee.

Procedure of Irritancy Assessment in Human Volunteers

On the basis of a low irritation potential as proven by animal or future validated in vitro methods, the skin tolerability of a substance can be confirmed by testing in human volunteers. A number of test protocols are available, such as open- and closed-patch tests, single- and repeated-exposure tests, and use tests. They should be chosen on the basis of the relevant use pattern of the ingredient (8).

- In the open test, the substance is applied on the skin without occlusion for time periods between 15 minutes and 24 hours. This test allows the assessment of concentrated products.
- In closed-patch tests, diluted or undiluted products are applied under occlusive chambers over 24 or 48 hours. The test allows the comparative study of substances in the same individual.
- Cumulative or repetitive closed-patch tests involve applications on the same test site between one and seven times per week over a period of one to five weeks. These repetitive tests allow the assessment of cumulative irritation that is missed by single application tests.
- Used or repeated open application tests (ROAT) imply the repeated application of a substance closely modeled to the use situation.

While these tests historically have been assessed by clinical methods, noninvasive bioengineering technology such as measurement of transepidermal water loss or of blood flow may provide higher sensitivity and objectivity to these tests, and thereby reduce the exposure and risk to volunteers.

However, neither the above confirmatory tests nor the use of bioengineering methods have been validated according to modern scientific criteria (9).

Assessment of Sensitization Potential in Human Volunteers is Discouraged

Sensitization potential has also been investigated using human volunteers, and the development of animal sensitization tests has been partly based on comparison with human tests performed with the same chemicals (10). Further, human testing has the advantage that extrapolation of the test results from one species to another is avoided.

Human predictive skin sensitization tests have been in use for the past 50 years. They have been used more widely in the United States than in Europe. Contract laboratories have performed the vast majority of human sensitization tests, and the scientific literature contains a limited number of publications giving results from tests with cosmetic ingredients as preservatives and fragrance chemicals. There are a number of different human sensitization tests available. They vary with regard to the number of induction patch tests, the placing of the patches, and the use of a maximization step. However, it is not entirely clear how useful these variations are because validation of the tests has not kept pace with development of new tests. The human sensitization tests require great experience in design and execution of the test, and a number of artifacts are possible.

Three different approaches, for predictive testing in man, have been in use:

1. A single induction/single challenge patch test
2. Human Repeated Insult Patch Tests (HRIPT)
3. Human Maximization Test

The performance of the different test methods depends on a number of factors, including type of test substance (ingredient or finished product), chemistry and animal toxicological data available, and intended use of the product.

Concerns Regarding the Use of Human Volunteers for Predictive Allergenicity Tests

Cosmetic ingredients identified as sensitizers in animal assays or other validated assays, when existing, should not be studied in humans. The human sensitization tests are time consuming and very expensive because a large number of volunteers (150–200) are required in each test; however, considerably less number of volunteers (25) are required for the human

maximization test, which, as the name says, maximizes the response to a certain degree. The argument for reducing the number of volunteers in the human maximization test is the amplifying step introduced by treatment with an irritant test product or sodium lauryl sulfate. Further, the selection of human volunteers usually results in the use of an inhomogeneous test group (compared with the more homogeneous group used for animal experiments). The large numbers of participants in most of these tests are necessary to reduce the 95% confidence interval for the test result, otherwise the likelihood of unpredicted responses in the consumers increase. If, for instance, no positive reaction occurred in 100 induced test subjects, then for statistical reasons up to 36 of 1000 consumers may react.

In any case, it is scientifically inadequate and unethical to perform predictive tests with a number of subjects insufficient to produce valid data.

The performance of human sensitization tests raises ethical considerations, in particular concerning the risk for the volunteers, especially the risk that a patch test sensitization elicits a clinical disease in the subject.

In the literature, there is no answer on the consequences of such testing on human volunteers. A request of information about the risk involved was sent to COLIPA in December 1998. The answer dated March 22, 1999 gave the following information provided by member companies:

- Dermatological testing to confirm skin compatibility is common practice; data on 470,000 human volunteers covering 2000 products did not reveal any positive results identified as due to sensitization.
- Reported data covering HRIPT tests carried out during the last 10 years and related to 2044 different products tested on a total of 136,765 persons showed 123 cases of probable/confirmed sensitizations.

In conclusion, a risk for human volunteers cannot be excluded. There is still a lack of information on the severity and frequency of adverse effects.

Minimal Requirements for Human Testing for Other Purposes

One or more clear hypotheses should be stated in the study protocol. As a result of the study, these hypotheses will be refuted or accepted. Only a clear a priori statement of hypotheses will allow the choice of an appropriate study design, an appropriate study sample, and choice of the appropriate statistical methods.

Study Design

The design of skin compatibility studies depends on the study problem investigated. Many study designs have been described and successfully used in the past, even though there are no protocols standardized and validated according to strict criteria. The study design will depend on

- kind of cosmetic ingredient or mixture of ingredients tested;
- anticipated use of the ingredient or mixture of ingredients in cosmetic finished products; and
- kind of skin compatibility problem to be assessed (e.g., immediate or delayed effects, acute or cumulative effect).

All valid study designs for compatibility studies have to include negative and positive controls and the vehicle (blank). The scientific criteria for the choice of the study design should be clearly stated in the protocol.

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76 Values and Limitations of Bioengineering Measurements

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In the dermato-cosmetic world, evaluation of the skin looks at first glance rather easy since the skin is very accessible for clinical evaluation (1,2). Visual examination reveals color, dryness, oiliness, roughness, ageing, etc. Tactile evaluation by feeling reveals also dryness and oiliness, plus roughness, firmness, laxity, ageing, etc. Even olfactory examination can be carried out, which reveals information on perspiration, microbial colonization, and antiperspiration. As an example, human vision is a very sensitive sensory and neurophysiological process. Experience shows that we have a high sensitivity and discriminative power at low intensities of luminosity. Our color discriminative power is so high that 0.5% to 1% differences in color can be easily detected. Our sensitivity at higher intensity of luminosity is much less present. On the other side, this neurophysiological process is very limited, subjective, mostly qualitative, highly observer dependent, and there is no precise memorization of color in function of time. The sensitivity in the visible region is not constant in function of wavelength with a maximum of sensitivity in the green color (490–550 nm). On the contrary, the use of tristumulus reflectance colorimeters using the CIELAB L*a*b* color system, which is internationally accepted, delivers a quantitative, accurate, and reproducible analysis of the skin color and can be calibrated with standard color charts (3).

Morphological and histological informations about the different layers of the skin can be obtained by the very invasive conventional biopsies that provide information of the full skin layers down to the hypodermis (4). Skin biopsies could be envisaged in dermatology but for ethical reasons are not acceptable in the cosmetic domain.

The development of novel, ingenious, noninvasive bioengineering measuring and imaging devices as tools in the evaluation of the skin has known an enormous development within these last years. They are used in clinical and fundamental dermato-cosmetic research. The aim of bioengineering measurement strategies is also to fullfil the legal requirements concerning safety and validation of claims, mainly with the ban of animal testing on finished cosmetic products. Directive 93/35/EEC requires that evidence is provided to support efficacy claims for marketed cosmetic products (5). Although the Directive does not specify how this kind of information should be obtained, more and more efficacy claims are supported with bioengineering measurements.

The advantage of bioengineering and imaging techniques is that almost all the aspects and properties of the living skin and also the changes of the skin in health (ageing) or in pathology (e.g., psoriasis) are now measurable and quantifiable based on objective techniques, and this is done in a noninvasive way, with no pain or inconvenience caused to the subjects (1). The noninvasive methods are by nature objective, quantitative, generally investigator independent, and they can be calibrated and validated in vivo and/or in vitro on skin model systems.

About 20 years ago, Lévêque (one of the founders of the noninvasive bioengineering) was already impressed by the availability of noninvasive devices (an estimation of about 20 different devices in 1989). Currently the number is probably far above 100 to 150. The second edition of the *Handbook of Non-Invasive methods and the Skin* edited by Serup, Jemec, and Grove was published in 2006 and counts more than 100 chapters devoted to bioengineering measurements and methods (6).

Many of these instruments or techniques are nowadays commercially available (not always at affordable cost) and adapted for routine measurements. However, some of the recent devices are still experimental, developed by skillful engineers in research laboratories, and sometimes requiring experienced scientific operators.

The aim of this chapter is to critically investigate not only the principle of bioengineering methods and the advantages of testing with a bioengineering instrument but also their

limitations in applications. Problems concerning the standardization and validation will be evoked using some specific examples such as in relation with hydration and mechanical properties of the skin.

ADVANTAGES IN USING BIOENGINEERING INSTRUMENTS

The noninvasive methods are by nature objective, quantitative, generally investigator independent, can be calibrated and validated, and they are also developed to work on human volunteers. If bioengineering measurements are properly performed, under standardized conditions, then they can lead to a harmonization of the obtained results and allow interlaboratory and interobserver comparisons (7). Another important advantage of bioengineering measurements is that some of them are able to detect subclinical effects not present visually. For example, the detection of an abnormally high level of transepidermal water loss (TEWL) that can be correlated with the appearance of skin irritation at a later stage (8,9).

STANDARDIZATION, CALIBRATION, AND VALIDATION OF THE INSTRUMENTS

Each bioengineering instrument delivers raw data, which become only valid through a process of validation involving calibration and comparison with other methods or with a reference method (2). This procedure of standardization, and calibration to validate the technique and the performances, is necessary before starting a dermato-cosmetic study. Serup (2) has defined and described some classical key terms regarding the performances and validation of any instrument: accuracy, precision, range, repeatability, reproducibility, sensitivity, etc. Each commercial instrument is in principle calibrated at the factory before reaching the customer. Furthermore, the recalibration of the instrument should be performed at regular intervals (ideally, if possible, before each set of measurements). It is strongly advised that a laboratory should use the instruments according to precisely described standard operating procedures (SOPs), which cover the calibration, validation, maintenance, performance, and condition of the equipment used with all the details of the operation procedure (10).

Most of the measurements on the skin are strongly influenced by variations in the environmental conditions. Therefore the temperature and the relative humidity in the experimental measuring room should be standardized and kept constant (11). The ambient air temperature should be kept between 19°C and 21°C and the relative humidity at 40% to 45%. The subjects should be acclimatized and conditioned in the standardized environmental conditions for at least 20 minutes. The skin test sites should not be covered with any clothes and exposed to the ambient air during this 20-minute period. The influence of seasonal variations (winter and summer periods) needs to be taken into account when performing longitudinal studies over many months. It is preferable to perform repeated measurements within one season. Finally the problem of cleansing the skin or not before starting the measurements on the skin must be considered (the presence of excess sebum could influence the measurements). If preliminary cleansing is carried out, it must be done under standard conditions and followed by sufficient acclimatization time for the skin to recover.

The elaboration of valid guidelines by independent and unbiased expert groups and the publication of relevant and reproducible test results according to these guidelines, such as those suggested by the European Group on Efficacy of Cosmetics (EEMCO) group (12–16), should be encouraged for each type of technique to promote the recognition and acceptance of specific bioengineering measurements.

DISADVANTAGES

In this paragraph, we will give an overview of some problems when investigating the skin with noninvasive bioengineering methods.

Quoting Kligman: “A fool with a tool still remains a fool” (1); actually with the marvelous development of bioengineering techniques, one should quote: “A fool with a marvelous array of tools still remains a fool.”

Single Limited Parameter

The noninvasive methods are by nature objective, quantitative, and generally investigator independent, and they can be calibrated and validated but are *narrow* based on a single physical modality of the propriety of the skin (2). As a consequence, each instrument is only able to detect and measure one single parameter of a complex skin structure, which is not sufficient for providing an overall clinical picture (7). For some investigations, it is necessary to combine two methods: the efficacy of an occlusive hydration cream can be evaluated using hydration and TEWL.

Measuring Units

The bioengineering instruments furnish as results either real physical units or arbitrary units.

Physical units are easily correlated with a physical phenomenon. For example: thickness of the dermis in millimeter; pressure applied in the suction method in millibar or Pascal, sebum quantity secretion rate in mg/hr, electrical capacitance in microfarad, etc.

Unfortunately many devices deliver nonphysical arbitrary units, which are much more difficult to correlate with a physical concept and to relate with other instruments. For example, laser Doppler and arbitrary flux/velocimetry units proportional to the speed of and the number of red blood cells. Comparing different instruments that deliver results expressed in *arbitrary units* is complex.

Calibration

The problem is that each company uses its own way to calibrate the device with its own proper standards. This procedure is not always transparent for the customers (sometimes the calibration is proprietary). Actually for many measuring techniques there is no *golden* universal standard, which can be used for standardization and validation for all instruments. Ideally, such a *golden standard* could be a simple artificial skin model whose properties are constant and known.

Claims Made by the Cosmetic Industry

When looking at the intensive advertisements and marketing done by the cosmetic industry, the very promising claims remain rather vague and are very difficult to substantiate by bioengineering methods. A few examples taken from the publicity in the cosmetic world: skin looks younger, rejuvenation of your skin, skin radiance, reenergizing the skin, the skin is invigorated, etc. How are we going to measure these claims? As an example, we will mention here the problem of the frequently mentioned cosmetic claim—skin tone or skin radiance.

Skin tone or skin radiance is an expression often used by the cosmetic industry in their publicity; more and more cosmetics are put on the market with the indication of skin radiance. The complex radiance is a reality that everybody is aware of, but difficult to describe. The evaluation of radiance is made by subjective visual observation either by the subjects themselves or by the investigators. Skin tone is the mirror of general good health, reflects emotional state (joy, sadness, stress, etc.), hormonal status (menopause), nutrition, fatigue, and age, and is also influenced by environmental factors (smoking, drinking, weather, etc.). Obviously we are faced with few objective criteria, and therefore a need exists for a bioengineering optical method of in vivo evaluation of the cosmetic radiance efficacy (17).

Mechanical Measurements on the Skin

The elastic and viscoelastic properties of the skin can be evaluated with different commercial devices: lateral torsion method (TorqueMeter®, Andover, U.K.) (18) and vertical suction method (DermaLab®, BTC-2000®, and Cutometer®) (19–24).

In the torsional method, the skin displacements in the horizontal plane can be small, and this lateral torsional movement involves only the epidermis and dermis (25). In the suction method, the mechanical stimulus is vertical to the skin surface. When applying a large probe aperture (1–2 cm) and working with a high negative suction pressure (400–500 mbar), the observed displacement involves epidermis, dermis, and may also include subcutaneous fat. Only the Cutometer presents a complete range of aperture diameter (2–8 mm diameter).

With all instruments, two type of curves can be obtained (see Fig. 1): the strain versus time curves, mode 1 (left, Fig. 1), which delivers elasticity, viscoelasticity, recovery parameters, and the stress-strain curves, mode 2 (right, Fig. 1), which allows to calculate the Modulus of

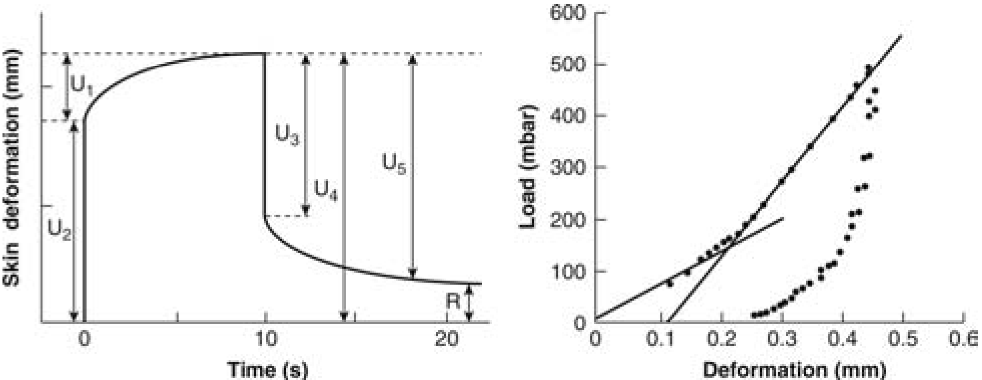

Figure 1 Strain-time curves (mode 1, *left*) and stress-strain curves (mode 2, *right*).

Young (firmness and stiffness). The Modulus of Young is normally not considered to be appropriate for a complex multilayer structure such as the skin (20). Both in vitro and in vivo uniaxial tension tests have shown that the amount of deformation grows steadily smaller so that the course of progressive extension cannot be represented by a straight line, but by a curve (Fig. 1, mode 2 in right side), (26). The calculation of Young's Modulus is complex because the skin shows a nonlinear stress-strain curve. However, this curve can be divided in three phases with more or less two linear portions. The first linear phase corresponds to the initial extension of the tissues at small loads, followed by the second linear phase corresponding to the stiffening of the dermis with increasing extension (24). The Modulus of Young can be either computed from the first linear phase (low stiffness) or from the second linear phase corresponding to higher stiffness. Considering the strain versus time curves (Fig. 1, mode 1), we are faced with the problem of the terminology of the different elastic and viscoelastic deformation parameters. A standardization in the terminology of these deformation parameters is strongly recommended (24,27). The development and further use of a universal in vitro calibration system using a simple skin model system (soft silicone polymers with known Modulus of Young and known viscoelastic properties) is strongly recommended in the future.

Hydration Measurements

Quantitative hydration evaluation of the upper layers of the skin, for example, horny layer and upper epidermis are numerous: electrical impedance and capacitance measurements (28), Fourier-transformed infrared spectroscopy with an attenuated total reflection unit, ATR-FTIR (29), and confocal Raman microscopy (30). The last two instruments, although they give quantitative data directly related to the amount of water present in the horny layer, are less used in routine clinical research due to the high price of purchase. Most routine hydration measurements are carried out using the electrical impedance/conductance properties: Dermalab (31) and Skicon® (Shizuoka-Ken, Japan) (32), impedance-based capacitive reactance: DPM Nova® (Portsmouth, U.S.A.) (33), or capacitance properties: Corneometer® (Köln, Germany) (34) and MoistureMeter® (Kuopio, Finland) (35), of an alternating electric current applied on the skin surface. It must be pointed out that the data of these instruments (electrical units or arbitrary units) are related to hydration, but not linearly proportional to the percentage of water present in the horny layer (28). Again, in vitro calibration can be carried out using simple model systems, such as cellulose filters impregnated with aqueous solutions and solutions of known dielectric or impedance properties.

Sensitive Skin

Despite the numerous different bioengineering instruments that are available and skin properties investigated, some dermato-cosmetic properties of the skin remain difficult to quantify. As an example, we would like to mention the concept of *sensitive skin*. The diagnosis of sensitive skin is defined by neurosensory hyperreactivity of the skin, and is essentially based on self-perceived sensations of people who report facial skin discomfort as stinging, burning,

and itching when their skin is exposed to some environmental factors (wind, sun, and pollution) or after application of topical products (hard water, soap, and cosmetics) (36). Epidemiological studies performed on large populations have shown that about 50% of women declare that they have self-perceived sensitive skin. Furthermore, the subjects with sensitive skin give a positive response profile, which is highly characteristic of a standardized *Sensitive Skin Questionnaire*. It appears from the literature that with the classical routine bioengineering devices it is very difficult to detect the presence of sensitive skin in subjects. Hydration, TEWL, and skin color are poorly correlated with sensitive skin (37). Only a very sophisticated device such as functional magnetic resonance imaging is capable to detect in the brain cortex specific sites involved with sensitive skin (36).

CONCLUSIONS

It is now obvious that noninvasive techniques have proven to be valuable tools for measuring objectively and quantitatively the biophysical and histological properties of the human skin; actually they are used more and more widely in dermatological departments for clinical diagnosis and therapy, and in pharmaceutical and cosmetic industries for safety and efficacy testing of topically applied product (38). Furthermore, bioengineering methods are becoming so sensitive that they are capable in well-designed cosmetic studies to show significant effects of topical products on the skin—effects that are not perceived by the consumers themselves. The noninvasive technology is harmless to the human volunteers and causes almost no discomfort. The eventual risks of bioengineering methods are concerning the substances applied on the skin: these topical ingredients could provoke irritant or allergic reactions. Most bioengineering instruments are commercially available at affordable cost. In addition, some of the recent devices are still experimental, developed by skillful engineers in research laboratories, and sometimes requiring experienced scientific operators. However, it is important to consider the limitations of the instruments used in bioengineering technology. These measurements are validated only if they are carried out under standardized conditions, if the devices have been calibrated, and if a certain number of precautions have been taken in account so that investigators in widely separated countries can obtain reproducible results and come to similar conclusions (1).

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77 The Current Regulatory Context in the European Union

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INTRODUCTION

Directive (Dir.) 76/768/EEC relating to cosmetic products (1) is a vertical legislation and every cosmetic product placed on the European market must fulfil its requirements. It would, however, be quite unrealistic to assume that this is a stand-alone piece of legislation that is not affected by other legal texts. In practice, Dir. 76/768/EEC forms part of a complex legislative process that was initiated more than 40 years ago to guarantee the free movement of goods within Europe while simultaneously ensuring the safety of the European citizens and their environment.

The current chapter provides an overview of the most relevant features of the Cosmetic Products Directive, after which the milestones depicted in Figure 1 are individually discussed in the light of their relevance to the cosmetic regulatory framework.

THE COSMETIC PRODUCTS DIRECTIVE

Definition of a Cosmetic Product

According to the European Commission Dir. 93/35/EEC, [Article 1], a cosmetic product is defined as *any substance or preparation intended to be placed in contact with the various parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance and/or correcting body odours and/or protecting them or keeping them in good condition* (2). This definition gives an indication on the target site of application of a cosmetic product and on its allowed functions (3). Thus, products such as skin creams, lotions, perfumes, lipsticks, fingernail polishes, eye and facial makeup preparations, soap products, shampoos, permanent waves, hair colors, toothpastes, and deodorants fall under the category of cosmetic products in the European Union (EU). More questionable product types such as suntanning preparations, antiperspirants, and antidandruff shampoos are also considered cosmetics within Europe, whereas this may differ in other parts of the world (4).

The Safety Prerequisite and Responsibilities

The current EU legislation on cosmetics literally states that *a cosmetic product put on the market within the Community must not cause damage to human health when applied under normal or reasonably foreseeable conditions of use, taking account, in particular, of the product's presentation, its labelling, any instructions for its use and disposal as well as any other indication or information provided by the manufacturer or his authorised agent or by any other person responsible for placing the product on the Community market* [Art.2]. The responsibility to ensure that cosmetic products are safe for consumer use is placed upon the manufacturer or his authorized agent or by any other person responsible for placing the product on the community market (2).

A qualified safety assessor, holding a specified diploma (5) in the field of pharmacy, toxicology, dermatology, medicine, or a similar discipline, undersigns the safety assessment of the cosmetic product under consideration and thus takes responsibility for the safety of the product when applied under reasonably foreseeable conditions of use.

By means of a post-marketing surveillance system the EU member states are on their turn expected to take all necessary measures to ensure that only cosmetic products that conform to the provisions of Dir. 76/768/EEC and its Annexes may be placed on the European market [Art.3] (2). Nevertheless, the ultimate responsibility for the safety of a cosmetic product resides with industry.

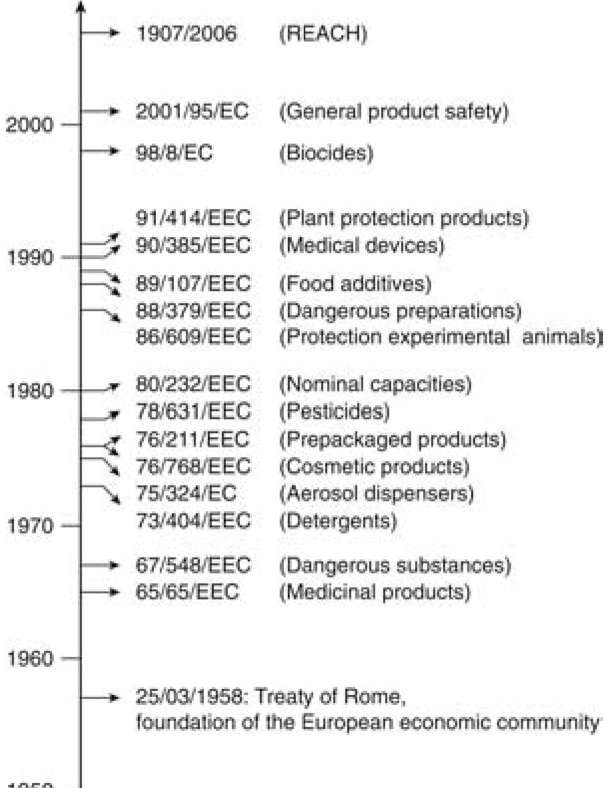

Figure 1 Overview of the major milestones in the EU chemical-related legislative process. *Abbreviation*: EU, European Union.

The Public Information Prerequisite

To optimally inform the consumer, every cosmetic product sold in the EU must contain the following information on its label [Art.6]:

1. name and address of the manufacturer or the responsible person for placing the product on the market within the EU;
2. nominal content of the finished product at the time of packaging (weight or volume);
3. date of minimal durability (products with a minimum durability less than 30 months) or an indication of the period of time after opening for which the product can be used without any harm to the consumer;
4. particular precautions to be observed in use, especially those indicated in the Annexes to Dir. 76/768/EEC;
5. batch number, enabling identification of manufacturing;
6. function of the product, unless evident;
7. a list of ingredients in INCI (International Nomenclature of Cosmetic Ingredients) in descending order of weight at the time they were added, unless they are present at a concentration below 1%, in which case they may be mentioned in any chosen order.

Moreover, the qualitative and quantitative composition of the cosmetic and the existing data on undesirable effects on human health resulting from use of the cosmetic product are enforced to be made easily accessible to the public by any appropriate means, including electronic means. Whereas the qualitative composition already features on the label (ingredient list mandatory), the quantitative composition is limited to "dangerous substances" according to Dir. 67/548/EEC (see section "Horizontal Provisions for the Protection of Animals").

The "Technical Information File" Prerequisite

For cosmetic products, the EU legislation does not foresee an extensive premarketing notification/authorization procedure involving a full toxicological dossier on the ingredients and the finished cosmetic product. Instead, the EU member states are charged with the

installation of a post-marketing surveillance system to check industry's compliance with the provisions of the Cosmetic Products Directive.

To this respect, Art.7a of the Cosmetic Products Directive imposes that the following information should be readily accessible to the member states' competent authorities (2,6):

1. Qualitative and quantitative composition of the product;
2. Physicochemistry, microbiology, and purity of the ingredients and the cosmetic product;
3. Manufacturing method;
4. Safety assessment of the finished cosmetic product;
5. Name and address of the safety assessor;
6. Existing data on undesirable effects on human health;
7. Proof of the effects claimed;
8. Data on animal testing.

The compilation of points (1) to (8) is commonly referred to as a cosmetic's technical information file (TIF) or product information requirement (PIR).

The Annexes to the Cosmetics Directive and the SCC(NF)P

Like the majority of EU Directives, Dir. 76/768/EEC is composed of the classical set of articles (definitions, responsibilities of the EU member states, safeguard clause, etc.), followed by a number of technical annexes. Five of them consist of ingredient lists:
Annex II: list of forbidden substances in cosmetic products;
Annex III: list of substances, which are not allowed to be used in cosmetic products outside the restrictions and conditions laid down;
Annexes IV, VI, and VII: lists of allowed colorants, preservatives, and UV filters, respectively, accompanied by their maximum levels and/or conditions of use in cosmetic products.

The content of these Annexes is regularly updated through amendments or adaptations to technical progress of the Cosmetics Directive. The cosmetic legislation charges the EU member states with the designation of a competent authority responsible for checking that every cosmetic product's composition complies with the provisions laid down in the above Annexes [Art.4] (2).

For the safety assessment of the ingredients appearing on the Annexes, the Commission is assisted by the Scientific Committee on Consumer Products (SCCP), previously called the Scientific Committee on Cosmetic Products and Non-Food Products intended (SCCNFP) for consumers. The SCCP forms part of DG SANCO[a] and owns the official mandate to provide opinions on questions concerning the safety of consumer products (nonfood products intended for the consumer). It is composed of independent scientists in the field of medicine, toxicology, pharmacy, dermatology, biology, chemistry, and other disciplines, collectively covering a wide range of expertize for this multidisciplinary committee (7). Together with the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), the SCCP provides the Commission with sound scientific advice needed when preparing policy and proposals relating to consumer safety, public health, and the environment. In addition, the Inter-Committee Coordination Group (ICCG), consisting of the chairs and vice-chairs of SCCP, SCHER, and SCENIHR, warrants harmonization of risk assessment and deals with questions, which are common to more than one committee, diverging scientific opinions and exchange of information on the activities of the three committees.[b]

In the EU, the safety of cosmetic ingredients is guaranteed by two operative channels, namely (8):

1. The safety evaluation of cosmetic ingredients to be taken up in the Directive's Annexes II, III, IV, VI, or VII, evaluated by the SCC(NF)P, and benefiting from an

[a]Directorate-General Health and Consumer Protection.

[b]http://ec.europa.eu/health/ph_risk/committees/committees_en.htm. Accessed February 2008.

extended physicochemical and toxicological data package as set out in the SCCP Notes of Guidance (7).
2. The safety evaluation of all ingredients present in finished cosmetic products is included in the product's TIF and carried out by a qualified safety assessor.

Since for substances not taken up in one of the Annexes to Dir. 76/768/EEC (1), no specific additional data requirements apply, the availability of data depends on data requirements and data accessibility measures laid down in the other legislation(s), with which these substances have to comply.

The SCCP specifically addresses questions in relation to the safety and allergenic properties of cosmetic products and ingredients with respect to their impact on consumer health, toys, textiles, clothing, personal care products, domestic products such as detergents, and consumer services such as tattooing (7,9). In this context, the committee also performs full risk assessments for candidate ingredients to be included in the Annexes to the Cosmetic Products Directive. The SCCP is not responsible for the safety assessment of cosmetic ingredients not taken up in the Annexes to the Cosmetic Products Directive (7).

Since 1997, the opinions of the SCCP and SCCNFP are made publicly available through the Committees' Web sites.[c]

The Animal Testing Ban for Cosmetics and Their Ingredients

Since the cosmetic field is often seen as a luxury area, posing no health benefits, being innocuous, and not needing any innovation, it turned out to be a fruitful battlefield for animal protection organizations, politicians, and parliament lobbyists to introduce an animal testing ban. Although, it was clear from the start that only a limited number of animals could be saved by banning animal tests for the safety of cosmetics and their ingredients (10), the "cosmetics case" became a remarkable example of how to introduce alternative methods into legislation in a politically driven and not scientifically driven way. The Sixth Amendment to the Cosmetic Products Directive for the first time introduced the concept of an animal testing ban on cosmetics and their ingredients. More specifically its Art.4 (1) stated that cosmetic products should not contain *ingredients or combinations of ingredients tested on animals after 1 January 1998 in order to meet the requirements of this Directive*. This statement was somewhat mitigated by the provision that *if there has been insufficient progress in developing satisfactory methods to replace animal testing, . . ., the Commission shall, by 1 January 1997, submit draft measures to postpone the date of implementation of this provision, for a sufficient period, and in any case for no less than two years, . . .* (2). The mentioned date of implementation was postponed twice (11,12).

Nevertheless, as a result of the limited progress in alternative method development and with the clear aim of pursuing the abolishment of animal testing for cosmetic products, the Seventh Amendment (6) to Dir. 76/768/EEC introduced explicit marketing and testing ban provisions for cosmetic products and their ingredients. More specifically, from 11 September, 2004, onward, animal experiments with finished cosmetic products are subject to an absolute ban, whereas a testing ban on ingredients or combinations of ingredients applies step by step as soon as alternative methods are validated and adopted, but with a maximum cutoff date of 11 March, 2009, irrespective of the availability of alternative nonanimal tests.

In addition, a marketing ban applies step by step as soon as alternative methods are validated and adopted in the EU legislation. This marketing ban will be introduced at the latest on 11 March, 2009, for all human health effects with the exception of repeated-dose toxicity, reproductive toxicity, and toxicokinetics. For these specific health effects, the deadline of 11 March, 2013, is put forward, irrespective of the availability of alternative nonanimal tests.

Proposal for a Recast of the Cosmetic Products Directive

Quite recently, the European Commission published a proposal for a regulation on cosmetic products (13), the so-called recast of the 32 year-old Cosmetic Products Directive (1). This recast is meant to bring together the original directive with all its amendments, simultaneously introducing some substantive changes to the individual texts when

[c]http://ec.europa.eu/health/ph_risk/committees/sccp/sccp_opinions_en.htm, http://ec.europa.eu/health/ph_risk/committees/04_sccp/sccp_opinions_en.htm, and http://ec.europa.eu/health/ph_risk/committees/sccp/sccp_opinions_en.htm. Accessed February 2008.

incorporated. Since the recast is at the Commission proposal stage, it requires extensive discussions between the member states and within the European Parliament, implying that it will not remain unchanged. Nevertheless, it is useful to provide an overview of the major changes that are currently introduced. It should, however, be noted that the list below is not exhaustive and that it cannot be foreseen which of the provisions will actually be taken up in the final version of the regulation.

Moving from a Directive to a Regulation

One of the main goals for the recast being simplification of the administrative procedures related to the Cosmetic Products Directive, the text proposed aims at becoming a "regulation on cosmetics." European regulations have the advantage that they are binding in their entirety and are directly applicable in all member states, whereas directives need to be transposed into the national legal frameworks of the individual member states. With the 27 member states Europe currently counts, regulations automatically represent a major administrative simplification for the member states.

The articles of the original directive have been reorganized into chapters displayed in a logical order.

Introduction of a Set of Definitions

The recast aims at clarifying a number of issues for which legal uncertainty exists. Therefore, definitions for terms such as "manufacturer," "importer," "placing on the market," "making available on the market," "harmonised standard," "traces," "preservatives," "colourants," "UV filters," "(serious) undesirable effect," "repeal," and "withdrawal" are introduced in Art. 2, and some definitions of different cosmetic product types, such as "rinse-off product," "leave-on product," "hair product," "skin product," etc., are included in a preamble to Annexes II to VI. This preamble would replace the original Annex I to the Cosmetic Products Directive (1), which contains a non-exhaustive list of possible cosmetic product types.

One Single European Notification and a Strengthened Market Control

The proposed recast introduces a single centralized electronic notification of certain information concerning the product placed on the market. Instead of having to notify in every individual member state and needing to comply with all the national provisions (e.g., communication to poison control centres), the recast now foresees one single notification and one single poison control communication at the European level.

The member states are responsible for in-market control and in case of noncompliance, some specific possibilities for actions to be taken are mentioned in the recast (e.g., the introduction of penalties).

New Provisions for CMR Substances

Substances classified as carcinogenic, mutagenic or toxic to reproduction (CMR) category 1 or 2 according to the principles of Dir. 67/548/EEC (14) are actually prohibited for use in cosmetic products (6). The basic principle would remain unchanged, but the recast opens more possibilities in the sense that *there should be a possibility, in the exceptional case where these substances are legally used in food and no suitable alternative substances exist, to use such substances in cosmetic products if such use has been found safe by the SCCP*.

Introduction of Harmonized Standards

Throughout the text, reference is made to the use of harmonized standards. This implies that the Commission considers further development of European standards for analytical methods, claim substantiation, etc., enabling insurance of product compliance in these fields.

Clarifications on the Safety Assessment of Cosmetic Products

The TIF or PI(F) would be called the "cosmetic safety report." A newly created Annex I to the regulation would contain some guidance on the content of this report. A responsible person ensuring that the cosmetic safety report is kept up to date is to be designated.

The qualifications of the safety assessor are specified within the text and allow safety assessors also from outside Europe to sign the cosmetic product safety assessment.

"INCI" Becomes "Name of Common Ingredients Glossary"
The recast replaces the original INCI[d] list by the so-called common ingredients glossary. This glossary is described to contain the names of relevant cosmetic ingredients (~10.000), but not to constitute a list of authorized cosmetic ingredients. This is the same definition as was given for the INCI list, meaning that only the name has changed.

However, it must be emphasized that this new regulation on cosmetics is in a preliminary stage. It still needs to be discussed by the EU member states and the European Parliament, meaning that some adaptations are expected. This brings the possibility of a final version beyond 2009. The only certainty seems to be that all statements related to the animal testing ban as mentioned in the current cosmetic legislation (6) are precluded to be changed.

RELEVANT "VERTICAL" EU LEGISLATIONS

In parallel to the Cosmetic Products Directive, some other important legal milestones deal with the protection of human health with respect to specific types of substances. These so-called "vertical" legislations are depicted in Figure 2.

Although they all appear to function independently from the cosmetic legislation, there are some chemicals, which are regulated by more than one directive. Because of these intersections between the Cosmetic Products Directive and other vertical legislations, the following points address the relevance of each of them. Previously, we placed it in the light of data generation through the provisions of the above directives/regulations (8). Here, we focus on the existing intersections with the cosmetic field.

The Dangerous Substances Directive and REACH

Over the past four decades, chemical substances have been regulated at the European level by Dir. 67/548/EEC (14), its amendments and adaptations to technical progress. In first instance, this chemical legislation covers the listing and review of existing substances in the EU, together with the notification of new chemicals. Basically, a new chemical substance could only be produced within or imported into the EU after having received a favorable judgment from the EU member state's competent authority to which a full notification dossier has been addressed.

Figure 2 Schematic presentation of "vertical" cosmetic-related legislations in the EU. *Abbreviation*: EU, European Union.

[d]International Nomenclature of Cosmetic Ingredients.

In a second stage, the Dangerous Substances Directive covered the rules for classification and labelling of chemical substances in the EU. This means that not only test descriptions for physicochemical (Annex V, Part A), toxicological (Annex V, Part B), and ecotoxicological (Annex V, Part C) studies were provided, but also some explicit rules to translate the results from physicochemical and/or (eco)toxicological studies into a classification involving appropriate risk and safety phrases to be mentioned on the label (Annex VI). The classification and labelling principles of Dir. 67/548/EEC are still referred to in many other EU legislative texts.

The recently published EU Regulation No.1907/2006 (15) concerning the registration, evaluation, authorisation, and restriction of chemicals, commonly referred to as "REACH," introduces some major changes in the EU regulatory framework for chemicals, some of which are as follows:

1. The reversal of the burden of proof: Manufacturers and/or importers become fully responsible for proving and ensuring that their substances are safe for use, whereas previously the member states' competent authorities equally expressed an approval for the safe use of the substance under consideration [Recitals 18,25,29, Art.4] (15).
2. The creation of a European Chemicals Agency (EChA), established for the purpose of managing and in some cases carrying out the technical, scientific, and administrative aspects of REACH and to ensure consistency at community level in relation to these aspects [Art.75] (15).
3. Protection of experimental animals: REACH intends to reduce testing on vertebrate animals as much as possible by imposing data sharing, prohibiting duplication of animal testing, and the promotion of 3R[e]-alternative methods [Recitals 1,33,36,40,47,49, Art.13(2),15,25,26(3),27, Annex VI(1.4)]. Moreover, for the highest tonnage levels (>100 tonnes/yr), testing proposals need to be officially approved before the animal experiments are initiated [Recital 64, Art.22(1h),40] (15).
4. "PBT," "vPvB," and "CMR" substances and the substitution principle: REACH describes specific procedures as well for environmentally persistent, bioaccumulative, and toxic (PBT) and very persistent and very bioaccumulative (vPvB) substances, as for CMR substances [Art.14(4),40(1),58(3)]. Moreover, every application for authorization for such a substance must include an analysis of possible substitute substances or procedures as well as an analysis of their technical and economic feasibility [Recitals 12,70,72, Art.55] (15).
5. Enforcement of restrictions: Prohibitions on substances or restrictions on certain uses were previously imposed through Dir.76/769/EEC (16), its amendments, and numerous adaptations to technical progress. They will now be taken up by REACH through a faster and simplified procedure [Recitals 23,80,84,85, Art.68] (15).
6. The flow of information up and down the supply chain: Suppliers of a substance or a preparation must provide to their customers a safety data sheet including information about any potential hazard in detailed exposure scenarios. To enable suppliers to draw up correct exposure scenarios, downstream users will need to ensure a good upstream communication on potential usage patterns [Recital 56, Art.31,32,36,37] (15).

Although most cosmetic ingredients by definition are chemicals (17), they are exempted from the classification, packaging, and labelling provisions of the Dangerous Substances legislation and REACH. Nevertheless, good knowledge on these legal texts significantly helps to estimate data availability for a cosmetic ingredient (8) and to know its legal status as a chemical in the EU.

The Dangerous Preparations Directive

As early as 1973 and 1977, solvents, paints, varnishes, printing inks, adhesives, and similar products were identified as requiring special attention, and thus rules on these categories of preparations were laid down (18,19). However, divergences in national legislations on the remaining types of preparations still constituted a significant barrier to trade within the EU and led to the publication of an overall Dangerous Preparations Directive (20,21).

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[e]Refinement, Reduction and Replacement.

Since "preparations" are defined as *mixtures or solutions composed of one or more substances* (21), numerous cosmetic ingredients fall under this category. Therefore, especially in negotiations with raw material suppliers, the cosmetic manufacturer benefits from a good understanding of the Dangerous Preparations Directive and its testing, classification, labelling, and confidentiality rules and provisions (8).

EU Legislation on Food Additives

Since food additives are intended to be ingested, this type of chemicals calls for a separate set of legal provisions and a risk assessment procedure. In Europe, food additives are regulated through a number of complementary directives (22–25) based upon the common principle that only those additives that are explicitly authorized and taken up in the official EU positive lists may be used and only subject to the specific restrictions laid down. In 2002, after a number of serious food crises in Europe (bovine spongiform encephalopathy, dioxins, and acrylamide), the general principles and requirements of food law were translated into a new regulation (26). The European Food Safety Authority (EFSA) was established to produce scientific opinions and advice for drawing European policies and legislation (inter alia the adaptations to the positive lists) and to support the European Commission, European Parliament, and EU member states in taking effective and timely risk management decisions with regard to food and food additives.

Since some cosmetic ingredients, such as flavoring and coloring agents, have also been accepted as food additives in the EU, their legal status in that field is useful to consult. In case the ingredients were found safe in the human food sector for daily ingestion, they usually make ideal candidates to be used in cosmetic products at comparable exposure levels (8).

The Biocidal Products Directive

Since biocidal active substances are intended to kill living organisms, they need to be accurately classified, labelled, and controlled to inform and protect the professional user and/or the general public. Therefore, the Biocidal Products Directive (27) deals with data requirements and risk assessments of active substances and ready-to-use end products. Herein, the classification and labelling provisions of the Dangerous Chemicals and Preparations Directives are taken over.

The intersection between the biocidal and cosmetic world mainly consists of preservatives used in cosmetics, which makes knowledge on the provisions of Dir. 98/8/EEC (27) relevant for that particular type of cosmetic ingredients (8).

The Medicinal Products Directive

The first version of a directive regulating the marketing of medicinal products in the EU was issued in 1965 (28). It has been repeatedly adapted and has been finally replaced by its current version in 2001 (29). The combination of the uncontested benefit and social value of medicines on the one hand and their potential side effects on the other hand leads to the necessity of extensive regulatory requirements.

However, in the EU, the use of medicinal active substances in cosmetics is strongly discouraged. A number of exceptions exist, but as a general rule, the intersection between active medicinal substances and ingredients allowed in cosmetics is kept very restricted (8).

The EU Legislation on Detergents

The legislation on detergents has been amended on several occasions until it was published in its final form in 2004 (30). Its focus resides on environmental aspects, viewing the chemical nature (many are anionic surfactants) of the substances concerned.

Cleansing cosmetic products typically contain different kinds of surfactants, which also form part of detergents. Therefore some knowledge on the detergents legislation may be of use, although to a more restricted level (8).

The Plant Protection Directive and the Legislation on Medical Devices

The EU Plant Protection Products Directive (31) and the Medical Devices Legislation (32,33) are taken up to complete the list, but are of inferior relevance for the cosmetic world. The intersection between cosmetic ingredients and chemicals involved in both fields is indeed very limited (8).

RELEVANT "HORIZONTAL" EU LEGISLATIONS

Besides the discussed "vertical legislations" coexisting in the EU, some horizontal directives also affect the regulatory background for cosmetics (as visualized in Fig. 3). In most cases, they are complementary to the Cosmetic Products Directive, but sometimes their provisions overrule the cosmetic legislation wherefore they certainly deserve to be mentioned.

Horizontal Provisions for the Protection of Animals

A directive commonly referred to by other pieces of legislation is Dir.86/609/EEC on the protection of animals used for experimental and other scientific purposes (34). Seeking to improve the controls on the use of laboratory animals in nearly all sectors, Dir. 86/609/EEC sets minimum standards for housing and care [Art.5] and the training of personnel handling animals and supervising the experiments [Art.7(1),14].

It also aims at reducing the number of animals used for experiments by requiring that an animal experiment should not be performed when an alternative method exists [Art.7(2)], and by encouraging the development and validation of alternative methods to replace animal methods [Art.23(1)]. The latter served as the basis for the Commission to set up the European Centre for the Validation of Alternative Methods (ECVAM) (35). Member states are imposed to collect statistical information on numbers and use of animals in experiments [Art.13].

It should be emphasized, however, that the scope of Dir. 86/609/EEC is restricted to (1) animal use in the framework of the development, manufacture, quality, effectiveness, and safety testing of drugs, foodstuffs, and other substances or products and (2) to the protection of the natural environment in the interests of the health or welfare of man or animal [Art.3]. Thus the fields of scientific research, education, and training and forensic research are not covered by this horizontal directive. Acknowledging this gap and to protect animals used in any procedure that may possibly cause pain, suffering, distress, or lasting harm, the Council of Europe published Decision 1999/575/EC (36). Herein, a number of conclusions of the 1986 European Convention for the protection of vertebrate animals used for experimental and other scientific purposes are officially approved. Basically, they defend the same principles as Dir. 86/609/EEC, but they additionally cover the neglected areas.

With the scientific progress made since 1986 and increasing political pressure on the development of alternative methods, a revision of Dir. 86/609/EEC was inevitable. Despite years of surveys and discussions on several aspects of the Directive such as scope, ethics, animal housing and care, statistical reporting, etc., allowing different parties to express their

Figure 3 Schematic presentation of "vertical" and "horizontal" cosmetic-related legislations in the EU. *Abbreviation*: EU, European Union.

opinions and concerns, a generally revised version of the Directive is not yet available. The most recent developments can be obtained through the EU Directorate General Environment (DG ENV) Web site.[f]

General Product Safety Directive

The aim of the General Product Safety Directive (GPSD) (37) is to establish a coherent level of consumer protection for all consumer products on the internal market. Thus, it automatically covers many products, which are simultaneously regulated by the provisions of the vertical legislations mentioned under 1.4. The legal provisions of Dir. 2001/95/EC are, however, intended to be fully complementary while conveniently taking up consumer products falling outside the scope of other community legislation (e.g., lighters) (38).

Out of the numerous provisions of the GPSD, the following ones deserve special attention due to their relevance to the cosmetic field (38):

1. The basic principle of the GPSD is that only "safe" consumer products are allowed to be placed on the European market [Art.3(1)]. A safe product is defined as *any product which, under normal or reasonably foreseeable conditions of use including duration and, where applicable, putting into service, installation and maintenance requirements, does not present any risk or only the minimum risks compatible with the product's use, considered to be acceptable and consistent with a high level of protection for the safety and health of persons* [Art.2(B)] (37).

   At first sight, this completely corresponds with the provision of the Cosmetic Products Directive that a cosmetic product must not cause damage to human health (1). Nevertheless, it should be noted that the GPSD goes further by also covering, e.g., mechanical injuries caused by packaging of cosmetic products.
2. The GPSD describes active post-marketing activities for producers as well as competent authorities. The producers are obliged to perform sample testing, keep a register of complaints, and inform their distributors. They also need to alert the competent authorities. The latter are expected to take the appropriate steps to coordinate market surveillance and report every consumer product health risk into the harmonized European rapid exchange of information (RAPEX) system. This allows other member states to take necessary precautions with regard to similar products. The Cosmetic Products Directive includes a market follow up requirement as part of the information that should be kept readily available to the member states' competent authorities, but does not include any mandatory filing.
3. The GPSD gives the member states the authority to withdraw products from the market in case they are found unsafe. This provision is not taken up in the Cosmetic Products Directive, which means that for a withdrawal of a cosmetic product from the EU market, reference will be made to the GPSD.

EU Legislation on Prepackaged Products and Nominal Quantities

The term *prepackaged product* covers not only a wide range of consumer products, among which a large variety of foodstuffs but also cosmetics products. As early as 1976, Dir. 76/211/EEC related to metrological requirements for prepackaged products introduced the concept of mentioning the EU-harmonized e-sign on the product label in case the metrological requirements specified in the Directive were respected (prepackages between 5 g and 10 kg) (39).

For example, the tolerated error between the actual content (measured weight/volume of product) and the nominal quantity (quantity indicated on the prepackage, i.e., the weight/volume the prepackage is deemed to contain) is not allowed to be exceeded, the nominal quantity needs to be preceded by the e-sign and displayed in correct metrological units and marked in figures of predefined sizes depending on the overall size of the package. It must be mentioned that this Directive is currently under revision.[g]

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[f] http://ec.europa.eu/environment/chemicals/lab_animals/revision_en.htm. Accessed February 2008.

[g] Details through http://ec.europa.eu/enterprise/prepack/metrol_requir/inmetrolog_requir_en.htm. Accessed February 2008.

In addition to the above-mentioned metrological requirements related to the use of the e-sign, Dir. 80/232/EEC imposes restrictions on the allowed nominal quantities for skin care and oral hygiene products, hair care and bathing products, alcohol-based cosmetics, deodorants, and personal hygiene products and talcum powders (40). However, this was considered to hamper the freedom of producers to provide goods according to consumer tastes and to hinder competition as regards quality and price on the internal market, wherefore Dir. 80/323/EEC is repealed.

From 11 April, 2009, onward, member states may not, on grounds relating to the nominal quantities of the package, refuse, prohibit, or restrict the placing on the market of prepackaged cosmetics (41).

EU Legislation on Aerosol Dispensers

In 1975, the Council of Europe drafted a Directive dealing with measures for the specific category of aerosol dispensers, independent of their content. The rationale was that viewing the presence of a gas compressed, liquefied or dissolved under pressure, aerosol dispenser's call for specific investigations. Capacities and volumes of individual powder, liquid or gas phases, flammability issues, coating of containers, and valve sealing are examples of aspects that need to be addressed before the European ∋-sign is allowed to be placed on the aerosol dispenser's label (42). Dir. 75/324/EEC (42) also covers deodorants and any other cosmetic spray.

However, it must be mentioned that this Directive is optional, meaning that member states can, under their national law, allow the marketing of aerosol dispensers not complying to Dir. 75/324/EEC, provided they do not bear the ∋-sign (43).

CONCLUSION

This chapter shows that the European cosmetic legislation foresees some clear duties, requirements, and prohibitions related to the placing on the market of finished cosmetic products. Simultaneously, however, it forms part of an extensive web of vertical and horizontal legislations intended to ensure the free movement and safe use of chemical-related substances within the EU. Basic knowledge on these individual legislative texts has become an essential tool to navigate within the European cosmetic world and understand some problems the concerned parties can be faced with today and in the near future.

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78 Trends in Cosmetic Regulations in the U.S.A.

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INTRODUCTION

The regulatory environment for cosmetics in the U.S.A. is rapidly evolving. This is not due to changes in the U.S. regulatory framework for cosmetics; rather it is the effect of globalization. Globalization impacts cosmetic regulation in two, somewhat related, ways. Firstly, as manufacturers of both cosmetic raw materials and finished products try to sell their goods in multiple markets, they must abide by the regulations in each market. The U.S. is just one of the major markets in which companies sell their products. Other large, key markets include the European Union (EU), Japan, and China. The framework developed by the EU is becoming the model towards which many other countries and regions are gravitating. Thus, if an ingredient or product is to be compliant on a global, rather than on a national or even regional basis, it must take account of EU requirements.

Secondly, another impact of globalization is rapid communication. News stories about issues and problems are rapidly transmitted between countries and regions. Unfortunately, the Internet does not assess the accuracy or validity of the information. Furthermore, interested parties such as nongovernmental organizations (NGOs) and activists in different countries rapidly communicate with and learn from each other and determine what works and what does not. The NGOs have been very effective at molding public opinion, especially in Europe, and building coalitions that can change the regulatory environment at the legislative level.

The U.S. cosmetics industry through its trade association, the Personal Care Products Council (PCPC), is working to meet these challenges. The PCPC is working in parallel with trade associations in other regions, such as Colipa in Europe, to provide a balancing opinion as well as to influence the legislative and regulatory processes. This will become an even more important initiative in the future, if the U.S. cosmetic industry is to remain innovative and, to a large degree, self-regulating.

U.S. FEDERAL COSMETIC LAWS AND REGULATIONS

The underlying regulatory framework for cosmetic products at the federal level in the U.S.A. has remained unchanged for 70 years. The Federal Food, Drug, and Cosmetics Act of 1938 (1) defines a cosmetic as:

> ...articles intended to be rubbed, poured, sprinkled, or sprayed on, introduced into, or otherwise, applied to the human body or any part thereof for cleansing beautifying, promoting attractiveness or altering the appearance.

It is the intent of the product, as defined by the claims made for its benefits, which determines whether the product is a drug or a cosmetic. One result has been the ability of marketers to sail closer and closer to this divide, making claims that suggest true physiological benefits, which would make them a drug, only to cleverly phrase the claim so it relates to appearance, making it a cosmetic. Frequently, these products are incorrectly termed "cosmeceuticals," which is a marketing term not a legal category. As one company makes a questionable claim, others rush to meet it and push the claim even closer to drug status. At some point, the Food and Drug Administration (FDA) recognizes that such advertising has crossed into drug claims and brings legal action or sends "Warning" or "Regulatory" letters. This happened in the mid-1960s with the "Wrinkle Remover" cases, and again in the late 1980s when the focus was on claims of antiaging and cell rejuvenation, and periodically since then. The cosmetic industry then recalibrates itself, learns where the new boundaries are, and

pursues other directions. The current interest in cosmeceuticals must be evaluated against the regulatory framework of only having drugs and cosmetics in the U.S.A. As there is no cosmeceutical category in the U.S., these products must fall into one of the two established statutory categories.

Another effect of the U.S. definitions of drugs and cosmetics is that a single formula can be either a drug or cosmetic, depending on the claims made. One good example is a soap containing triclocarban. If it is sold as a deodorant soap, it is cosmetic as deodorancy improves attractiveness. In contrast, the same formula being sold as antimicrobial soap, making disease prevention claims, is a drug (2). Another example is a clear antiperspirant gel made by Gillette in 1995, which was originally marketed as a deodorant. It made the claim of higher deodorancy than other deodorant products. Gillette successfully defended this claim with the National Advertising Division (NAD) of the Better Business Bureau on the basis of the antiperspirant technology being more effective at reducing body odor than that used in deodorants, which do not contain aluminum salt (3). The one exception to that general rule is where the included therapeutic ingredient, such as penicillin, is so well known that its very presence in a product would imply therapeutic, i.e., drug benefits (2).

There is no premarketing review or approval required before selling a new cosmetic in the U.S. Thus the FDA only regulates cosmetic products once they have reached the marketplace. Last year, in their review of nanotechnology, the FDA confirmed that this approach was appropriate for cosmetics (4). The FDA does have the ability to exclude ingredients that they believe to be harmful. In the past they have declared that the presence of halogenated salicylanilides such as tribromsalan (TBS, 3,4′,5-tribromosalicylanilide) or hexachlorophene in a cosmetic would result in an adulterated product. In 2005, the FDA issued a "guidance" to the effect that cosmetic products containing α-hydroxyl acids (AHA) should carry the sun burn alert, unless the manufacturer had data that showed that the increased AHA-induced UV damage to the skin would not occur (5).

There are many consumer products that carry both drug and cosmetic claims and are therefore governed by both sets of regulations. In practice, as the drug regulations are more stringent for many aspects including manufacturing (Good Manufacturing Practices, GMP) and adverse event reporting, these combination products are effectively regulated as drugs. Many of these combinations are over-the-counter (OTC) drugs, and as long as the monograph requirements are met, preapproval to enter the marketplace is not required. OTC regulations continue to slowly evolve. Some of the monographs that impact personal-care products have been finalized, such as antiacne and antidandruff. Others are still at the tentative final stage, such as antimicrobial. The monograph for sunscreen products is still a work in progress. In August 2007, the FDA proposed changes to the monograph related to UVA testing, labeling, and use instructions (6). It is not clear when these amendments will be finalized.

Like the personal-care industry, the FDA has recognized the increasing concern of some of the general public regarding the safety of cosmetics. In 2004, The Environmental Working Group (EWG) petitioned the FDA to take a more active regulatory role, including the preapproval of cosmetics before they were marketed. The FDA rejected the petition, believing that it already had sufficient authority to regulate cosmetics and ensure their safety (7). This is similar to the position that the agency took on nanotechnology. Since then, the FDA has strengthened the voluntary registration of manufacturing premises and ingredient statements, which has existed since the 1970s. The voluntary annual reporting of adverse events was discontinued in the mid-1990s and has not been reintroduced, except for those that are "Serious and Unexpected." At the same time the personal-care industry has proactively introduced a program, the Consumer Commitment Code, to make the safety assurance process for cosmetics more complete and transparent. It is described in more detail in the section "U.S. Cosmetic Industry's Response to the Changing Regulatory Environment," and is in part based on the work of the Cosmetic Ingredient Review (CIR), a board of independent dermatologists and toxicologists that reviews the safety of cosmetic ingredients. The FDA has a nonvoting liaison status with the CIR.

Over the last 40 years, several legislators have attempted to increase the role of the federal government, especially the FDA, in the regulation of cosmetics and their safety. The U. S. cosmetics industry has successfully remained, mainly, self-regulatory by continuing to develop comprehensive safety programs that meet the government's and the public's expectations, while maintaining an excellent safety record in the marketplace.

The FDA is not the only federal agency that has jurisdiction over cosmetics and the personal-care industry. The Consumer Product Safety commission regulates some aspects of product labeling under the Fair Packaging and Labeling Act (FPLA). The Bureau of Alcohol, Tobacco, and Firearms (ATF) also regulates the packaging and labeling of those cosmetic products that contain ethanol (8). The Federal Trade Commission (FTC) has jurisdiction over the fairness of advertising, especially as it relates to false or misleading claims. Recently, the FTC has become more active in ensuring compliance with advertising rules for both cosmetics and dietary supplements, especially in the areas of weight loss and hair growth where there has been a long history of questionable claims.

STATE LAWS AND REGULATION OF COSMETICS IN THE UNITED STATES

For many years, individual states in the U.S. have had laws and regulations that impact cosmetics. Primary examples of this are from California, where the volatile organic compound (VOC) regulations have changed the composition of many cosmetic products such as hairsprays. The California Proposition 65 limits the level of potential carcinogens and reproductive toxicants that a person can be exposed to each day; otherwise warning labeling is required. Such regulations can impact personal-care products across the U.S. It is difficult to control distribution of a product once it leaves the manufacturer's warehouse and reaches mass merchandisers. Therefore, many manufacturers follow the requirements of California across the entire U.S.A.

Recently, California passed the Safe Cosmetics Act (2005) (9). This requires manufacturers to disclose to the State of California the intentional addition of potential carcinogens and reproductive toxicants. These would be posted on a Website that the public can access. Currently, the State of California is working with the various stakeholders to determine the best way to implement the law. We have yet to see the law's impact on the cosmetics industry.

Frequently, other states have discussed new cosmetic laws, but most have not been enacted into law. For instance, Massachusetts is considering a law saying that one cannot sell a cosmetic product with an ingredient that the CIR has said is unsafe. In the first year, companies will have the opportunity to remove the unsafe ingredient; the following year the product will be considered adulterated. Again, modern distribution systems for their products prevent companies from segregating products and preventing their sale in a specific state. Hence, such a law will have national or at least regional impact in the U.S. This does not take account of any publicity that may arise when a product cannot be sold in a specific area because of safety concerns.

Obviously, the personal-care industry prefers to have a single set of laws and regulations to follow, rather than separate requirements in each state. Furthermore, since many of the laws related to product safety utilize information and analysis published by panels or committees of experts, industry is trying to ensure that a few lists of the highest scientific quality are used as the basis of the regulations.

IMPACT OF EU LAWS AND REGULATIONS ON U.S. COSMETICS INDUSTRY

Although they do not have the force of law in the U.S., EU (and Canadian) cosmetic regulations are having a great impact on the development of raw materials and cosmetic products in the U.S. This is because cosmetics manufacturers want to be able to develop global formulas or, at least, ones that can be sold in a major market of the U.S. and EU. To do this, the products must be compliant with both sets of cosmetic regulations, and in this way, the EU impacts cosmetics produced in the U.S. Furthermore, Canada and Mexico are beginning to take more account of EU regulations as they develop their own. In this way, a Pan-North American personal-care product, will be impacted by the EU, even if the product is not intended for distribution in Europe. Indeed, Association of South East Asian Nations (ASEAN) has modeled their new cosmetic regulations on the EU.

For ingredient safety, there has already been a degree of harmonization. The science of toxicology is the same around the world. Thus, the criteria in evaluating safety that are applied by the advisory bodies in the U.S. (CIR) and in the EU (the scientific committee on cosmetic

products, SCCP) tend to be similar. This contrasts with labeling regulations where there is far less commonality among different regions on the basis of language requirements, as well as national sensibilities and expectations around product performance claims. The exception to this is the ingredient declaration, where the INCI nomenclature is becoming the global standard.

In the future, the regulation burden on cosmetic ingredients will increase in the EU with the introduction of the REACH (Registration, Evaluation, and Authorization of Chemicals) legislation and the animal testing and marketing ban, which commences for single dose methods in March 2009. It is not clear whether the effect of REACH will be as great in the U.S. as it will be in Europe. Many raw materials will have to be REACH compliant; they will have to meet EU regulations to be sold in Europe, and precluding the EU market will significantly reduce ingredient manufacturers' return on their investments. The same will hold true for the animal testing ban and its impact on the development of new raw materials in the cosmetic industry. Even with fragrances, EU regulations can impact formulas sold in the U.S. The EU requires labeling in the presence of fragrance ingredients that are putative allergens when they exceed specific thresholds. Many cosmetic manufacturers want to avoid such labeling for, at the minimum, it can significantly lengthen the ingredient statement. If a cosmetic manufacturer is going to have a global fragrance for the global product, again, EU regulations will have an impact.

In the past, the attempts to harmonize cosmetic regulations at the governmental level have been unsuccessful. However, industry pressures such as global economy of scales have led to a de facto harmonization of ingredients in products. Currently, the International Committee on Cosmetic Regulations (ICCR), which includes industry as well as governments and other stakeholders, is working to harmonize many of the tests, methods, and procedures so single methods and assays can be used across different jurisdictions and regulatory frameworks. Issues discussed at their first meeting (September 2007) include GMP, ingredient statements/INCI, nanotechnology, in-market surveillance as well as animal testing and alternative methods. A summary can be found at http://www.cfsan.fda.gov/~dms/cosiccr.html.

U.S. COSMETIC INDUSTRY'S RESPONSE TO THE CHANGING REGULATORY ENVIRONMENT

In the U.S., the cosmetic industry association (PCPC) is responding to the pressures of the activists and others concerned with the safety of cosmetics. Traditionally, the Council, when it was known as the Cosmetic Toiletry and Fragrance Association (CTFA), focused on its relationship with the U.S. federal and state governments. However, in the last five years, the NGOs in the U.S. have been having a greater impact on state legislators and in the court of public opinion. An example of the former is the pressure that was exerted in California that resulted in passage of the Safe Cosmetic Act (2005). Seeing this trend and recognizing what has already happened in the EU could portend what happens in the U.S.; the U.S. cosmetic industry is becoming more proactive in meeting this challenge. It has realized that it needs to increase its efforts to provide accurate information to the public and the press and use this as the starting point in influencing public opinion. The result is several initiatives from the council, which include

- The Consumer Commitment Code: industry is working to make the safety assessment process more transparent to the press and public in general. The code emphasizes that cosmetic manufacturers have a solid scientific basis for the safety of both the ingredients that they use and the final product.
  - For ingredients, it is recommended that companies use the assessments of expert authoritative bodies such as the CIR, FDA, SCCP, or NICNAS (Australia's National Industrial Chemicals Notification and Assessment Scheme) as the basis of the safety assurance process. Indeed, ingredients that the CIR finds to be unsafe should be excluded from products. Ingredients with a CIR-"insufficient" data finding should only be used if the company has enough scientifically valid data to support their safe use.

    - Serious and unexpected adverse events (reactions) should be reported to the FDA in a timely manner.
    - The FDA can make written requests to review the data that form the basis of a product's safety assessment.
- The council has set up a Website to give accurate information to the press and public in a way that is easier for them to understand. Its address is www.cosmeticsinfo.org.

These efforts are a start in the industry's goal to remain self-regulating. To do this successfully will require that industry effectively manage attacks from the activist NGOs. They have been active and effective in the EU. Their counterparts in the U.S. have been following their lead. The U.S. industry must watch what is happening in the EU, because with the rapid communications across the Atlantic, those regulations will be proposed in the U.S. very quickly.

CONCLUSIONS

Although the legal framework for cosmetic regulation has not significantly changed in the U.S. for 70 years, in practice, the U.S. regulatory environment is evolving rapidly. With globalization of raw materials and cosmetic formulas, the U.S. cosmetic industry is impacted by regulatory regimes from the different regions in which the ingredients and products are sold. This is especially true for the framework developed in the EU, which appears to be becoming the international benchmark. Many countries and regions are following the EU's model, or at minimum, incorporating some of the EU's approaches in their regulatory framework. Therefore, it is important for U.S. companies and industry to work closely with, and support their EU counterparts, as the Europeans try to influence their legislative and regulatory processes.

Green and other activist groups have been more politically influential in Europe than they are in the U.S. However, in the last few years, the U.S. NGOs have become more vocal and successful in influencing public opinion and legislators. This resulted in the passage of the California Safe Cosmetics Act (2005). The U.S. personal-care industry has not been willing to cede the "court of public opinion" to the activists. The PCPC (industry association) has developed a website (www.cosmeticsinfo.org) to provide accurate information to the public, and especially to the press. Additionally, the PCPC is working to make the product safety assurance process more transparent, through the introduction of the Consumer Commitment Code. In contrast, the FDA is not changing its approach; instead it is using the current regulatory structure to respond to scientific advances such as nanotechnology and to pressure from the activist NGOs.

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