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LIFE-LIKE FORMS IN METEORITES AND THE PROBLEMS OF ENVIRONMENTAL CONTROL ON THE MORPHOLOGY OF FOSSIL AND RECENT PROTOBIONTA

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BARTHOLOMEW NAGY

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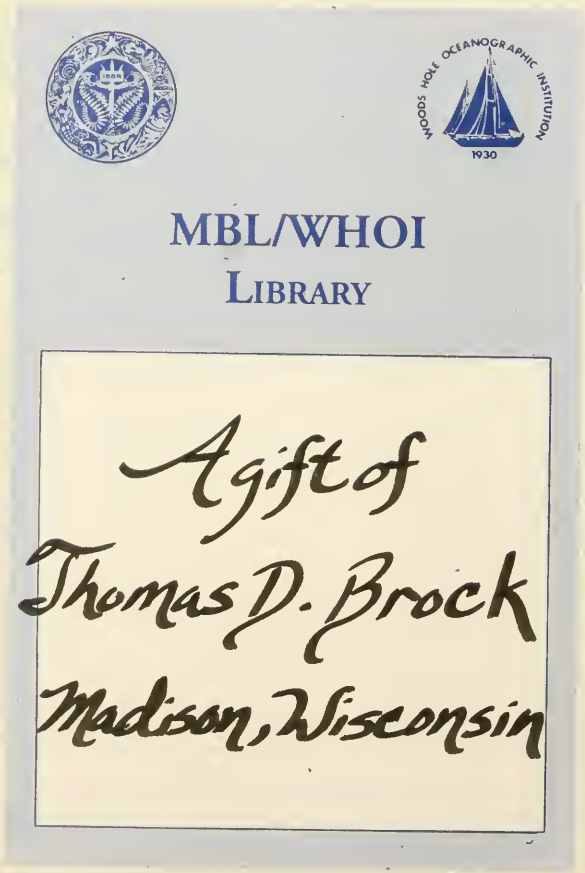
* This series of papers is the result of a conference on *The Problems of Environmental Control on the Morphology of Fossil and Recent Protobionta* held by The New York Academy of Sciences on April 30 and May 1, 1962.



INTRODUCTORY REMARKS

J. Joseph Lynch, S. J.

Seismology can contribute nothing to the problem of extraterrestrial life. One naturally wonders then why a seismologist should be called upon to open this symposium. Dr. Nagy must be blamed for that. He and I occupy offices in adjacent buildings and when either of us has a problem in Earth science we mull it over together. When Dr. Nagy first found evidence of organic fossils in the Orgueil meteorite he came to me and discussed the evidence with me. He thought that somehow I had helped him by my encouragement and as an acknowledgment insisted that I give these opening remarks.

The possibility of life outside of our planet has been a question in man's mind almost as far back as man himself. The divergence of views on the matter is about as broad as it could be. Only a century and a half ago the great English astronomer, Sir William Herschel, first President of the Royal Astronomical Society and discoverer of the planet Uranus said in one of his Presidential addresses that he was convinced that life existed within the Sun. Unfortunately he did not elaborate upon what kind of life he had in mind. The present Secretary of the same Royal Astronomical Society, Michael Ovenden, in his recent book, *Life in the Universe*, as his view states that life is probably possible anywhere in the universe except within a Sun! It would be hard to imagine two more divergent views on the same subject by members of the same society. It has even been suggested that life is older than Earth itself and came to us from another galaxy. However, confining ourselves to our own solar system, most thinkers on the subject would restrict the possibility of life—for reasons of temperature—to that part of our solar system between Venus and Mars. Beyond Venus the temperature would be too hot—beyond Mars and some of the asteroids, the temperature would be too cold. Where within this region did the fossils on the Orgueil meteorite originate?

Dr. Nagy and his co-workers in presenting their evidence for organic fossils on the Orgueil meteorite have adequately ruled out the possibility of their origin by contamination since the meteorite fell to Earth. How and where the organisms—if they were organisms—originated, are questions that this symposium should throw much light on. Did they originate on Earth and later return to Earth *via* the moon? Or did they originate on an asteroid or a planet outside of the Earth? The organizing committee deserves great credit for having brought together such a distinguished group of experts. They cover not only every phase of the subject, but represent the views of almost every country. Because you are gathered to hear their evidence and not any rambling conjectures of mine, I shall cut my remarks short and let the session chairman get the program started.

The which if you with patient ears attend,
Whence came these forms, you'll find out at the end.
(*With apologies to William Shakespeare*)

ENVIRONMENTAL BIOPHYSICS AND MICROBIAL UBIQUITY

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Since the downfall of the near-collision theory of the origin of the solar system and the revival of the dust cloud hypothesis it has generally been assumed that planetary systems must be common in the universe. There has also been a strong tendency to regard the formation of life within a planetary system as the probable outcome of a series of nonbiological events operating within a restricted range of physicochemical conditions. These points of view contrast markedly with those held even as little as 30 years ago. Few persons today would attempt to maintain that Earth is the sole place in the universe where life resides.

In spite of this drastic change in attitude and the recent reports of organized matter in carbonaceous chondrites (Nagy *et al.*, 1961; Claus and Nagy, 1961), there are still many who hesitate to believe that life within the solar system can exist beyond the confines of Earth. In relation to the question of life on Mars, for example, it is customary to find opinions clouded in a mass of delicately phrased intellectual jargon that is designed to be all inclusive and noncommittal. Much of the criticism levelled against the notion of life on Mars is made from what the self styled Soviet astrobotanist, G. A. Tikhov (1955), would term a geocentric point of view. Thus, it is often questioned whether organisms could survive the rigors of a Martian climate: an average temperature 50° C. below that of the earth; daily temperature fluctuations of about 60° C. at the equator; an atmosphere richer in CO_2, and decidedly lower in O_2 and total pressure than that characteristic of Earth; an environment in which water is scarce and in which the level of ultraviolet radiation may reach "lethal" proportions.

This, however, is absolutely the wrong approach to the question. The whole approach assumes a curious lack of adaptation on the part of the presumed Martian organisms, almost forcing them to adapt to terrestrial conditions in a Martian locality. At least two assumptions seem to be involved in the reasoning: (1) that a complete body of information exists defining the environmental limits beyond which life, as known on Earth, is impossible; and (2) that these geoenvironmental limits of life are not exceeded on a cosmic scale. The first of these assumptions is clearly erroneous as the present paper will show, and the second seems rather questionable.

My main purpose here is to summarize current knowledge and ignorance regarding the environmental boundaries that delimit the "stability field" of living matter. The problem is approached purely on an empirical basis. Most of the discussion is limited to conditions that permit growth and reproduction because this is the central question that has to be faced; however, some remarks are made concerning survival because of its pertinence to life in fluctuating environments. The review is not intended to be exhaustive, nor comprehensive in anything other than a qualitative sense; only to serve as a reminder of forgotten or little known facts concerning some of the extreme types of environment inhabited by living organisms. Attention is focussed on microorganisms

because of their great environmental and physiological diversity as compared to the so-called "higher" forms of life.

Temperature

The temperature range for growth and reproduction of different microorganisms extends from −18° to 104° C. These limits exceed those defining the stability field of pure water under one atmosphere of pressure, but they do not exceed the stability field of water in the liquid state when it is impure and under variable pressure.

Let us first consider some cases of microbial activity at temperatures below 0° C. It is important in this connection to realize that ice does not form in sea water with a salinity of 35 per thousand until the temperature drops below −1.9° C., and also that 90 per cent of all sea water has a temperature less than 5° C. It is thus not surprising to find that many marine bacteria will grow at subzero temperatures. Bedford (1933) was able to culture 65 of 71 marine bacteria from the north Pacific at subzero temperatures, and ZoBell (1934) independently showed the same for 76 out of 88 marine bacteria in his collection. Ten of the taxa cultured by Bedford (1933) were capable of growth and reproduction in nutrient-enriched salt solutions at −7.5° C. Twelve others grew at −5° C. Horowitz-Wlassowa and Grinberg (1933) found 5 bacteria that would grow at −5° C., and 14 others that grew at −3° C. Bacteria are known to multiply in ice cream stored at −10° C. (Weinzirl and Gerdeman, 1929) and on fish stored at −11° C. (Redfort, 1932).

Fungi, and probably algae as well, also multiply at these low temperatures. Thus, the mold *Sporotrichum carnis* grows at −7.5° C. and very slowly even at −10° C. (Haines, 1931). *Choetostylum fresenii* and *Hormodendron cladosporoides* also grow at −10° C. (Bidault, 1921). Tchistiakov and Botcharova (1938) similarly found several different fungi that were capable of growth at −8° C., although none of these would grow at −12° C. The flagellate *Pyramimonas* (*Pyramidomonas*?) has been observed swimming in saline water at −7.7° C. under the cover of ice in Lake Balpash, Kazakh S.S.R. (Zernow, 1944). Populations of 12 other photosynthetic forms were found in the same water, presumably also alive and metabolizing. Zernow (1944) even observed swimming *Pyramidomonas* and *Dunaliella* in drops of Lake Balpash water derived from soft ice that had formed at −15° C.

The most extreme cases of growth at low temperatures are those referred to by Borgstrom (1961) who states that some molds and pseudomonads will grow in concentrated fruit juices and sugar solutions at temperatures of −18° to −20° C. He has also observed the growth of *Aspergillus glaucus* kept in glycerol at −18° C. A report of pink yeasts growing on oysters at temperatures of −18° to −30° C. (McCormack, 1950) needs independent verification.

No experiments seem to have been undertaken on the possibility of algal photosynthesis in saline media at subzero temperatures, but such a result would not be unexpected. Although slightly out of context, it is worth noting that some terrestrial plants are able to carry out a limited photosynthesis at −2° to −3° C., and respire down to −7° C. (Zeller, 1951). In the last century, Jumelle (1891) reported that certain lichens and conifers could photosynthesize at temperatures between −20° and −40° C., but modern studies have failed

to corroborate these findings (Rabinowitch, 1945; Zeller, 1951). Before leaving the subject of growth at low temperatures it must be stressed that *in all cases the growth is slow, usually requiring weeks and sometimes months before definitive results are obtained.*

At the upper end of the temperature scale it has long been known that some bacteria and blue-green algae exist in hot springs with temperatures in the range of 80° to 88° C. For summaries of existing information the works of Copeland (1938), Precht *et al.* (1955), and Allen (1960) should be consulted.

Baker *et al.* (1955), have cultured a strain of *Bacillus stearothermophilus* at 80° C. No attempt was made to determine whether growth would still occur at higher temperatures. According to ZoBell (1958) thermophilic sulfate reducing bacteria isolated from subterranean deposits have been cultured in the laboratory at temperatures to 65° to 85° C. These forms were originally obtained from depths of 6000 to 12,000 feet, at which temperatures *in situ* ranged from 60° to 105° C. and hydrostatic pressures from 200 to 400 atmos. ZoBell (1958) also states: "The maximum temperature at which the thermophilic cultures are active is increased by compression. At 1000 atmospheres one culture reproduced and produced H_2S at 104° C. No attempt has been made to ascertain whether bacteria will grow at temperatures higher than 104° C. when compressed, but indications are highly suggestive of the possibilities in view of the protective effect of high pressure on the thermal tolerance of bacteria." The case referred to represents the highest temperature so far recorded for the growth and reproduction of any organism.

Eh and pH

The best general treatment of the environmental limits of Eh and pH for growth and reproduction is that given by Baas Becking *et al.* (1960). These workers have summarized paired Eh-pH data for the growth of diverse microorganisms in natural environments and laboratory cultures. Although the Eh values may in some cases not represent truly reversible potentials they at least give a reproducible and reasonably accurate picture. Their results are shown graphically in FIGURE 1. When the data for all microorganisms are combined and compared to Eh-pH measurements in natural surface waters of the earth, a complete overlap is observed. This suggests that there is probably no major aqueous environment that cannot be colonized by some microorganism. The range for growth and reproduction of microorganisms was found to lie between 850 mv. and −450 mv. on the Eh scale (when expressed as Eh at the prevailing pH); and between values of 1.0 and 10.2 on the pH scale. These, however, do not represent the true extremes because the authors considered only data for which paired measurements of Eh and pH were available.

Some environmental extremes of pH that can be tolerated by reproducing populations may now be cited. Thiobacilli are well known for their ability to grow in acid solutions. In fact, they tend to show optimal growth in the pH range of 1 to 3, many growing poorly above pH 7. Carbon dioxide is the sole carbon source, and energy is obtained from the oxidation of reduced forms of sulfur to sulfate under aerobic conditions. Growth and reproduction can occur at pH values in the neighborhood of 0, and cultures receiving no initial

supply of H_2SO_4 can contain concentrations up to 2.08 N H_2SO_4 at the end of growth (Starkey, 1925).

Several molds are capable of growth at a pH of 1.7 (Johnson, 1923). The most acid tolerant fungi known are *Acontium velatum* and fungus D (an unidentified member of the Dermatiaceae), originally isolated from strong acid

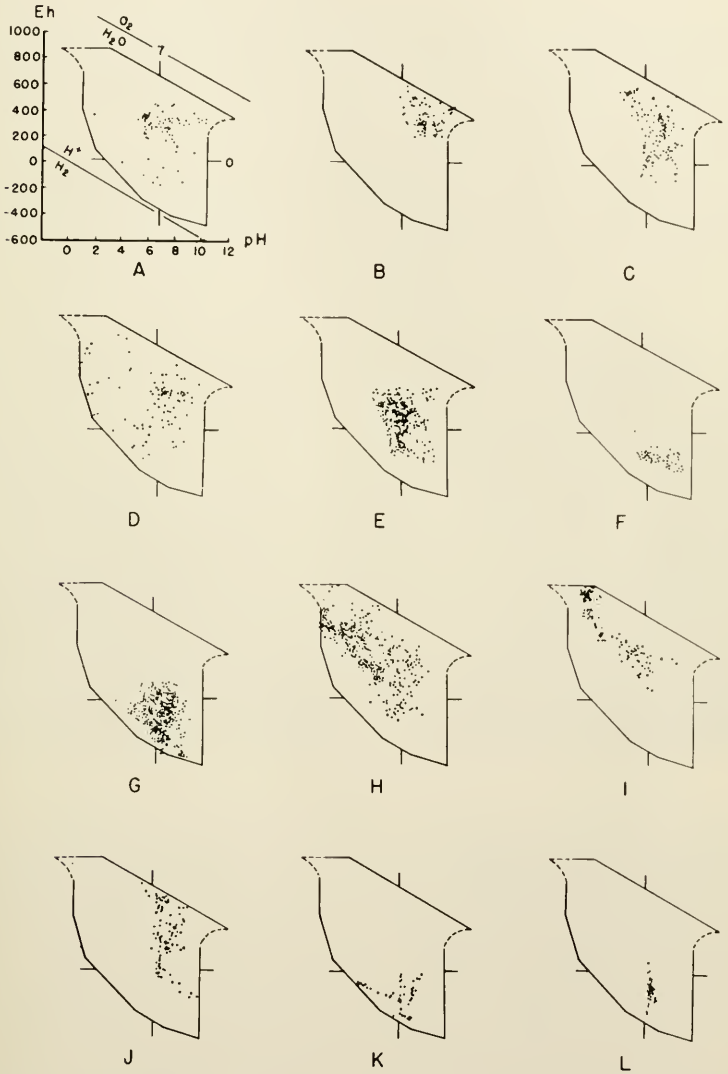

FIGURE 1. Eh-pH characteristics of diverse microorganisms. *A*, green algae and diatoms; *B*, *Dunaliella; C*, *Enteromorpha; D*, blue-green algae; *E*, photosynthetic purple bacteria; *F*, photosynthetic green bacteria; *G*, sulfate reducing bacteria; *H*, thiobacteria; *I*, iron bacteria; *J*, denitrifying bacteria; *K*, three species of heterotrophic bacteria; *L*, methane producing bacteria. Redrawn from Baas Becking *et al.* (1960). Eh is expressed in millivolts.

solutions containing 4 per cent $CuSO_4$ in an industrial plant (Starkey and Waksman, 1943). These forms grow well when submerged in nutrient-enriched sulfuric acid solutions at pH values between 0.4 and 7.0. Some growth occurs at pH 0 (2.5 normal H_2SO_4) even when solutions are saturated with $CuSO_4$. No study was made of the permeability of the cells to copper and hydrogen ions, but presumably there was little to no penetration.

One alga is notable for its growth in acid solutions, a strain of *Cyanidium caldarium* originally isolated from a hot spring containing 0.1 N H_2SO_4. Allen (1959) has cultured this form in 1 N H_2SO_4. No attempt was made to determine whether growth would still occur in more concentrated solutions or acid solutions at elevated temperatures.

At the upper end of the pH scale many microorganisms are known to grow actively at a pH of 10, some at a pH of 11, and a few others possibly at still higher pH values. Johnson (1923) reported that limiting growth of *Penicillium variabile* occurred in the pH range of 10.1 to 11.1. Two other fungi, *Fusarium bullatum* and *F. oxysporum*, were limited by pH values in the range of 9.2 to 11.2. Many alkaline lakes are known with pH values in the range of 9 to 11, and these are by no means sterile. Jenkin (1936) found populations of 13 algae, 4 rotifers, and 2 copepods living in the alkaline lakes of Kenya. In lakes Elementeita and Nakuru, in which the pH was commonly in the range of 10 to 11, large concentrations (10^3 individuals per ml.) of the blue-green alaga *Arthrospira platensis* were found (Jenkin, 1936). Still more extreme cases of growth at high pH have been reported by Meek and Lipman (1922) for *Nitrobacter* and *Nitrosomonas*. They state that these forms multiplied in solutions with initial pH values of 13.0, although not when the initial pH was as high as 13.4. These results, however, seem rather surpising because of the apparent lack of a toxicity effect due to ammonium hydroxide which would be expected for these forms under the culture conditions used. Other workers have failed to corroborate the findings of Meek and Lipman for *Nitrobacter* and *Nitrosomonas*. Kingsbury (1954) has reported that the blue-green alga *Plectonema nostocorum* will grow in solutions of Ludox (a DuPont 30 per cent SiO_2 solution) adjusted to an initial pH of 13, however the growth in this case was apparently limited to the surface.

Salinity

The range of salt concentrations tolerated by microorganisms during growth and reproduction is enormous. Kalinenko (1957) has shown that some heterotrophic bacteria will multiply in double distilled water. (The water in this case contained only 70 μg. of organic matter per liter.) On the upper side it is known that the fungi *Aspergillus oryzae* and *A. terricola* will grow in 4.1 M $MgSO_4$, a concentration equivalent to about 500 g. of salt per liter of solution (Johnson, 1923). Halophilic bacteria in nature grow abundantly in salt limans, saturated brines, and on animal hides dried with concentrated salt solutions. Even the Dead Sea with its salinity of 280 to 320 per thousand and high bromide concentration is not sterile. A small gram negative rod, a yeast-like form, and a green filamentous form were all found to grow and reproduce in Dead Sea water enriched with 1 per cent peptone (Wilkansky, 1936). Other bacteria and algae were also present. Some of the bacteria failed to grow in

media containing less than 15 per cent salt. See Clifton (1958, p. 262) for a summary of Volcani's study of the Dead Sea biota.

Solar evaporation ponds are often discolored by the growth of halophilic bacteria and algae. According to Carpelan (cited by Gibor, 1956) photosynthetic production rates in such environments are comparable to those in the most productive parts of the oceans. Gibor (1956) has shown that the osmotolerant brine flagellate, *Dunaliella salina*, grows well in 10 × concentrated artificial sea water. Some halophilic bacteria isolated from salt brines fail to grow in salt solutions containing less than 16 per cent NaCl, and will survive on dry crystals of salt obtained by the evaporation of brines (Browne, 1922). According to Gibbons and Payne (1961) the most rapid growth rates of several halophilic bacteria (*Halobacterium spp.* and *Sarcina littoralis*) occur in solutions containing 20 to 25 per cent NaCl at temperatures in the range of 40° to 45° C. ZoBell (1958) states that sulfate reducing bacteria grow naturally and can be cultured in waters with salinities up to 300 per thousand.

Pressure

The effect of varying atmospheric pressure on the growth and reproduction of microorganisms seems not to have been investigated in much detail. Strughold (1961), however, passingly refers to the cultivation of soil bacteria under an atmosphere with the composition and total pressure (0.1 Earth atmos.) of that presumed to exist on Mars. The existence of barophilic bacteria in subterranean deposits and deep sea sediments has been demonstrated by ZoBell *et al.* Most organisms living in the surface regions of Earth fail to grow and are killed by hydrostatic pressures of a few hundred atmospheres. In contrast to these, barophilic bacteria isolated from the deep sea bottom can be cultured *only* under hydrostatic pressures comparable to those in their natural environment, *i.e.*, pressures of 1000 atmos. or more (ZoBell and Morita, 1956). The viability of some barophiles is unaffected by alternate compression and decompression between 1 and 1000 atmos. of hydrostatic pressure when applied 10 times within 10 minutes (ZoBell, 1958). ZoBell (personal communication) has cultured deep sea bacteria under 1400 atmos. of hydrostatic pressure.

Water

Water is the most concentrated single molecule in protoplasm. Its depletion can therefore be expected to restrict growth and reproduction. Most organisms, microbes included, survive periods of extreme drought in dormant states, often as spores. On the other hand, in the case of *Pleurococcus vulgaris* slightly modified vegetative cells suffice to withstand prolonged drought (Fritch, 1922; Fritch and Haines, 1923). According to Zeuch (1934) cell division of *Pleurococcus vulgaris* can still occur at relative humidities of 68 per cent at 1° C., 55 per cent at 10° C., and 48 per cent at 20° C. *Aspergillus glaucus* is well known for its growth on substrates where the activity of water (a_w) is as low as 0.65 to 0.70 (Scott, 1961). Kordyum and Bobchenko (1959) hold the opinion that many microorganisms can actually use air as a habitat for growth and reproduction. The growth of lichens on bare rock surfaces, bacteria and fungi in flour, and many microorganisms in strongly saline media represent ecological instances of

growth in environments in which the chemical potential of water is low. Nothing more than speculative attention has been given to the possibility of microbial growth in nonaqueous media. It should not be forgotten, however, that the water dependent metabolism of all living organisms that are known must be at least to some extent the end result of selection on a water rich earth. It is not known whether life could form on a planet on which the predominant liquid was some other compound than water. One should also remember that under aerobic conditions of metabolism water is one of the main excretory compounds formed by living organisms. Mechanisms for the selective retention of metabolically formed water might enable some organisms to persist and grow in liquid media with low water contents.

Other Factors

In relation to natural radiations, direct sunlight is known to be lethal for many microorganisms, but the effects probably result from dehydration and high temperatures in most cases. ZoBell and McEwen (1935) were unable to detect any lethal effect when marine bacteria were exposed in layers of water greater than 5 mm. in thickness to full noon sunlight on a roof top in La Jolla, California. Two halophilic bacteria isolated by Browne (1922) withstood indefinite exposure to "the brightest sunlight."

The effect of ultraviolet light on microorganisms has been studied by many workers; however, most of the data refer to high dosages for short times. It would be of much interest to know the maximal levels of continuous ultraviolet radiation that can be tolerated by actively growing cultures. Although ultraviolet light in high doses is harmful to all organisms, it must be remembered that deleterious effects are much less pronounced above 300 mμ. than below for equal energies of incident light (Meier, 1936). There is also a great variation in the sensitivity of different microorganisms to ultraviolet light. Siliceous tests of diatoms apparently afford no protection (Ursprung and Bloom, 1917). Because the possibility of shielding and the well known photoreactivation phenomenon, whereby the lethality of ultraviolet light is partly reversed by later application of visible light, it is probably incorrect to assume, as many have done, that an ozone free earth would necessarily be sterile.

The biological effects of gamma- and other types of ionizing radiations have also been studied by many investigators. Single large doses have usually been used. Populations of many microorganisms will survive single doses in the range of 10^6 r. (Shields *et al.*, 1961). *Saccharomyces cerevisiae* has been cultured under continuous exposure to 50 mr. per day of radium emanations (Maisin *et al.*, 1960), however, this is doubtlessly far below the maximal level that can be tolerated. According to Prince (1960) a good place to look for radiation resistant microorganisms would be in nuclear reactors. He states that it is "common knowledge that some bacteria can adapt even to the water in a swimming-pool-type nuclear reactor."

A few other case histories will serve to round out the picture that has been presented. Some of these refer to survival rather than to growth and reproduction. The cases are as follows.

(1) The growth of several bacteria and fungi in concentrated $CuSO_4$ solutions. The subject has been reviewed by Starkey and Waksman (1943).

(2) Bacteria that grow actively in solutions containing 1 g. of phenol per liter (Putilina, 1959).

(3) Growth of the fungus *Aspergillus* in a 40 per cent solution of citric acid (Johnson, 1923).

(4) An *aerobic* bacterium (*Hydrogenomonas*?), originally isolated from sewage sludge that shows poor growth in air, but develops well in an atmosphere containing 20 per cent by volume O_2 and 80 per cent by volume CO (Kistner, 1953).

(5) Heterotrophic growth of algae in lakes during the sunless arctic winter (Rhodhe, 1955) and reproduction of algae in subterranean caves (Claus, 1955).

(6) The survival of some bacterial spores after 5 hours' immersion in non-aqueous media at temperatures approaching 140° C. (Rodenbeck, 1932).

(7) The survival of bacterial and fungal spores, and even vegetative cells of *Mycobacterium smegmatis*, after 5 days' exposure to ultrahigh vacuum at pressures below 10^{-9} mm. of Hg. (Portner *et al.*, 1961).

TABLE 1

ENVIRONMENTAL LIMITS OF TEMPERATURE, Eh (AT THE PREVAILING pH), pH, HYDROSTATIC PRESSURE, AND SALINITY FOR GROWTH AND REPRODUCTION OF MICROORGANISMS

Factor	Lower limit	Upper limit
Temperature	−18° C. (fungi, bacteria)	104° C. (sulfate reducing bacteria under 1000 atmos. hydrostatic pressure)
Eh	−450 mv. at pH 9.5 (sulfate reducing bacteria)	+850 mv. at pH 3 (iron bacteria)
pH	0 (*Acontium velatum*, fungus D, *Thiobacillus thiooxidans*)	13 (?) (*Plectonema nostocorum*)
Hydrostatic pressure	Essentially 0	1400 Atmos. (deep sea and bacteria)
Salinity	Double distilled water (heterotrophic bacteria)	Saturated brines (*Dunaliella*, halophilic bacteria, etc.)

(8) Survival of many microorganisms after prolonged exposure to temperatures approaching absolute zero (Bêlehrádek, 1935; Becquerel, 1950). Life may, in some cases, be capable of almost infinite preservation under such conditions.

One could multiply the examples at greater length, but those already presented suffice to make the point.

General Remarks

In TABLE 1 are summarized the ranges of temperature, Eh, pH, hydrostatic pressure, and salinity that still permit growth and reproduction of one or more microorganisms. It is not maintained that growth is anywhere near maximal under the extreme conditions referred to, merely that it does occur. Selection and mutation over long periods of time could doubtlessly result in a further widening of the observed limits. It should also be stressed in this connection that scientists are inclined to study single factors taken one at a time. When two or more environmental factors show antagonistic effects, as is the case with temperature and pressure, one can expect to find an increased tolerance to each factor using combined action.

The microorganisms referred to in this paper are peculiar in that they grow in environments that are lethal to most other forms of life. One can instructively reverse the point of view that has been taken here and ask why it is that most organisms live under "common" conditions. The answer is, of course, because life as a whole is selectively adapted to growth in common environments. If the waters of the earth were predominantly acid, growth at neutral pH values would be regarded as an oddity. Thus, the fact that most living species conform physiologically and ecologically to average Earth conditions should not be taken to indicate any inherent environmentally based physicochemical conservatism of living matter. Adaptation has taken place.

Environments of the Earth that are sterile or nearly so mostly fall into one of two categories: nonaqueous environments, and noncirculatory aqueous environments. The first category is so obviously restrictive in a biological sense that it requires no further comment. The second refers to rock-enclosed waters that do not readily enter into the hydrological cycle. Oil brines, for example, that are perfectly sealed in place, seem to be sterile (Shturm, personal communication), and deeply buried wet sediments usually have low to negligible bacterial populations. In small enclosed systems extinction becomes increasingly probable with time because of the small numbers of organisms involved, the accumulation of metabolic waste products, and the general decrease in free energy of the system as a function of time. Continuous circulation negates these factors and in addition permits occasional injections of diverse microorganisms into new environments, to which they may become adapted over many generations. Given the presence of circulating water, it seems rather unlikely that any aqueous environment could remain indefinitely sterile over geologically long periods of time. The powers of microbial reproduction and variation are so immense in an evolutionary sense as to make this a virtual impossibility. This assumes, of course, that some energy source is available for metabolism in the environment concerned; but this is not a restrictive limitation either biologically or geochemically.

Returning to the question of extraterrestrial life, the problem involved seems not so much to be whether organisms could live elsewhere under conditions that we would regard as unusual on Earth, as it is to account for the origin of life itself. In relation to the possibility of life on Mars, for example, the questions should be of two types: (1) whether conditions there were ever favorable for the origin or introduction of life; and (2) whether subsequent conditions have been favorable for the persistence of such life as might have been formed. The second question is far less critical at the present time than is the first. To appreciate the potentialities of adaptation one need only contemplate how an Ordovician observer might have viewed the likelihood of birds flying in the air, the possibility of an animal maintaining a temperature of $37° \pm 1°$ C. for virtually all of its lifespan over a period of 100 years, or the existence of plants that trap and feed on animals. What can the leper know of the scorpion's sting? And what does the blind man know of the firefly's light?

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THE INFLUENCE OF WATER CURRENTS ON THE LIFE FUNCTIONS OF ALGAE*

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Selective effects by the current. Of the many habitats on Earth which are colonized and exploited by sessile organisms, those which are in contact with a mass of air or water in relatively rapid movement are likely to constitute a convenience or a necessity to the uptake and excretory systems of the organism but to represent, at the same time, a major threat to the organism's security. Metabolizing organisms as we know them are inhabitants of fluids. These fluids when laden with small quantities of nutrients and motionless may or may not be suitable for successful growth and reproduction. When the fluid is in unidirectional or turbulent motion and the organism remains in place, the possibilities for successful growth of many sessile organisms are greatly enhanced, but security is likely to be threatened by factors like evaporation or physical buffeting by the current, and by the molar agents which are flung at the organism. Areas where surface or subsurface currents run in close proximity to the bottom or other stable objects are successfully exploited by numerous sessile marine plants and invertebrates; in fresh water currents sessile invertebrates are relatively few and inconspicuous, but the algae have successfully colonized what to most animals is a peculiarly dangerous spot, the rapids of streams both large and small. So unique is this habitat that some of the algae which are found in the rapid water habitat are seldom if ever found anywhere else.

The present paper concerns algae which inhabit and are essentially limited to fresh water currents, that is, algae which have moving water all around them or in very close proximity; but inasmuch as the current has varied influences as well on organisms which are in it only temporarily, I shall make occasional mention of other river algae. The true current-inhabiting species are not adequately described by the term "river algae" because the latter category includes many forms which cannot attach and which are often unable to remain in place in a strong current. Essentially all surface streams are inhabited by some such forms, many of which are found as commonly or more commonly in standing water.

From source to mouth a freshwater stream consists of alternating shallow (riffle) areas and pools. These respective habitats differ in many ways and it is usual to find that each is inhabited by a distinctive assemblage of animals and plants. Current rate is influenced by a number of well known variables; in small streams these variables act so as to subject different but adjacent points to quite different pressures. Such pressures are likely to fluctuate greatly from moment to moment but minute differences in depth and presumably in average current rate between points distant by only a few millimeters on the stream floor make of each shallow area a mosaic of differing

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microhabitats whose existence and individuality is attested by striking differences in the algal populations which colonize them at certain seasons.

A primary influence of current on algae, therefore, is the exclusion of certain species from pool areas or other places where current is minimal, or the enhancement of growth of such species in the most favorable, frequently the fastest current. The fact that algae colonize so dangerous a habitat as flowing water suggests that they can be provided some unique service by this habitat. The relationship of algal photosynthesis and respiration to water movement has been discussed by various investigators including Gessner (1937) and Steeman-Nielsen (1944). Oxygen consumption in the dark and the photosynthetic rate are increased in moving water above the respective values for standing water. More recently, respiratory rate and P uptake by *Oedogonium kurzii* Zeller have been studied by Whitford (1961). Radioactive P uptake in water moving at 18 cm. per second was found to be over 10 times that in still water. He concludes that the cause for "inherent current demand" by lotic organisms is the need for rapid exchange of materials with the water and that the steep diffusion gradient in a current satisfies this demand.

This inherent current demand and the gradients involved may be of significance to algae in two ways: for materials which are brought to the algae by the current and for removal downstream of substances which might be harmful. At least some algae are known to excrete substances which eventually retard their own growth rate. That such materials would be flushed away from an alga growing in a current is evident, and may explain the limitation of at least certain species to rapid water. It may also explain the high cell density achieved by many current algae.

Effects of current on algal size or shape. Precisely how current influences the structure of an individual algal cell or thallus has received relatively little attention. Many benthic stream algae are so flexible that the current continually bends and twists them without visible damage or effect. Unlike a tree which bends permanently under the influence of prevailing winds, there is nothing about their structure which would even betray the usual direction of the current if by some means the current were suddenly averted or brought to a stop. The same is true of certain less flexible bottom-inhabiting forms. The *Phormidium-Audouinella-Schizothrix* community which is known from streams of the North Temperate Zone (Blum, 1956) does not, in the surface topography of its crust, show any very evident polarity with respect to the current. Others—and relatively few cases are known—show by the form or orientation of their thallus the effects of unidirectional current as in the Phormidium community described by Wehrle (1942), a composite community of Vaucheria and Plectonema described by Wallner (1934), or in the colonies of Cocconeis growing on a vertical cylindric stake as described by Gessner (1955).

How the current controls the size of certain benthic algae is shown by work done by Picken on the alga Rivularia. In regions of relatively rapid flow thallus size was found to be proportional to the size of the stones to which the thallus was attached. In slower water, however, thallus size was independent of stone size. The bulk of this alga increases more rapidly than the area of its attachment, and the current limits the maximal size of the thallus, either

tearing the thallus away from the stone, or transporting both stone and thallus to a slower part of the stream (Picken, 1936).

Influence of the current on algal reproduction. In their reproduction current algae take full advantage of the medium of dispersal which is at their doorstep. It is commonly observed that many algae which colonize stream bottoms achieve in certain seasons almost saturation coverage of available and favorable sites. Thanks to the mixing done by the current these algae are able to introduce their reproductive units into what must be a very high percentage of rock fissures, cracks, scratches, and roughened areas, into enough, at least, of such depressions to permit subsequent growth from the colonizing cells to cover close to 100 per cent of the available surface. In southern Michigan streams which I investigated colonization of rock surfaces is very rapid, and successful in very high percentages of the space available. The winter dominant diatoms *Gomphonema olivaceum* and *Diatoma vulgare*, for example, achieve good growth in winter on newly submerged rock surfaces in as little as 10 days. Both of these forms were at the same time colonists and seasonal dominants, no evidence being found of succession before the establishment of the communities they represent. The period within which *G. olivaceum* colonized bare rock surfaces extended from late November to early April, and colonization seemed to be possible at any time within this period (Blum, 1954).

Evidence that planktonic forms reproduce as they are carried downstream has been presented by various workers but there remains the suspicion that much of the actual cell division occurs on the bottom and that the apparent increase in phytoplankton downstream is largely the result of more extensive nutrient beds there and of more dense populations of benthic individuals, many of which rise every day into the plankton. I observed the vegetative dissemination of Spirogyra and Oscillatoria communities on warm summer days in the Saline River in southern Michigan. These communities were especially characteristic of quiet shoals or bays. Here the algae remained on the bottom in contact with nutrient-rich silt deposits, as masses of filaments easily visible from a distance. The surface waters of such shoals or bays is usually in slow circular movement set up by the main current of the stream, which by-passes the shoal or the bay in a tangent to the circular current which it produces there. At times of rapid photosynthesis, individual masses of the algal filaments are detached and buoyed upward by trapped oxygen bubbles. Once the algal mass has quit the floor of such a shoal, it is carried slowly along in the eddying surface water. After moving for some time in this circular manner it may eventually be picked up by the tangential current of the main stream which removes it definitively from the shoal. As the algal mass travels downstream, it disseminates live filaments along the way. The progress of these filaments is arrested on obstructions or on new shoal areas or other sediments downstream, which in this way are themselves colonized. The elevation of algal masses by entrapped bubbles can be observed from about noon until about 2 to 3 P.M. on sunny days in summer and the movement downstream of these floating masses can be observed throughout an entire afternoon.

The evolution of current-inhabiting algae. I believe the first attempt to classify the body types of current algae was made by Cedergren (1938). His

classification included 4 groups, namely (1) richly branched thalli; (2) long, flexible cylinders, (3) spherical cushions, and (4) simplified platelike forms. The second of these groups should probably be modified to include forms with laciniate, reticular or lacunate bodies which float downstream from a point of attachment; it should also be pointed out that certain algae with short unbranched filaments, although they indeed qualify as cylinders, nevertheless have a somewhat unique superficial form since, as in Vaucheria, they frequently constitute a virtual turf but do not become interwoven to form massive skeins as in the first group. If body form is a major criterion for these groups, at

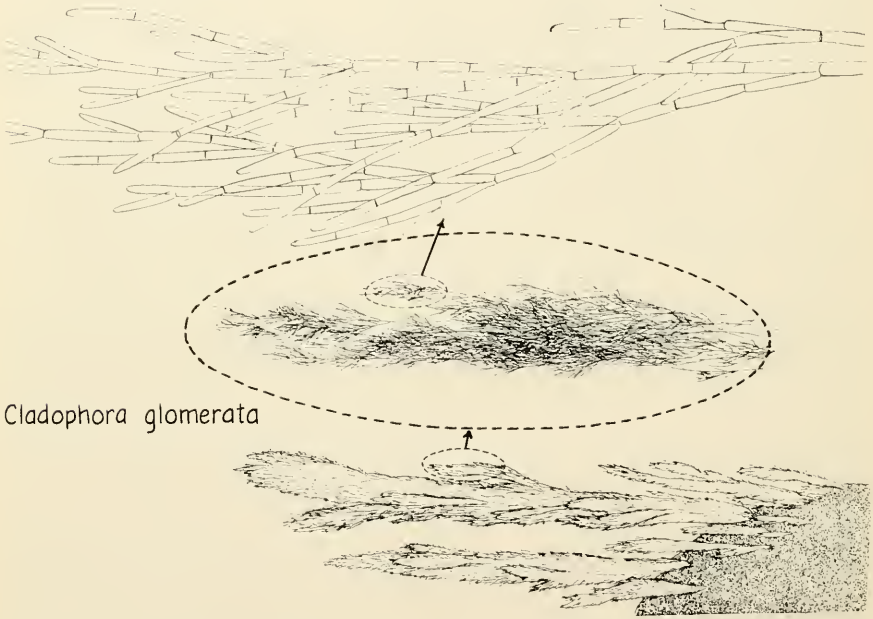

FIGURE 1. *Cladophora glomerata.* The illustration at bottom represents several algal thalli X^1_2 attached to a portion of rock (stippled). The upper drawings represent increasing magnifications of small portions of the thallus.

least one other category should probably be added for forms with a rigid, cylindrical, but pseudoparenchymatous body like Lemanea.

The first 2 groups as outlined by Cedergren can be summarized by the qualification that they live in the current and permit water to run among their filaments or at least on more than one side of the thallus. Hence they expose a large surface area directly to the surrounding water. A common example of this type is *Cladophora glomerata* (L.) Kütz. (FIGURE 1). These groups can be further subdivided into gelatinous and nongelatinous types. The gelatinous types in general have relatively small filament or trichome diameter.

The last 2 groups of Cedergren can be qualified by virtue of their position mostly below the current — in other words they become a part of the stream bottom. The current does not flow among their filaments but only in their

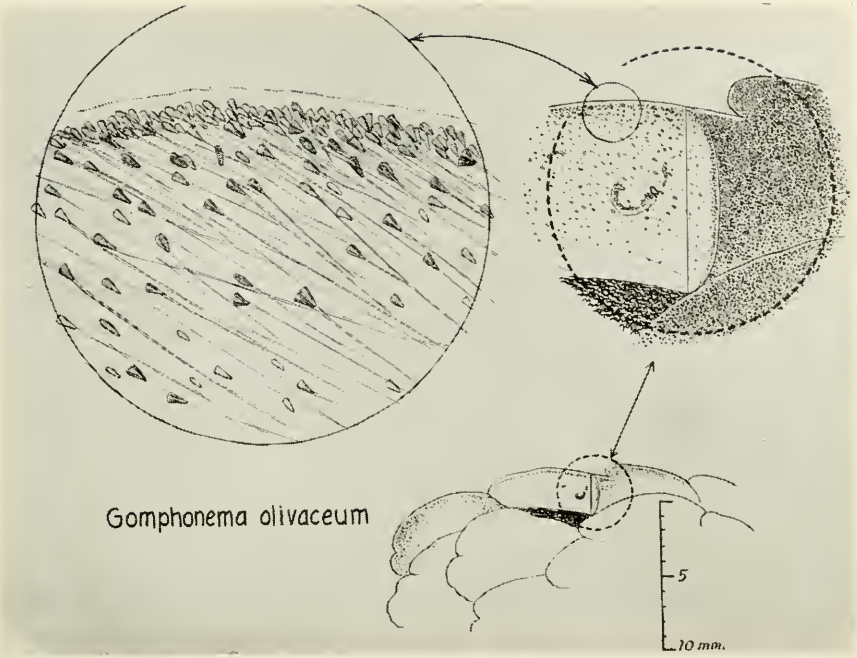

FIGURE 2. *Gomphonema olivaceum*. The illustration at the bottom represents several soft thalli attached to and completely covering a rock. The mottled dark area represents bare rock at a point where an algal thallus has been cut away. The upper drawings represent successive magnifications of the area cut away. Insect larvae which feed on these diatoms are shown within the algal mass.

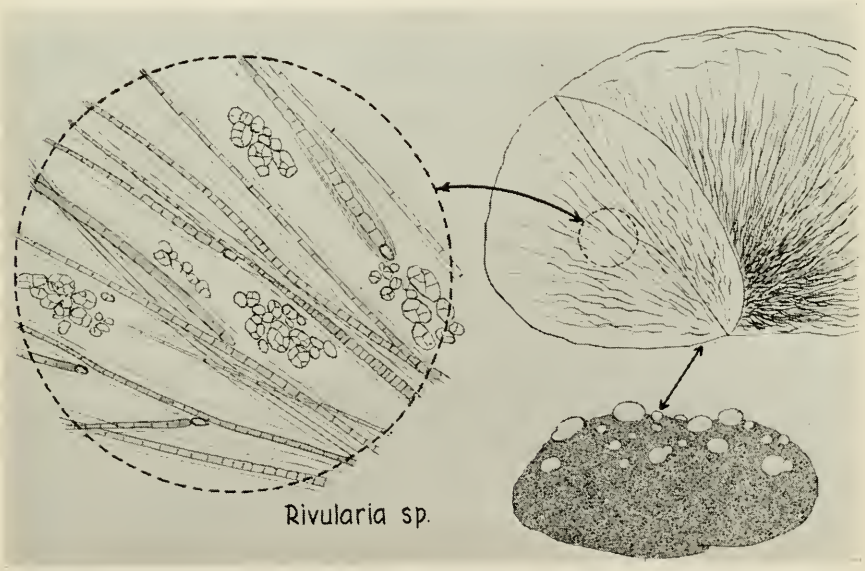

FIGURE 3. Rivularia sp. Stippled portion represents a rock to which several subspherical colonies of Rivularia are attached. The upper drawings show increasing magnification of a single colony or thallus. Calcium carbonate crystals are shown as they appear in the gelatin between adjacent algal filaments.

vicinity. These include massive sheets which cover rocks in the current and may extend partly into the current as in Phormidium spp.; parenchymatous or pseudoparenchymatous collections of cells; soft, gelatinous masses that move slightly in the current (some diatoms such as *Gomphonema olivaceum* (Lyngb.), Kütz. (FIGURE 2); and firmer, spherical, or hemispheric masses which are frequently gelatinous as in Rivularia spp. (FIGURE 3). The gelatin in these types serves to lubricate the alga-current interface and to reduce friction and injury to the plant but it also serves to separate adjacent trichomes or filaments and to keep, in many algae, a rather precise spatial relationship between filaments as they lie in their intercellular material (FIGURE 3).

When fresh water algae, generally, are compared and contrasted with marine algae, the essential absence from the former of massive plant bodies, leathery and foliose types which are so common in the marine Rhodophyta and Phaeophyta is noteworthy. Although the Phaeophyta have proven generally unsuccessful in fresh water and would not really be expected to produce such plant forms in any event in fresh water, the same is not true of the Rhodophyta or of the Chlorophyta. Nevertheless, the latter groups are not represented in fresh water by forms more massive than Tuomeya, Lemanea, Chaetophora, or Monostroma.

The evolution of fresh water algae has thus been successful largely for the smaller, more delicate forms which are characteristic of standing water rather than of currents. If we suppose that the rather specialized current algae have evolved at least in part from their fresh water relatives that are tolerant of standing water, it must be granted that their form has not been greatly modified by the change in habitat.

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THE STRUCTURE OF DIATOM COMMUNITIES UNDER VARYING ECOLOGICAL CONDITIONS

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During the preceding 15 years we at the Academy of Natural Sciences of Philadelphia have spent a great deal of time studying the composition of diatom communities in the eastern and southern United States. The findings from sections of streams characteristic of this area which have not been adversely affected by pollution are discussed in this paper. To understand these communities of diatoms not only the species which compose them but also the sizes of the populations of these species must be known. This necessitates collecting species from all types of habitats in the community. It also necessitates counting sufficient specimens to determine most of the species composing the community. Obviously, when studying a community which has one or two species with large numbers of individuals, many more specimens must be counted to discover the species composed of small populations.

From our studies it is evident that 7000 to 8000 or more specimens usually must be counted before a reliable picture of a diatom community can be obtained if one wishes to compare the quantitative characteristics of communities. From TABLE 1 it is evident that only a small percentage of the species composing the community are seen when only 200 or 500 specimens are counted and approximately 50 to 75 per cent of the number of species are seen when 1000 specimens are counted when compared with the number seen when several thousand specimens are counted. Similarly, the percentage of the population composed of specimens of dominant species in some cases varies greatly when based upon counts of a few hundred specimens as compared with counts of a few thousand specimens. As seen in TABLE 2 the numbers of species composing the diatom community remain fairly similar when similar segments of the communities are analyzed if no serious change in the environment occurs. As seen in TABLE 3 the percentage of the population composed of dominant species does not vary greatly for similar environments when similar segments of the community are studied.

When the structure of these populations was plotted by representing the number of species as the ordinate and the number of individuals composing each species as the abscissa, the data approached the shape of a truncated normal curve, FIGURE 1. To determine what mathematical formula might best express the results of these studies several formulae were tried (Patrick *et al.*, 1959) and the truncated normal curve provided a little better fit than the other methods investigated. The use of a truncated normal curve to express the structure of communities of organisms has been supported by the work of MacArthur *et al.*

By using this method we objectively compared similar segments of diatom populations. For example, if enough specimens are counted and enough species are identified to always place the mode in approximately the same interval,

a similar segment of the community will have been studied regardless of the dominance of any species that may be present.

We have found in natural rivers which are relatively free from pollution the communities are composed of many species with most of them having relatively small populations. These findings support the theory set forth by Thienemann (1939) that optimal environments support many species composed of relatively small populations. Furthermore, the numbers of species do not change greatly from season to season in the same area nor do they change very much from area to area collected at the same time. For example, in TABLE 2

TABLE 1

THE NUMBER OF SPECIES AND THE PERCENTAGE OF THE SPECIMENS IN POPULATIONS OF DOMINANT SPECIES OBSERVED WHEN VARYING NUMBERS OF SPECIMENS ARE COUNTED

River	Specimens counted	Number of species	Percentage of dominance*
Wateree River, South Carolina, September 22, 1961	200	33	62.5
	558	52	62.2
	1009	81	51.2
	5970†	117	27.2
Assunpink Creek, New Jersey, September 19, 1959	200	35	51.5
	569	65	31.6
	1219	97	61.5
	12,584†	178	39.5
Potomac River, Maryland, October 18, 1960	206	24	89.3
	558	37	87.3
	1637	76	71.5
	17,911†	148	62.2
Sabine River, Texas, October 18, 1960	211	24	75.8
	511	39	72.2
	1348	68	54.7
	7369†	105	60.0

* The percentage of specimens counted composing the dominant species. A dominant species is one that is represented by 1000 or more specimens when 5000 or more specimens are counted.

† These are the number of specimens which had to be counted to place the mode in the second interval when a truncated normal curve is constructed from the data.

are shown the data for these statements derived from studies of the Savannah River.

When the numbers of species found in different natural soft water rivers, for example the Savannah River, the Red Clay Creek, and the Wateree River, are compared, they do not vary greatly. The total number of species for the Savannah River (South Carolina) was 188; Red Clay Creek (Delaware), 145; Wateree River (South Carolina), 181. Considering only those species represented by more than 6 specimens when 7000 or more specimens are counted, we find Savannah River, 85; Wateree River, 89; Red Clay Creek, 76. The reason that 6 or more specimens have been used for estimating that a species is established in a given area is that if a truncated normal curve is constructed those species represented by 4 to 8 specimens will have better than a 50 per

cent chance of not shifting their position in the curve (Preston, 1948) and, therefore, will remain a part of the community.

However, if the kinds of species in similar sections of various rivers are examined, a great variation as to the kinds of species is seen as described by Patrick (1961). Also, in studies of the same area of the Savannah River at about the same season (late August, early September) of the year in different years, only 34 per cent of the species were common to both studies. A similar, but not as great, variation is seen when two different areas in the same

Table 2
Savannah River
Summary of Catherwood Diatometer Readings at Station 1
October 1953 to January 1958

Date	Specimen number in modal interval	Species in mode	Species observed	Species in theoretical universe
Oct. 1953	4–8	22	150	178
Jan. 1954	4–8	19	151	181
Apr. 1954	2–4	24	169	200
July 1954	2–4	23	153	193
Oct. 1954	4–8	21	142	168
Jan. 1955	4–8	19	132	166
Apr. 1955	2–4	25	165	221
July 1955	2–4	20	132	180
Oct. 1955	2–4	27	171	253
Jan. 1956	2–4	30	185	229
Apr. 1956	4–8	35	215	252
July 1956	2–4	24	147	185
Oct. 1956	2–4	23	149	206
Jan. 1957	2–4	29	177	233
Apr. 1957	2–4	21	132	185
July 1957	4–8	29	181	203
Oct. 1957	2–4	25	157	232
Jan. 1958	2–4	27	152	212
(Apr. 1954–1958 averages)		24	151	194

Table 3
Dominant Species in Two Areas, Guadalupe River

	Station 1 9 Sept. 59	Station 2 9 Sept. 59
Gomphonema affine var. *insigne*	1272	2850
G. parvulum	4346	2700
Navicula sp.		1900
N. tripunctatus var. *schizonemoides*	30,634	23,750
Nitzschia palea		2400
Percentage of total count composed of dominant species	95	90

river which have the same types of ecological habitats are studied at the same time. The kinds of species in common are more variable than the numbers. For example, Stations 1 and 6 on the Savannah River which are about 30 miles apart were studied in June of 1960 and 187 species were identified at Station 1 and 54 per cent of these were found at Station 6. At Station 6146 species were identified and 75 per cent of these were found at Station 1. In October of 1960 when these two areas were studied the number of species at Station 1 was 184 and the number at Station 6 was 185. However, 75 per cent of the species at Station 1 were at Station 6 and 75 per cent of the species at Station 6 were at Station 1.

This same principle as to similarity of numbers of species but differences in kinds of species also holds for the hard water rivers we have studied. Often the numbers of species are slightly less in natural hard water rivers than in

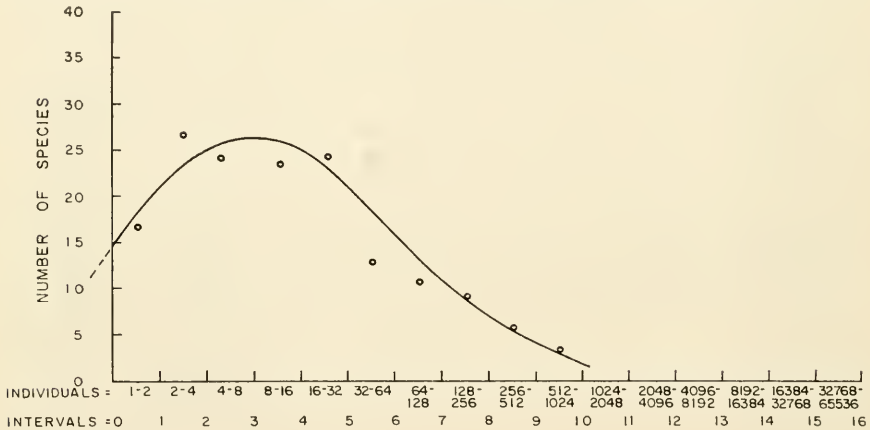

FIGURE 1. Ridley Creek, Pennsylvania

soft water rivers. For example, in the Potomac River, a hard water river, from April of 1959 to October of 1960 in one area studied the observed species varied from 130 to 148 (average 144) as contrasted with a variation from 118 to 185 (average 161) in the Savannah River which is a soft water river, over a similar period of time.

In brackish waters such as the estuary of the York River the numbers of species composing a diatom community sometimes are a little less than in a soft water river. From November of 1956 to May of 1959 the number of observed species varied from 108 to 147 (average, 130). However, in all of these three types of rivers—soft, hard, and brackish water—the communities are made up of many species most of which have relatively small populations if the rivers are natural and not polluted.

A different picture is found when the structure of diatom communities in dystrophic streams is examined. In these there is a restricted diatom flora which can live in these naturally acid streams high in humates. They are species largely confined to the genera *Eunotia* and *Frustulia* and certain species

of genera such as *Pinnularia, Actinella, Anomoeoneis* and *Surirella*. Thus, we have a community composed of fewer species with populations that are much more variable in size (FIGURE 2). The truncated normal curve representing the structure of the community has a much lower mode, fewer observed spe-

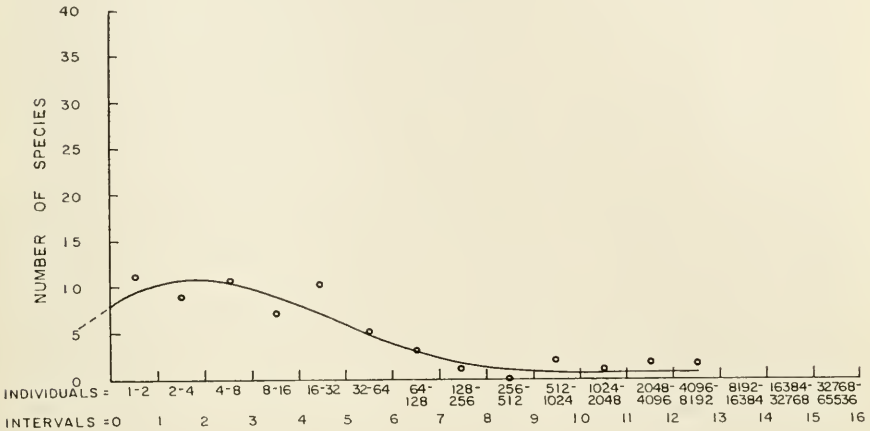

FIGURE 2. Egg Harbor River, N.J.

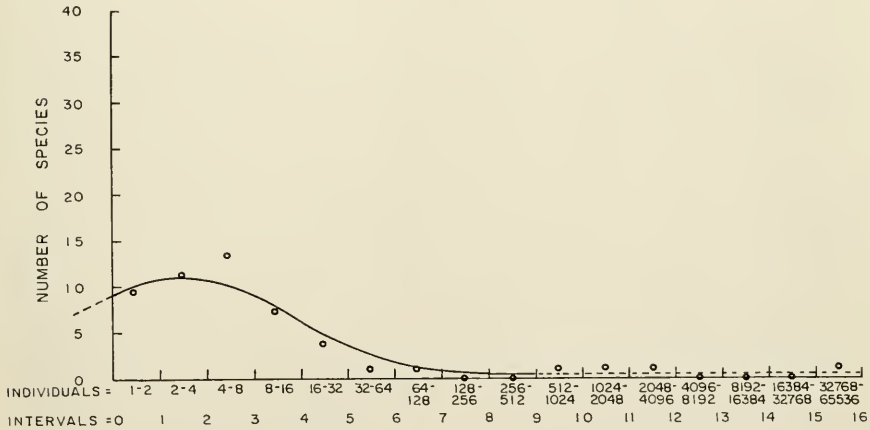

FIGURE 3. Back River, Maryland

cies, a much greater σ^2, which means more variability in the sizes of the diatom populations, and covers more intervals, because a few species have very large populations.

This is the type of curve often found for the structure of diatom populations which are subjected to pollution (FIGURE 3). In both cases one or more eco-

logical factors have operated to greatly limit the numbers of species which can survive in these particular kinds of ecological conditions.

A few studies which we have done indicate that in springs in which the environment is fairly constant the numbers of species composing a diatom community may be much less than in the very variable environment of an eutrophic or mesotrophic, natural river. It seems that it is the highly variable, yet continuously favorable, environment of natural rivers of these types that is largely responsible for the great diversity of species that make up these communities.

The fact that the numbers of species remain fairly similar, although the kinds of species vary considerably, suggests that there are a similar number of niches for diatom species in ecologically similar natural areas and more species are available than there are niches for them. Thus, each niche is occupied by a different species. The lack of similarity in kinds of species present is probably in part due to the highly variable environment in a natural river and the availability of species which have their best development in different variations of the environment. Because diatoms have very rapid reproduction under favorable conditions the populations of certain species can quickly increase, whereas populations of other species decrease beyond the limits of collectability or disappear.

Another important consideration in the study of diatom communities is the kinds of species composing the communities. By careful consideration of the kinds of species associated together, a qualitative evaluation of many of the characteristics of the environment can be made. However, because of a lack of data as to the complete physiological requirements of any species in nature, it is very dangerous to say that the lack of any species indicates that the specific characteristic of the environment under consideration is not there, because the lack of any factor essential for the life of an organism may eliminate it, although all other factors of the environment may be favorable to it. Also, it is hazardous to use changes in the population sizes of specific species as a basis for saying that the quantitative nature of a given environmental factor has changed. For example, we studied two areas in the Guadalupe River which were not over 500 yards apart. The structural environmental characteristics of the two areas were very similar. Because no tributaries or pollution entered the river between these two areas during the time of this study, the characteristics of the water were very similar. This was substantiated by chemical analyses. When similar segments of the communities of diatoms were studied the percentages of the community composed of specimens of dominant species were very similar, 95 and 90 per cent, respectively (TABLE 3). However, the sizes of the populations of the dominant species and the kinds of species varied considerably. At Station 2, the population size of *Gomphonema affinis* var. *insigne* was twice that found at Station 1. The population of *Gomphonema parvulum* was 38 per cent larger at Station 1 than at Station 2. At Station 2 *Navicula* sp. had a population of 1900 specimens and *Nitzschia palea* had a population of 2400 specimens yet neither of these species were present at Station 1. Only one of the dominant species, *Navicula tripunctata* var. *schizonemoides* had populations of similar size at the two stations.

It is only as a result of thorough and continuous study of an environment and the species living in it that one can venture to describe the quantitative

changes in the natural environment of a river by changes in the quantitative abundance of specific kinds of species.

In conclusion, our studies have shown that diatom communities can be best characterized by consideration of the kinds of species, the numbers of species, and the relative sizes of the populations of the species that comprise the community. An excellent way to consider the relative sizes of the populations of all the species studied is by the construction of a truncated normal curve. The presence of certain kinds of species may tell us much as to the qualitative characteristics of an environment. The best means for determining quantitative shifts in the environment is by considering the shift in numbers of species and the ratio of the number of species with small populations to those with large populations. Perhaps the reasons that the numbers of species do not vary greatly is that there are similar numbers of niches for species occupancy in ecologically similar types of streams. Also, at any one time there are probably more species available to inhabit natural eutrophic or mesotrophic areas of streams than there are niches available for species occupancy, thus, each niche is filled with a different species. The reasons that the kinds of species vary considerably in streams of these types are the continually varying yet favorable environment; the availability of species which have their best development in different conditions of the environment; and the ability of diatom populations to quickly expand or contract with changes in the environment.

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CELL STRUCTURE AND ENVIRONMENT

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During the so-called classical period of the study of cells, algal cells were frequently used in cytological investigations. The great discoveries of Weismann, Bütschli, Ramón y Cajal, and Flemming were made possible with fixed and stained objects, but as the equipment and the microscopical methods then available were unsuitable for living and especially for unstained objects, living algal cells were only rarely used for cytological purposes. Instead, methods were developed which were supposed to leave the fixed protoplasm unaltered, and differential staining procedures were used which rendered visible to the human eye structures which, it was believed, occurred in the living cell. Violent, but barely scientific controversy which often led to personal insults and verbal battles ensued, during which the living cell was more and more forgotten. This was also due to the exemplary, or not so exemplary preparations which were made to support sophisticated hypotheses which arose from staining techniques. These techniques often resulted in works of art rather than impressions of the living cell.

No matter how perfectly fixation for specific purposes has been accomplished, the living constituents of the cell must necessarily undergo alteration when fixed (otherwise they would continue to live), and minute changes in the protoplasm due to environmental factors cannot, therefore, be detected. The difficulties were increased because colloidal physics had not yet been developed, and because the changes were generally of a submicroscopical nature. Investigations of these changes in the living protoplasm, therefore, were only later tackled.

Seen against this background, the accidental discovery by Benecke (1901) of the reduction in size and the ultimate disappearance of the chromatophores in *Nitzschia putrida* (*Synedra hyalina* Provazek), by means of which he sought to show a clear connexion between the size of the chromatophores and the pollution (as he called it) of coastal waters, was surprising and also important.

The approach adopted by Benecke was, however, soon abandoned, and the observation of living algal cells continued only for the purpose of systematics and morphology. The observed structures, cell components, etc., were regarded as something rigid and unchangeable, or, as we would express it today, genotypically determined. Consequently, the results of these observations were used only as characteristics: they were used, and frequently misused, for describing species, and as a result, plant physiologists and the early ecologists did not want to do anything with them.

On the other hand, investigations into the causes of adaptation of algae have begun. These investigations at first pursued a course which was of importance to Man but not to the algae. Apart from the vague conjectures of the 19th century, which were mainly concerned with descriptions of the habitat or with plant geography, the first ecological study of algae was the so-called Saprobic

System of Kolkwitz. This was, however, not based upon precise observations or experiments but on an untenable hypothesis (Kolkwitz and Marsson, 1902, 1908, 1909; Kolkwitz, 1950; Liebmann, 1951, etc.). As a basis for the hypothesis it was assumed that the substances (of which no one then bothered to ascertain the chemical nature) which were responsible for the pollution of waters could be removed first by reduction and subsequently by oxidation. The so-called reduction phase was called polysaprobic and the oxidation phase mesosaprobic. Naumann (1932), however, had shown that this hypothesis was untenable: he demonstrated the nonexistence of a reduction phase and consequently it was found impossible to judge the quality of waters, let alone to purify them, on Kolkwitz principles. Under these circumstances it was not surprising that cytologists found no reason to study protoplasmatic changes attributable to "pollution" according to Saprobic System concepts.

It was only much later that greater stimulus was given by the ecological work of Kolbe (1927, 1932), who showed that certain diatom species are better adapted to a high salt concentration than others. In his opinion it was the chloride ion of sodium chloride which was responsible for the phenomenon of adaptation. He also attempted to prove that in the absence of the aforementioned ions (oligohalobic conditions), a moderate concentration (mesohalobic conditions), or a high concentration (polyhalobic conditions) simulating salt water was responsible for the distribution and adaptation of certain species.

Almost at the same time it had been shown (Cholnoky, 1929) that the diatom associations of the soda lakes of Hungary (which contain carbonates and not chlorides) were identical with those of Kolbe's mesohalobic waters of Sperenberg. From these observations it was possible to deduce the fact that primarily it was not the chemical composition of the salt molecules but rather their concentration which was responsible for the halobic phenomena. In other words, it was not the chloride ion at all, but the prevailing osmotic pressure, *i.e.*, molarity. It also became clear very soon after that the prevailing osmotic pressure in the Hungarian soda lakes can be as high as, or even higher than, that of the sea (a concentration of 2 mol. sodium carbonate is not exceptional in the lakes), and that these high values do not necessarily give rise to the growth of typical marine algae. It was recognized that it was not the absolute salt content or molarity, but the variation of osmotic pressure which produces the necessary conditions for the so-called brackish water species; or put more precisely, the ability to withstand the molarity variations gives advantages to these brackish water species.

Because the variation of osmotic pressure mainly affects the protoplasm of the brackish water organisms, it was clear that protoplasmic differences must exist, and that these differences could only be discovered by studying the living cells.

After the classical studies of de Vries (1871, 1885), one could assume, as a matter of course, that an increase in osmotic pressure would cause plasmolysis, and also that plasmolysis could be neutralized by permeance to, or active uptake of, the plasmolyzing substances. Höfler showed (1918, and more accurate concept 1931) that de Vries's concept of semipermeability was untenable.

Thus, the causes of adaptation to the conditions of brackish water, *i.e.*, the considerable variation of osmotic pressure, were to be found in plasmolysis which must necessarily and at least temporarily occur.

The experiments which were undertaken (Cholnoky, 1928*a*, 1930*b*, 1932) showed that apart from certain fundamental morphological features which seem to be genotypically determined, and which are characteristic for the various algal groups during plasmolysis, there are large morphological differences between the plasmolyses of freshwater and brackish water species. Among other things, the distribution of viscosity of the protoplasmic colloids is characteristic for the species. It was equally evident that the brackish water species poses a high degree of permeability in regard to the salts in solution in their habitat. In Hungary the high degree of permeability is confined to the carbonates, and only to a lesser degree to the chlorides, although the cells show only slight or nonpermeability to such plasmolytica as nitrates, sugar, urea, etc. The same species when found along the South African coast are mostly permeable to the chlorides, whereas when they occur in the South African sodium carbonate rich waters of the Jakkals River for instance, the same permeability to carbonates as in Hungary was observed.

These observations forced me to the conclusion that owing to the high degree of permeability of the protoplasts, the brackish water species are ecologically favored. It follows that if such an assumption were true, there would be far-reaching colloid-physical effects. The permeating salt molecules would, under normal circumstances, alter the electrical charge of the mono- or polymolecular micelles and thus be the cause of coacervation and ensuing coagulation and death. The protoplasm of brackish water species appears to be extremely well protected against such alterations of electrical charge, and further study will probably provide important information on the submicroscopical structure of the protoplasm.

Typical freshwater algae which were treated with a plasmolyticum consisting of some partly evaporated brackish water from another habitat speedily died as a result of permeation (Cholnoky, 1930*b*, 1931*a*, 1931*b*). Others, however, remained plasmolysed for an extraordinarily long time without showing any sign of protoplasmatic damage and without the least trace of permeation. Other chemical compounds for which the protoplasts of the investigated species were more or less permeable, acted immediately on permeation as poisons, during which it was seen that the gradual destruction of the protoplasm indicated an unequal resistance of the protoplasmic components of the cell (Cholnoky, 1953).

Höfler (1951) obtained similar results and found that Na_2CO_3 acted in a specific manner on the diverse species of bog algae (Desmidiales). The cells of some species were slightly permeable, others were barely permeable. The nonpermeable ones were able to survive plasmolysis lasting several days without sustaining any visible protoplasmatic damage. (It was possible completely to deplasmolyse *Euastrum* after plasmolysis lasting 72 hours.) I was able to confirm that certain brackish water algae were even more resistent to plasmolysis than *Euastrum*. These species built a superficial inner cell wall on the site of positive plasmolysis; *i.e.*, at those places where the protoplasm body had withdrawn from the original cell wall. As a result of the possible

repetition of this operation, the formation of the so-called inner cell wall is explained: it arises from an increase in the osmotic pressure of the environment during the gradual drying up of the waters in the summer (Cholnoky, 1928*b*, 1954; Kamija, 1938; Küster-Winkelmann, 1949). These phenomena are clear proof that the otherwise generally fatal plasmolysis does not alter, or alters only to a limited extent, the structure of the protoplasmic colloids in the brackish water species which are adapted to variations in osmotic pressure. These species not only survive the ordeal but actually build a cell wall during the process—a procedure which would be hardly thinkable if the metabolism had been upset.

The repeated reductions in pressure due to variations of osmotic pressure do not occur without having any side effects. With brackish water diatoms the reductions in pressure are the triggers for sexual reproduction (Cholnoky, 1929*b*), because the dilution caused by the culture medium always gives rise, in the diatoms, to sexual propagation, *i.e.*, auxospore formation.

A sudden dilution causes plasmorhexis in brackish water species. This is evidence for their having comparatively many free salt molecules in the water mantles of the micelles of their colloids, which can cause an osmotic pressure (Cholnoky 1928*a*). Such phenomena do not occur in brackish water species if the dilution is carefully made. But with marine species, a dilution, no matter how carefully made, causes plasmorhexis and death. This can be accepted as proof that the salts causing isotonia, are indispensable to the protoplasmic colloids, and are structurally part of, and inseparable from, the micelles. But further experiments will be necessary to be able to evaluate the position fully.

Without further experiments it will be equally impossible to explain the mechanism of the phenomena which Lenk (1953) called Seasonal Variation of Permeability. Variation of permeability without change of protoplasmic structure is unthinkable. Consequently it can be assumed that submicroscopical protoplasmic structure is also subject to seasonal variation which can only be due to adaptation to altered conditions of the habitat.

As I have already suggested, the behavior of freshwater algae which have been killed by the permeation of salt molecules, indicates that they undergo coacervation and lethal coagulation due to the penetration of the molecules. These protoplasmic changes are a kind of a poison effect and leads from a study of adaptation to the important study of resistance (Biebl, 1937, 1952). It would, however, be inappropriate here to discuss fully all of the hitherto known cytological resistance phenomena.

From the point of view of cytophysiology, a study of the poisonous effects of salts and the resulting cytomorphological changes (which are often submicroscopical) is all the more important, as far reaching deductions regarding adaptation phenomena will be possible. The studies on *Melosira arenaria* (Cholnoky, 1934) may be regarded as a beginning; and subsequent work on cellular changes in other species and other algal groups (*Ulothrix*, *Oedogonium*, Zygnemales, Desmidiales, and Siphonocladiales) led to important ecological and cytological regularities being discovered. The notes, manuscripts, and data, however, remained unpublished as they were lost at the end of the war.

The poisonous effects of some salts (*e.g.*, sodium carbonate) could only be

characterized if the cytomorphological changes which they caused could be compared with the poisonous effects of other substances. For this purpose cultures were utilized of which the culture fluids were displaced by cocaine and colchicine, both of which are known to be cell toxic to a high degree. The effect of cocaine on Cladophora (Cholnoky, 1930*a*) showed that this alga was able to tolerate appreciable concentrations and that it can react in a very characteristic manner. Without any microscopically visible protoplasmatic changes occurring, resting stages developed, which were independent of the seasons, and appeared to be completely resistant to cocaine so that when removed to a normal habitat (*i.e.*, cocaine free) they were able to germinate. That these observations remained comparatively unknown, may be due to the title of the paper having been arbitrarily changed by the editor of the journal to which the paper was sent. The observations made may explain how algae are able to survive temporary poisoning, as a result, for example, of industrial effluents.

As is well known, colchicine affects the development of the spindle during nuclear division and is, therefore, often used for obtaining polyploids. This substance was also used for culture experiments. Surprisingly, only a high concentration of colchicine (10 ppm) resulted in damage to the nuclear division, but no polyploids were obtained. With *Cladophora* the number of nuclei in the polyenergid cells was reduced. With *Spirogyra*, etc. pseudosexual conditions quickly developed which often became lethal after only a lapse of several weeks. The observed phenomena may explain why certain industrial wastes produce no poisoning of the cocaine type (certain cells become impermeable to poisons), but many abnormalities instead. The results of this series of experiments were also entirely lost owing to the war, and as no further opportunity to repeat them has been given me, it is up to some other researcher to undertake this work. Nevertheless, they do seem to elucidate the effects of the waste products produced by human activities as far as the terms "pollution" and "poisoning" of natural waters are concerned.

I am familiar with only a few of the cytological effects of other poisons: among these are the studies on aluminium salts and "cramp" plasmolysis (Weber, 1924, 1933; Höfler, 1958) which clearly show that the salts have rendered impossible the functions of the investigated cells through colloidal changes etc., and very probably also through interference with the electrical charge of the micelles. Although the quoted papers do not mention the colloid-physical significance of the phenomena, it seems to me that they must be due to coacervation and coagulation.

Colchicine as well as cocaine cause radical changes in the structure of the protoplasm which are to a certain extent discernible by experiment, but the mechanism of the effects of poisoning can be better seen microscopically if the poisons can be seen or can be made to be seen. When the first algal investigations were started, the results of the experiments which had been made with the cells of the higher plants led one to suspect that the process known to the workers during the classical period of cytology as vital staining was actually a microscopical manifestation of poisoning and destruction of the cells. It was, therefore, possible without further ado to use stains which were formerly regarded as harmless, *i.e.*, which did not kill the protoplasm suddenly.

As this exposition is mainly concerned with the effects of the environment on the structure of the protoplasm, I shall have to omit a detailed description of what is known about the general principles of stain uptake and storage or changes in the stain molecules, *e.g.*, ionization in the cell or its environment. It would also go beyond the scope of this paper to draw attention to the present state of our knowledge derived from investigations with the fluorescence microscope. As far as I am aware, those studies have hitherto only been made with material divorced from its natural habitat, and have, in many cases, degenerated merely into a study of stains, without reference to colloidal structure or the changes it undergoes. Such work often led Bütschli *et al.*, into fruitless hypothetical discussions.

This scarcely scientific approach is regrettable because even the first experiments on stain uptake in algal cells (Cholnoky, 1934, 1935*a*, 1935*b*, 1935*c*) showed that uptake and storage of the stain molecules, or the ion gradients in the protoplasm was far reachingly dependent on the conditions under which the algae lived before the experiments. When stained with methylene blue or neutral red, the disassociation of the stain molecules remained dependent upon the conditions of the culture before the staining experiments were done. Also in those cases in which the stain fluid (unlike the culture fluid) possessed constant physicochemical characteristics (*e.g.*, stains dissolved in distilled water, buffer solutions or plasmolytica) the effects on the protoplasm of increased osmotic pressure, pH, and light conditions could be clearly proved.

The environmental conditions before the staining experiments generally having remained neglected; this explains why so many apparently contradictory results were obtained. The use of fluorochromes increased still further the complexity of an already complicated position, as conclusions were drawn relating to the storage of stain molecules and ions which incorporated many hypothetical assumptions, such as "full" and "empty" cell-sap (Höfler and Schindler, 1955), which did not attempt to reconcile observed facts with the environmental factors under which the algal cells were living before the experiments.

This change of concept became apparent as preliminary work (Cholnoky and Höfler, 1950) had already been done which went so far as to enable one to distinguish between the cytological behavior of species (Loub, 1951).

Regarding Loub (1951), it should be remembered that his material came from ecologically dissimilar environments. After arrival in the laboratory they were rough cultured and only examined after a more or less lengthy period. Apart from the fact that the culture conditions were uncontrolled in the rough culture, Loub did not investigate the natural conditions of the habitat. By his method he was able to examine only adapted associations. He thus lost the opportunity to investigate the protoplasmic changes caused by ecological factors.

It will be clear from what has been said that most protoplasmic experiments (such as plasmolysis and staining) were done without reference to the conditions in which the algae lived in nature or in cultures. Although the cytological results obtained are of very great value, it is indispensable that the methods so far used should be thoroughly changed. Ecological studies have shown on the one hand that not only the conditions of life prevailing at the time of the experiments but also their fluctuations must affect protoplasmic structure. On the other hand, it now seems certain that Naumann's trophic conditions of the

waters (1932) play a much greater part in cell protoplasm than was formerly believed. All future experiments must, therefore, take place under rigid control of the culture conditions. Only in this way shall we discover protoplasmic adaptation phenomena.

We shall first have to consider the possible effects of changes of pH and the nitrogen content of waters, the latter having a direct bearing on trophic conditions enabling one to distinguish between autotrophic and heterotrophic algae (Algéus, 1946; Fogg, 1953; Saubert, 1957).

It seems obvious, finally, that the permeability and uptake of dissolved compounds depends principally upon the structure of the protoplasm, so that one can no longer think in terms of a specific filter system. Such hypothetical systems are, however, still accepted by some, although Seifriz (1936) has indicated that the permeation of the whole protoplasm was responsible.

The correctness of this concept was confirmed by later experiments (Cholnoky, 1952*a*, 1952*b*; Höfler, 1959). On this basis, it seems to me highly probable that the structure of the protoplasm (after obligatory or optional nutrition of the algal cells) is subject to changes which are also necessarily manifest in the uptake of stain molecules. As the protoplasm of the purely autotrophic algae must be adapted to small molecules and even ions, its microstructure must be very different from that of the nitrogen heterotrophic species, the protoplasm of which can take up amino acids or even bigger molecules (protein particles, amino acid groups). These differences in protoplasmic structure, which are due to the nutritional requirements of the cell and must also be manifest in the uptake and storage of such substances as stains, seem to me so probable that I am presently engaged in appropriate culture experiments. These experiments will include the uptake of stains and fluorochromes in algae of the same species which have been given different nutrients and also with algae which are genotypically different for a study of their metabolism.

Owing to circumstances beyond my control, these experiments have just begun. It has, however, been supposed that the uptake of dissolved substances represents an active function on the part of the protoplasm, *i.e.*, that it must be a dynamic process, and not one influenced by static structures such as lamellae or filters. That is why it is hardly likely that the results of these experiments will ever be reconcilable with the static concepts of such researchers as Frey-Wyssling (1955). Electron microscopical observations cannot be regarded as a basis of research on the living substances concerned with the uptake of dissolved molecules that Frey-Wyssling called "Grundplasma".

I would like to recall what I said when I referred to the classical period of cytology. The fixing and staining procedures then used could not lead to a knowledge of protoplasmic structure, let alone changes due to physiological causes. Electron microscopy must of necessity use similar, if more refined, methods, as it is technically impossible to study living protoplasm with this kind of microscope. The images obtained with the electron microscope are only of static structural elements, and not of dynamic functions and changes in the protoplasm. More succinctly, fixed protoplasm under the electron microscope is at least partially an artificial product, as otherwise it would continue to live unchanged.

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THE MORPHOLOGY OF PPLO AND BACTERIAL L FORMS*

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The smallest organisms growing without the help of other cells are found in the cultures of pleuropneumonia-like organisms (PPLO). Some are as small as 0.15 to 0.25 μ. The majority of the organisms in the culture is considerably larger. Size is only one of the distinctive characteristics of these organisms. Their structure, the appearance of their colonies, their chemical makeup and their reproductive processes also differ at first sight from those of other microorganisms. However, many similarities to bacteria are present. Their organization is as simple as is that of the bacteria. They do not have distinct nuclei. Their growth requirements, metabolism, and sensitivity to antibiotics are quite similar to those of the bacteria. An important exception is that the PPLO are not sensitive to penicillin. The basic difference between PPLO and bacteria is the absence in PPLO of a rigid cell wall, and most of the distinctive properties of PPLO are the consequence of the lack of this structural property characteristic of bacteria. The organisms are soft, fragile, and easily distorted. Their size varies within wide limits from 0.15 μ to 10 μ, or larger. On agar media the structure and appearance of the colonies of PPLO are characteristic and differ markedly from those of bacteria. Finally, the method of reproduction seems to be more complex than that of bacteria, although basically it is probably similar. In the light of these similarities and differences some authors propose to create a special class for PPLO,[1] while others regard them as a subdivision of the class of bacteria.[2,3]

The PPLO were discovered as parasites causing disease in animals or living on their mucous membranes. They were isolated also from sewage, well water and soil. The saprophytic strains differ in some respects from the parasitic, but we have no information to suggest that they are part of the microflora other than those related to animal organisms.

The suggestion that the PPLO might be an independent subdivision of microorganisms is made unlikely by the observation that bacteria under certain conditions assume a growth form which presents all the distinctive properties of PPLO.[4] These bacterial forms, usually designated as L forms, like the PPLO are soft and fragile, lack a rigid cell wall, and are considerably smaller than the usual bacteria. The appearance of the colonies, the morphology of the organisms, their reproductive processes and their sensitivity to antibiotics are similar to that of PPLO, and include resistance to penicillin. The best illustration of the similarity between the two groups is the fact that 15 years passed before it was generally recognized that the L forms were growth forms of bacteria and not PPLO mixed with the cultures and thus foreign to the bac-

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teria with which they were associated. At present the impression of the majority of bacteriologists interested in these organisms is that, although they are fixed in their form of growth, PPLO derived from the bacteria at some time in the past. This status would correspond to that of the *fungi imperfecti*. The impression of some investigators is that PPLO may represent a primitive stage in the phylogenetic development of bacteria to which under certain conditions bacteria may return. It should also be mentioned that some authors[3,5,6] regard the similarity between PPLO and bacteria as superficial and without significance.

Information on morphology and reproductive processes of PPLO has been confused for a long time, and to some extent it still is today. This confusion exists not so much because of their small size but because of their fragility and the ease with which they may be distorted. For these reasons, use of the electron microscope thus far has yielded hardly more information than a better definition of the smallest elements in the cultures of PPLO.

In 1935, Turner[2] gave an excellent description of the morphology of the organism of bovine pleuropneumonia in broth cultures with dark field illumination. His basic observations are as follows: "An old broth culture contains only small granules less than 0.5 μ in diameter. Transferred to fresh media these granules increase in size to about 1 μ. One or more areas appear on their peripheries from which short filaments may grow out. The structures thus formed suggested the first name of the organism "Asterococcus." The ends of the short filaments grow to a larger size and repeat a similar reproductive process. The filaments may grow longer and either differentiate into small granules or develop swellings from which filaments again grow out. In addition, rather large spherical or irregularly-shaped bodies, several μ in diameter appear in the culture. Under appropriate conditions these reproduce the granules and filaments. Very long straight filaments, sometimes visible in the cultures, are apparently artefacts."

The development of colonies of PPLO in agar cultures was carefully studied by Liebermeister.[7] With the phase microscope he examined several strains. Like Turner, he observed the extrusion of short filaments from the granules and the development of new organisms at the end of the filament. It is characteristic that the smallest organisms seem to divide but that the daughter organisms usually are not closely associated but seem to be at the ends of a short rod. Liebermeister did not observe the development of multiple filaments from a granule nor the development of long filaments in the strains which he studied.

The size of the organisms, especially on the surface of agar colonies, varies in the cultures, and the smallest forms visible with the light microscope usually make up a very small fraction of the culture. Autolysis and deformation of the larger forms often produce a bewildering pleomorphism in aging cultures.

Klieneberger[5] has published beautiful photographs indicating that the larger organisms are aggregates of small ones enclosed in a common envelope. This structure of the large forms is clearly visible in electron micrographs. Under appropriate conditions granules grow out from the large bodies.

From this short discussion it seems that the morphology and reproductive

processes of the organisms are very simple. The basic elements are small granules between 0.15 and 0.3 μ in diameter that multiply by fission after elongation. Somewhat larger forms may divide by extruding short filaments. In addition the granules may form more or less large aggregates enclosed in a common envelope out of which they again grow. The structure of such large bodies is essentially similar in cultures of bacteria, L forms, and PPLO. In the organisms of bovine pleuropneumonia, and possibly in a few other strains, the granules also can grow into thin filaments. This form of growth was not observed in most strains.

L forms, like PPLO, do not have rigid cell walls. This lack of a rigid cell wall is demonstrated in thin sections of L forms examined with the electron microscope. Chemical studies indicate that the L forms do not have the chemical complexes that are responsible for the rigidity of bacterial cell walls. A large part of the similarity of L forms to PPLO is the consequence of this lack. However, some of the similarities to PPLO do not seem to be the immediate consequence of the absence of a rigid cell wall. One of these is the small size of both PPLO and the L forms. According to filtration measurements by Klieneberger, the size of viable granules in the L forms of *Streptobacillus moniliformis* is similar to, or only slightly larger than, the size of PPLO. The electron microscope shows granules of similar size in both groups. Another property not directly connected with the cell wall, common to both groups, is the tendency of the growing organisms to embed themselves in agar. Multiplication in agar cultures occurs mainly inside the agar. The characteristic appearance of the colonies in both groups is the consequence of this tendency. Both groups of organisms invade agar only and not other solid media. Finally, a remarkable property of both groups is the tendency to grow into large bodies. This tendency is greater in the L forms than in PPLO. The L forms in broth or in gelatin multiply only by the growth of granules to large bodies and by the liberation of granules from the large bodies.

As noted above, bacteria also tend to grow into large bodies. Transformation of bacteria to L forms is always preceded by the appearance of large bodies, and the L forms grow out of the large bodies. In a few instances large bodies were observed during formation from bacteria,[8] and like bacterial filaments, these bodies developed by multiplication without separation of the bacteria. In the early stages large bodies disintegrated into a group of bacteria by the development of cell walls between the constituent organisms. After this period, the large bodies reproduced bacteria for a certain length of time. Later, an increasing number lost the ability to develop or they produced L forms. Some of the L forms so produced, like the large bodies developing from bacteria, return immediately to bacterial form when the influence resulting in these transformations, *e.g.*, penicillin, is eliminated. Most L forms revert to a bacterial form of growth only occasionally and after long cultivation may lose this ability completely.

The large bodies are formed in these instances, under conditions which inhibit the multiplication of the single organisms, by multiplication of organisms possessing the full potentialities of bacteria. After some time the ability to return to bacteria is lost, but the organisms are able on agar media to multiply

outside the large body. The agar seems to offer a suitable physical environment similar perhaps to that present in the large bodies and necessary for multiplication of L forms.

Bacterial large bodies have been known since the beginning of bacteriology and are usually referred to as involution or dying forms. They are produced by a great variety of influences on the bacteria that prevent normal multiplication. Large bodies occur in the natural environment of bacteria. In some cases, in contrast to older opinion, it is apparent that they remain viable and able to reproduce for a longer period than single bacteria. Hence, the formation of large bodies is probably a useful process for bacteria in their natural environment and can be thought of as a phenomenon of adaptation and not merely the result of degeneration.

At present, L forms can not be regarded in the same light. In most instances they develop and can be propagated only under artificial conditions. It seems likely that they represent the growth of forms in the laboratory that naturally occur only in the large bodies derived from bacteria. Small size, growth into agar, and a tendency to produce large bodies (characteristics of L forms) may be the result of this natural site of growth.

It is remarkable that bacteria cultivated directly from pathological processes relatively often have the tendency to grow into large forms and to produce L colonies. This may be the result of injury to the organism by the defensive forces of the host. On the other hand, it may be an adaptation of the bacteria to parasitism. In one case of peritonitis, for example,[8] it seemed that a bacteroides strain continued to multiply in the L form inside the phagocytic cells of the host. Such an observation suggests that although L forms may be produced under artificial conditions, this process might occur naturally and thus might have been the origin by stabilization of strains of PPLO that have continued life in this form. The PPLO not only appear to be bacteria without the usual cell wall but also bacteria that have passed through the processes involved in the growth of the large bodies. The most marked difference between L forms and PPLO is that PPLO are better adapted to grow in artificial media and especially to grow in the small granular form. The L forms grow usually only from large inocula and have a pronounced tendency to grow into large bodies as well as to undergo autolysis.

At present PPLO do not seem to be of the main stream of phylogenetic development or to be a link in it. These organisms probably represent the result of the simplification of the structure of bacteria as a consequence of parasitism. They are not complex and occasionally are very small but, like the viruses, they offer no direct clues for the origin of life.

For illustration of the morphology of PPLO and L forms we refer to articles previously published in the Annals of this Academy.[9,10]

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AXENIC CULTURE OF *PARAMECIUM*—SOME OBSERVATIONS ON THE GROWTH BEHAVIOR AND NUTRITIONAL REQUIREMENTS OF A PARTICLE-BEARING STRAIN OF *PARAMECIUM AURELIA* 299λ

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The genus *Paramecium* comprises a group of free living ciliates noted for their morphological and genetical complexity. For these reasons and because the organisms represent an end point in a divergent course of evolution, this genus has been an object of interest. Certain members of this group exist in association with self reproducing, intracytoplasmic particles.[1,2] Recent advances in the knowledge of the nutritional requirements of *Paramecium* has made it possible to cultivate these particle-bearing paramecia in sterile medium. The purpose of this paper is to summarize the present state of knowledge of the nutrition of *Paramecium* and to present the results of some detailed studies on a particle bearing strain, *Paramecium aurelia* 299λ.

Nutrition of Paramecium. In the past, *Paramecium* was cultivated in a medium consisting of plant extracts, notably cerophyl and lettuce infusion, supplemented with living bacteria, usually *Aerobacter aerogenes*.[3] The first successful report of axenic cultivation was made by Johnson and Baker in 1942.[4] These workers grew *Paramecium multimicronucleata* in a medium consisting of pressed yeast juice and proteose peptone. Two components of the pressed yeast juice were required for growth. One proved to be heat labile which they assumed to be a protein, but was later replaced by a mixture of ribosidic derivatives of a purine and a pyrimidine; the other was a heat stable component. In 1949, van Wagtendonk and Hackett successfully established *P. aurelia* in a medium composed of equal parts of 0.5 per cent yeast autolysate and a 24-hour culture of *A. aerogenes* in lettuce extract.[5] This medium could be heat sterilized and provided the basis for later work which led to the development of a more complex bacteria free medium.[6] Folic acid, riboflavin, thiamine, and a steroid proved to be absolute requirements for the growth of stock 51.7 of *P. aurelia*; the steroid requirement could be satisfied by β- and γ-sitosterol, fucosterol, brassicassterol, stigmasterol, and $\Delta^{4,22}$-stigmastadienone.[7,8] Miller and van Wagtendonk found that *P. aurelia* required 11 amino acids, nicotinic acid, panothenic acid, and possibly, pyridoxal.[9] Also, one or more essential growth factors remained in the yeast. Miller and Johnson studied further the nutrition of *P. multimicronucleata*, and demonstrated, in addition to the purine and pyrimidine requirement for that organism, a need for an exogenous source of a fatty acid.[10-13] Recently Lilly *et al.* cultivated *Paramecium caudatum* in a medium chemically defined, except for a single component.[14] Their medium was similar to the one used for the cultivation of *P. aurelia* and *P. multimicronucleata*, except that it was necessary to add microgram quantities of a protein concentrate obtained from dried green peas.

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Purification of the protein factor and subsequent analysis led to the qualitative identification of 16 amino acids. The nutritional role of this protein has not been satisfactorily explained. In TABLE 1 is given the composition of a typical medium which supports the growth of most strains of *Paramecium*.

Axenic cultivation of λ-bearing Paramecium. Lambda particles were discovered in the cytoplasm of stock 299λ of *P. aurelia* by Schneller, in 1958.[15] She noted that animals containing these particles possessed the ability to kill sensitive or particle free animals when members of the appropriate types were placed in the same container. In this respect, this particle-protozoan system is similar to the well known κ system.[16]

TABLE 1
AXENIC MEDIUM FOR *PARAMECIUM*

Amino acids	μg./ml.	Vitamins	μg./ml.
L-Alanine	25	Biotin	0.125
*L-Arginine·HCl	100	*Ca-pantothenate	5
L-Aspartic acid	50	*Folic acid	2.5
L-Glutamic acid	75	α-Lipoic acid	0.05
Glycine	25	*Nicotinamide	5
*DL-Histidine	50	*Pyridoxal·HCl	5
*DL-Isoleucine	150	Pyridoxamine·HCl	2.5
*DL-Leucine	150	*Riboflavin	5
*L-Lysine·HCl	125	*Thiamine·HCl	15
*DL-Methionine	150	*Inorganic salts*	
*L-Phenylalanine	75	$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$	15
L-Proline	50	$ZnCl_2$	2
*DL-Serine	200	EDTA	5
*DL-Threonine	150	$MnSO_4 \cdot 4H_2O$	2
*L-Tryptophan	50	$CuSO_4 \cdot 5H_2O$	0.3
*L-Tyrosine	50	$CoSO_4 \cdot 7H_2O$	0.5
DL-Valine	75	$MgSO_4 \cdot 7H_2O$	50
Purines and pyrimidines		*Other factors*	
*Guanylic acid	500	*Stigmasterol	1
*Uridylic acid	500	*Na oleate	40
		Na acetate	500
		*Yeast factor†	50–500

* Components known to be absolute requirements for the growth of one or more species of *Paramecium*.

† For preparation see (9). May be replaced by Pea factor for *P. caudatum* (14).

Efforts to cultivate λ-bearing animals in media used for the growth of particle free strains were unsuccessful. It was necessary to supplement a crude medium consisting of proteose peptone, a dialyzable component of hot water extract of Baker's yeast and salts, with Edamine S, an enzymatic digest of lactalbumin.[17] This medium supported the growth of the protozoans and maintenance of the particles through serial subcultures for a period of 2 years.

Particles of axenically cultivated animals number several hundred per cell, contain RNA, little or no DNA, and are similar in size to the bacterium, *Escherichia coli*.[18] They are gram-negative and may be stained with most bacteriological dyes. Examination under phase microscope reveals a rod or diplorod type structure. A furrow which divides the particle into almost

equal halves suggests that the particles may reproduce by longitudinal division; occasionally they appear to be vacuolated.

Particle reproduction is synchronized with the division of the protozoan.[18] Further evidence in support of this view is given in FIGURE 1. Animals re-

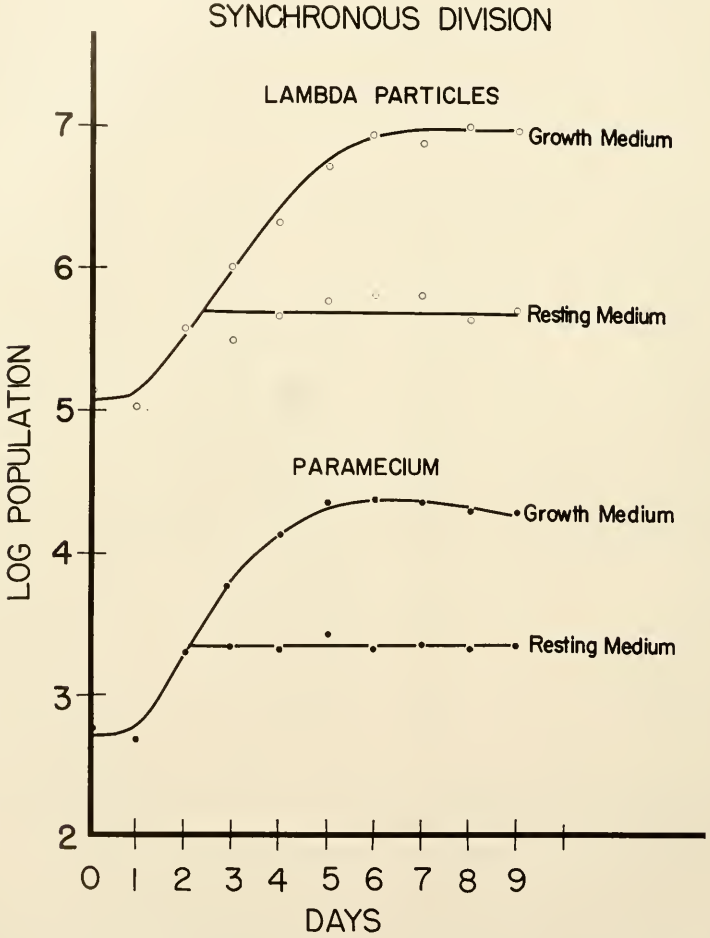

FIGURE 1. Growth medium: see TABLE 1. Resting medium: isotonic saline, 0.01 M phosphate buffer, pH 7.0.

moved during the log phase of growth, washed to remove all traces of the original medium and resuspended in a buffered salt solution, "resting medium," failed to multiply. Estimates of the particle population revealed that they, too, did not increase in number. Synchronous division may account for the ability of the particles to keep pace with the host, although it seems likely that this may be only a partial answer to the phenomenon. It has been observed that occasionally one or more of the animals loses all its particles. Clones

derived from these animals are also particle free. It has not yet been possible to rule out mutation as an explanation for this phenomenon.

The existing synchronism between the particles and the host cell makes it possible to quantitatively evaluate agents that may selectively inhibit the particles themselves. Of interest here is the number of antibiotics that possess this capability (TABLE 2). ID_{50} values, derived in a manner previously described,[18] reflect the relative effectiveness of these substances to inhibit particle populations. This selective action correlates with the toxicity produced by these agents in man. Antibiotics such as penicillin and tetracycline which exhibit the least toxicity in man prove to be excellent particle inhibitors; those,

TABLE 2

A COMPARISON OF THE ACTIVITY OF ANTIBIOTICS IN THE λ SYSTEM WITH CHRONIC TOXICITY IN MAN*

Antibiotic tested	ID_{50} ratio protozoan particle†	ID_{50} protozoan only	Toxicity in man
	λ		
Actinomycin D	0.9	1	Very high toxicity.
Actidione	0.9	>1,000	Toxic—fatal to rats—1 mg./kg. orally.
Bacitracin	0.9	370	Nephrotoxicity, proteinuria.
Neomycin	1.1	32	Nephro- and ototoxicity.
Polymyxin	1.2	32	Causes renal damage.
Candicidin	0.9	>1,000	Toxic—used topically.
Streptomycin	1.8	350	Low toxicity—damage to eighth cranial nerve on prolonged therapy.
Cephalosporin C	10	>1,000	Low toxicity—mice tolerate 5,000 mg./kg. intravenously.
Novobiocin	10	320	Low toxicity—7 mg./kg. intravenously tolerated in man.
Oleandomycin	14	450	Low toxicity—40 mg./kg. orally tolerated in children.
Chloramphenicol	22	220	Low toxicity—30 mg./kg. tolerated in man.
Aureomycin	39	40	Low toxicity—15–30 mg./kg. tolerated in man.
Terramycin	116	370	Low toxicity—15–30 mg./kg. tolerated in man.
Penicillin	312	>1,000	Very low toxicity—very well tolerated.
Tetracycline	930	220	Very low toxicity—very well tolerated.

* Toxicity data obtained from Spector, W. S. 1957. "Handbook of Toxicology," vol. II.
† ID_{50} ratios of greater than 1.2 indicate selective inhibition.

such as actinomycin and neomycin produce varying degrees of toxicity in man and are not selectively inhibitory for the particle. Thus, the particle-*Paramecium* system might be useful in predicting chronic human toxicity of potentially useful antibiotic substances. In FIGURE 2 the effectiveness of penicillin in reducing the λ population is shown. Under the conditions of the experiment complete destruction of the particles is achieved in 1 day at a concentration of 100 units per milliliter of the antibiotic.

Nutritional requirements of λ-bearing Paramecium. A nutritional study was made with particle-bearing and particle-free strains. The latter were obtained by treating axenically cultivated, λ-containing animals with penicillin to remove the particles. Both require a factor (or factors) present in a nondialyzable aqueous extract of Baker's yeast. Chemical fractionation resulted in a

partially purified material which is not absorbed on anion or cation exchange resins; the material may be precipitated with 67 per cent ethanol in the cold, contains carbohydrate, protein, and no nucleic acid or lipids. Attempts to replace this fraction with known substances, thus far, have been unsuccessful. However, it has been possible to demonstrate a purine and pyrimidine requirement for the organisms, as well as their need for a number of vitamins, in a medium (TABLE 1) supplemented with this factor.

Purine requirements for particle containing and particle free animals are summarized in TABLE 3. The need for exogenous source of a purine derivative

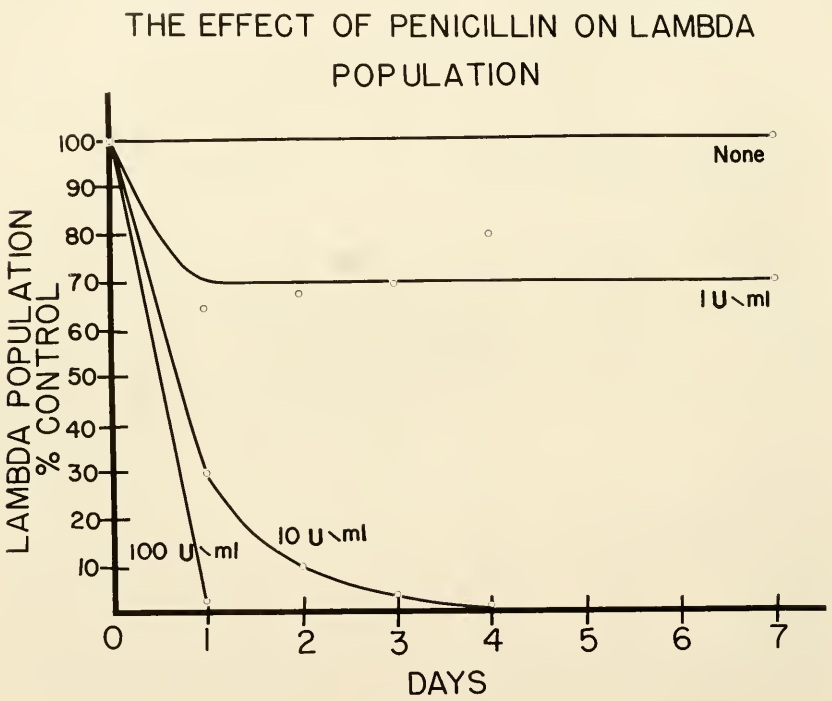

FIGURE 2.

is apparent and may be met by guanosine and guanylic acid. The free base, and adenosine and its derivatives, do not replace the purine. Apparently, *Paramecium* converts guanosine to adenosine and its derivatives, whereas the reverse reactions do not occur. Inosine, its derivatives, and xanthosine and its derivatives failed to replace guanosine as a growth requirement.

The pyrimidine requirements may be satisfied by uridine, cytidine, uridylic and cytidylic acids (TABLE 4). The free bases uracil, cytosine, and thymine, as well as thymidine and thymidylic acid were not effective in replacing uridine or cytidine. These data confirm earlier work with *P. multimicronucleata*, *P. caudatum*, and other strains of *P. aurelia*.

By means of C^{14}-labeled purines, it has been shown that adenosine is incorporated into nucleic acid adenine only, whereas exogenously supplied guano-

sine is incorporated into both nucleic acid-guanine and -adenine.[19] These data confirm the nutritional findings. Similar data obtained with isotopically labeled pyrimidines are in agreement with the nutritional evidence that cytidine and uridine are interconvertible and serve as precursors for thymidine and thymidylic acid. The data further illustrate that similar pathways exist for

Table 3
Purine Requirements of *Paramecium*

Purines tested (2 μM/ml.)	Population density* (No./ml.)	
	299λ	299 S
Adenine	0	0
Guanine	0	0
Hypoxanthine	0	0
Xanthine	0	0
Adenosine	0	0
Guanosine	9200	10,200
Inosine	0	0
Xanthosine	0	0
Adenylic acid (5′)	0	0
Guanylic acid (5′)	8700	9200
Inosinic acid (5′)	0	0

* Values obtained after first transfer.

Table 4
Pyrimidine Requirements of *Paramecium*

Pyrimidines tested (2 μM/ml.)	Population density* (No./ml.)	
	299λ	299 S
Cytosine	0	0
Uracil	0	0
Thymine	0	0
Cytidine	6600	5800
Uridine	8200	7600
Thymidine	0	0
Cytidylic acid (5′)	5200	7200
Uridylic acid (5′)	4800	6500
Thymidylic acid (5′)	0	0

* Values obtained after first transfer.

purine and pyrimidine utilization in both particle free and particle bearing animals.

Generally, the requirements for vitamins for particle bearing and particle free animals are similar (TABLE 5). The need for nicotinamide, riboflavin, and thiamine becomes apparent in the second transfer, whereas the requirement for pyridoxal is evident only after three or four serial subcultures. An absolute requirement for calcium panthentate has not been shown. Some degree of growth, approximating 10 per cent of the control, remains even after several transfers. Biotin and lipoic acid are not required. Particle bearing animals,

in the absence of folic acid, may be subcultured indefinitely. Particle free animals, on the other hand, show an absolute requirement for this substance, as judged from their inability to grow beyond the second transfer.

Particles may produce sufficient quantities of folic acid to provide for the nutritional needs of the protozoan. To test this possibility, particle bearing animals were treated with penicillin in the presence and the absence of folic acid (TABLE 6). As expected, particle free animals did not grow in the con-

TABLE 5
VITAMIN REQUIREMENTS OF *PARAMECIUM*

Vitamin	Population (% control)									
	299λ					299 S				
	Serial subculture					Serial subculture				
	1	2	3	4	5	1	2	3	4	5
Biotin	105	123	94	99	105	110	69	77	112	105
Ca pantothenate	95	38	38	7	12	105	73	74	36	8
Folic acid	100	71	71	69	75	40	0			
α-Lipoic acid	101	103	99	85	95	87	57	96	121	112
Nicotinamide	97	0				75	0			
Pyridoxal	110	98	82	0		75	48	37	26	0
Riboflavin	35	0				0				
Thiamine	25	0				12	0			

TABLE 6
THE EFFECT OF PENICILLIN UPON THE FOLIC ACID REQUIREMENT OF *PARAMECIUM*

Addition	Population density (No./ml.)			
	Medium plus folic acid		Medium minus folic acid	
	299λ	299 S	299λ	299 S
None	5200	7400	3800	0
Penicillin, 1000 U/ml.	9000*	10,200	0	0

* Animals particle free.

trols, or in penicillin-treated tubes in the absence of folic acid. Addition of folic acid to the medium restored the ability of these animals to grow in the presence or absence of penicillin. Particle containing animals, on the other hand, failed to grow in folic acid free medium containing penicillin, whereas animals under similar conditions retained their particles and grew well in the absence of penicillin. Growth of particle bearing animals in which folic acid was present in both the control and penicillin-treated tubes was good. Penicillin-treated animals contained no particles. Subsequent deletion of folic acid from the medium containing these penicillin-treated animals resulted in the death of the protozoan. These data support the view that folic acid pro-

duction is dependent upon the presence of the particle in the cytoplasm; implicit here is that the vitamin is produced by the particles themselves.

Discussion

The symbiotic association between λ particles and the host *Paramecium* is an example of what is doubtless a widespread phenomenon in nature. *Paramecium bursaria* harbors an alga of the genus *Chlorella* in its cytoplasm in what has been described as a symbiotic system.[20] Colicins in bacteria,[21] extranuclear particles responsible for cytoplasmic inheritance in yeast,[22] and particle-like inclusions found in many insect tissues[23,24] may be further examples. A well documented case of an endosymbiote has been found for the flagellated protozoan, *Strigomonas*.[25] This organism, apparently, exists in association with cytoplasmic bipolar-like bodies. The presence of these particles, as with *Paramecium*, alters the nutritional requirements of the host.

A distinctive feature of the λ system is the ability of particle bearing animals to release a toxin which causes the death of certain particle free detector strains, but is without effect upon the λ bearers themselves. In this respect the λ system bears a striking resemblance to colicin producing systems.[26] Colicins are antibiotic substances produced by certain bacteria, notably members of the family Enterobacteriaceae. The ability of these bacteria to produce these substances is believed to be due to the presence of a transmissible pathogenic agent which is regarded as a bacterial virus. The analogy serves to illustrate the degree to which the λ particles may have incorporated themselves into the genetic structure of the protozoan.

Yet λ particles, unlike viruses, are highly complex structures which resemble bacteria in size, morphology, staining characteristics, chemical composition, and, possibly, manner of reproduction. Studies concerning the chemistry of the particles reveal the presence of protein, carbohydrate, phospholipid, and nucleic acid (W. J. van Wagtendonk and R. Tanguay—personal communication). Moreover, antibiotics are particularly effective in reducing or eliminating the particles from the cytoplasm of the protozoan. (These data, obtained for the first time with axenically cultivated animals, provide the strongest evidence to date on the action of antibiotics on particles in *Paramecium*.) Finally, the finding that λ particles produce amounts of folic acid sufficient to support the growth and reproduction of the protozoan carries with it the implication that the complex enzymatic machinery necessary for the synthesis of this compound is present in the particles themselves.

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THE EFFECT OF POLLUTION ON RIVER ALGAE

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A large majority of algae are affected adversely by the gross pollution of streams with organic wastes such as domestic sewage. After partial self-purification of the stream has occurred, however, the populations and kinds of algae become much more numerous than are present in the clean portion of the stream above the area of pollution. This increase is due to the nutrients that are made available from the decomposing organic wastes.

The undecomposed organic wastes affect the algae by causing chemical and physical changes in the stream. Increased turbidity reduces the light available for photosynthesis. Increased organic content in the water stimulates saprophytic and saprozoic organisms which then compete for space with the algae. Certain constituents of the waste are toxic to many algae. Thus, many factors of the environment that are changed by the organic wastes have an effect on the algae.

Information on the physiological and morphological effects of organic pollution on algae is very limited at present. There have been, however, many studies of the change in the algal flora as a result of pollution. Gross pollution causes a great reduction in the number of kinds of algae in the stream. Those able to remain have frequently been called "indicators" of pollution, but no specific kinds individually are reliable indicators of grossly polluted water. Polluted water varies too much to ensure an environment satisfactory for the growth or persistence of any one particular algal species. Any individual species tolerant of pollution may also be found in unpolluted areas of a stream or may be absent in some areas of pollution.

When a number of the tolerant genera and species are considered, it becomes likely that a high percentage of these will be present in all areas of streams grossly polluted with organic wastes. The presence of such a community of algae in a stream, therefore, is a reliable indicator of the condition of the water.

Many workers have listed the genera and species of algae found in polluted waters, particularly in the United States and in Europe. The number of kinds which they have considered to be pollution tolerant is generally quite limited for any one area or survey, but becomes very large when all of the results of many investigators are combined.

The lists of pollution-tolerant algae reported by 110 workers have been examined by the writer to date. The genera and species of algae tolerant to sewage or to related conditions have been recorded, and a total of more than 600 species and varieties has been compiled.

To tabulate the information, the writer has allotted arbitrary numerical values to each author's record of an alga. A value of 2 was given to each alga reported as *very highly tolerant*, and a value of 1 to each alga *highly tolerant* to the presence of organic matter. Lightly tolerant and nontolerant algae were not recorded in the compilation. The total points from all of the 110

authors were then determined for each genus and species. The algae were arranged in the order of decreasing emphasis by the authors as a whole as indicated by the comparative total scores for each alga. Theoretically an alga considered as very highly tolerant by all 110 authors would have had a perfect score of 110 multiplied by 2, or 220 total points.

For studies in sanitary science the algae are frequently placed into four groups. All flagellates containing photosynthetic pigments constitute one of the four groups. The other three groups are the blue-green algae, the diatoms, and the green algae, the last group including all of the nonflagellated green, yellow-green, and other related forms.

TABLE 1
POLLUTION ALGAE

Most tolerant genera, by groups

	Highest		
	4	10	50
Blue-greens	1	2	8
Greens	1	3	15
Diatoms	0	3	15
Flagellates	2	2	12

TABLE 2
POLLUTION ALGAE

Most tolerant species, by groups

	Highest		
	4	10	50
Blue-greens	1	3	15
Greens	1	4	10
Diatoms	1	1	11
Flagellates	1	2	14

All four groups are well represented among the genera and species with high scores as pollution-tolerant algae. For example, of the 10 genera with the highest scores, 2 are blue-green algae, 2 are flagellates, 3 are diatoms, and 3 are green algae (TABLE 1). Of the four species with the four highest scores, each belongs to a different group. Among the 50 most tolerant species, the range in number per group is from 10 to 15 (TABLE 2).

The 52 most tolerant genera are listed in TABLE 3. Leading the list, in order of decreasing total scores, are *Euglena*, *Oscillatoria*, *Chlamydomonas*, *Scenedesmus*, *Chlorella*, and *Nitzschia*. The first two were considered as tolerant genera by 62 and 61 authors and rated 110 and 105 total points, respectively. These are in contrast with the fiftieth genus, *Cocconeis*, which was referred to by only 8 authors for a total score of eight.

TABLE 3

POLLUTION TOLERANT GENERA OF ALGAE LIST OF 52 MOST TOLERANT GENERA IN ORDER OF DECREASING EMPHASIS BY 110 AUTHORITIES

	Genera	Group	No. of authors	Total points*
1	*Euglena*	F	62	110
2	*Oscillatoria*	B	61	105
3	*Chlamydomonas*	F	42	70
4	*Scenedesmus*	G	40	65
5	*Chlorella*	G	36	63
6	*Nitzschia*	D	38	63
7	*Navicula*	D	35	55
8	*Stigeoclonium*	G	34	50
9	*Phormidium*	B	30	45
10	*Synedra*	D	25	33
11	*Phacus*	F	23	32
12	*Ankistrodesmus*	G	19	31
13	*Gomphonema*	D	20	30
14	*Spirogyra*	G	19	29
15	*Cyclotella*	D	22	29
16	*Pandorina*	F	18	25
17	*Closterium*	G	19	25
18	*Lepocinclis*	F	14	24
19	*Melosira*	D	18	24
20	*Chlorogonium*	F	14	23
21	*Anabaena*	B	17	23
22	*Ulothrix*	G	17	23
23	*Micractinium*	G	13	21
24	*Fragilaria*	D	15	20
25	*Anacystis*	B	16	20
26	*Trachelomonas*	F	16	20
27	*Arthrospira*	B	11	19
28	*Carteria*	F	12	19
29	*Surirella*	D	14	19
30	*Cryptomonas*	F	15	19
31	*Agmenellum*	B	11	18
32	*Lyngbya*	B	11	18
33	*Eudorina*	F	12	18
34	*Pediastrum*	G	14	18
35	*Oocystis*	G	12	16
36	*Pyrobotrys*	F	10	15
37	*Cymbella*	D	10	14
38	*Stephanodiscus*	D	10	14
39	*Coelastrum*	G	12	14
40	*Cladophora*	G	13	14
41	*Golenkinia*	G	9	13
42	*Spondylomorum*	F	9	13
43	*Achnanthes*	D	11	13
44	*Actinastrum*	G	11	13
45	*Hantzschia*	D	9	12
46	*Spirulina*	B	9	12
47	*Pinnularia*	D	8	11
48	*Stauroneis*	D	9	11
49	*Tribonema*	G	6	10
50	*Cocconeis*	D	8	10
51	*Selenastrum*	G	8	10
52	*Cosmarium*	G	9	10

* Tolerance by author, "Very High," 2 points. Tolerance by author, "High," 1 point.

TABLE 4

POLLUTION TOLERANT SPECIES OF ALGAE: A LIST OF THE 60 MOST TOLERANT SPECIES IN ORDER OF DECREASING EMPHASIS BY 110 AUTHORITIES

	Species	Group	No. of authors	Total points*
1	*Euglena viridis*	F	34	63
2	*Nitzschia palea*	D	30	46
3	*Stigeoclonium tenue*	G	17	26
4	*Oscillatoria tenuis*	B	17	25
5	*Oscillatoria limosa*	B	14	21
6	*Scenedesmus quadricauda*	G	12	18
7	*Chlorella vulgaris*	G	11	17
8	*Pandorina morum*	F	12	17
9	*Arthrospira jenneri*	B	9	16
10	*Ankistrodesmus falcatus*	G	11	16
11	*Cyclotella meneghiniana*	D	12	16
12	*Chlorella pyrenoidosa*	G	8	15
13	*Gomphonema parvulum*	D	8	15
14	*Euglena gracilis*	F	9	15
15	*Oscillatoria chalybea*	B	10	15
16	*Synedra ulna*	D	12	15
17	*Oscillatoria chlorina*	B	9	14
18	*Nitzschia acicularis*	D	10	14
19	*Oscillatoria formosa*	B	10	14
20	*Oscillatoria princeps*	B	10	14
21	*Oscillatoria putrida*	B	8	13
22	*Euglena oxyuris*	F	9	13
23	*Navicula cryptocephala*	D	9	13
24	*Phormidium uncinatum*	B	11	13
25	*Agmenellum quadriduplicatum*	B	7	12
26	*Chlorogonium euchlorum*	F	7	12
27	*Hantzschia amphioxys*	D	9	12
28	*Phormidium autumnale*	B	9	12
29	*Surirella ovata*	D	9	12
30	*Euglena acus*	F	10	12
31	*Lepocinclis ovum*	F	7	11
32	*Micractinium pusillum*	G	7	11
33	*Eunorina elegans*	F	8	11
34	*Euglena deses*	F	8	11
35	*Oscillatoria splendida*	B	9	11
36	*Oscillatoria lauterbornii*	B	6	10
37	*Euglena polymorpha*	F	7	10
38	*Lepocinclis texta*	F	7	10
39	*Spondylomorum quaternarium*	F	7	10
40	*Actinastrum hantzschi*	G	8	10
41	*Closterium acerosum*	G	8	10
42	*Anabaena constricta*	B	6	9
43	*Anacystis montana*	B	6	9
44	*Phacus pyrum*	F	6	9
45	*Scenedesmus obliquus*	G	6	9
46	*Cocconeis placentula*	D	7	9
47	*Achnanthes minutissima*	D	8	9
48	*Coelastrum microporum*	G	8	9
49	*Melosira varians*	D	8	9
50	*Chlamydomonas reinhardi*	F	5	8
51	*Pediastrum boryanum*	G	5	8
52	*Scenedesmus dimorphus*	G	5	8
53	*Chlorogonium elongatum*	G	6	8
54	*Euglena intermedia*	F	6	8
55	*Euglena pisciformis*	F	6	8
56	*Phacus pleuronectes*	F	6	8
57	*Tetraedron muticum*	G	6	8
58	*Anacystis cyanea*	B	7	8
59	*Melosira granulata*	D	7	8
60	*Phormidium foveolarum*	B	8	8

* Tolerance by author, "Very High," 2 points. Tolerance by author, "High," 1 point.

The 60 most tolerant species are given in TABLE 4. *Euglena viridis*, followed by *Nitzschia palea*, are at the top of the list with total scores of 63 and 46, respectively.

The names and total points for the 10 most tolerant species of a genus are shown for the two leading genera, *Euglena* and *Oscillatoria* (TABLES 5 and 6). In the former genus, the first species, *E. viridis*, is far ahead of the other nine species. In the latter genus there is a more gradual change in total points from

TABLE 5
SPECIES OF EUGLENA: TEN MOST TOLERANT OF POLLUTION

	Authors	Points
E. viridis	34	63
E. gracilis	9	15
E. oxyuris	9	13
E. acus	10	12
E. deses	8	11
E. polymorpha	7	10
E. intermedia	6	8
E. pisciformis	6	8
E. proxima	5	7
E. spirogyra	6	7

TABLE 6
SPECIES OF OSCILLATORIA: TEN MOST TOLERANT OF POLLUTION

	Authors	Points
O. tenuis	17	25
O. limosa	14	21
O. chalybea	10	15
O. chlorina	9	14
O. formosa	10	14
O. princeps	10	14
O. putrida	8	13
O. splendida	9	11
O. lauterbornii	6	10
O. brevis	6	7

one species to the next. Eight of the 10 species of *Euglena* and 9 of *Oscillatoria* are among the 60 most tolerant forms as noted in TABLE 4.

It would be interesting to know what species of *Chlamydomonas* was considered most tolerant of organic pollution, but unfortunately very few of the 110 investigators have determined and recorded the species for this genus. For the genus *Navicula*, numerous species have been recorded by the investigators, but there is little indication that there may be one or two species which are much more tolerant than others that they have named.

Additional records by other workers would undoubtedly change the comparative total points and the relative positions of the algae in both the genus and species lists. This is particularly so for the algae near the low ends of the lists where a relatively few reports are responsible for their present positions.

The lists of algae in the tables are meant to be aids for persons engaged in stream pollution surveys or related projects. They give a general consensus of opinion as to the relative significance of the many algae tolerant of organic wastes which have been reported. Particular care can thus be taken in biological surveys to check for the presence of these genera and species of algae during the microscopic examination of samples.

The references given represent many of the more exhaustive studies that were included in the preparation of this report.

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ULTRASTRUCTURE RESEARCH AS AN AID IN THE CLASSIFICATION OF DIATOMS

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Present Knowledge of the Ultrastructure of Diatoms

The frustules of diatoms were among the first biological objects to be examined with the electron microscope (Mahl, 1939), and in the preceding 20 years a large number of works dealing with the subject have appeared. These have been listed comparatively recently by Hendey (1959), and at the time at which he wrote information about some 300 species was available. Although it is not important to review the results of these studies in detail, there are two points about them which need to be emphasized here. The first is that none of this work has been done with any particular taxonomic problem in mind. For the most part it would seem that investigators took the material which came readily to hand, mounted drops of it on electron microscope grids, and took pictures of the forms they found there. This has on occasion led to doubt as to the true identity of the species studied, as in the case of the illustrations published by Kolbe (1951, plate 2, fig. 4, plate 3, figs. 5 and 6) as *Navicula subtilissima* Cleve, but said by Hustedt (1952, 1955) to be of *Anomoeoneis exilis* (Kütz.) Cleve or *A. serians* var. *brachysira* (Bréb.) Cleve (Kolbe, 1954, 1956, 1959). A more important consequence, however, is that there is not any group of supposedly related species of which more than a small proportion have been studied with the electron microscope. In no case do we know the patterns of similarity and difference and the range of ultrastructure to be found within a single genus, with the possible exception of *Pinnularia* Ehrenb., of which electron micrographs of some 15 species in a genus totaling at least 250 suggest that the ultrastructure is as uniform as that revealed by the light microscope.

The other important point is that the interpretation of electron micrographs is by no means easy, and also that, in some cases, those published do not give an adequate picture of the structure of the species illustrated, either because the specimen was damaged in preparation or because the resolution is insufficient. Interpretation is difficult because of the great depth of focus of the electron microscope and the considerable opacity of silica to electrons. Even in pictures of complete frustules, the whole is equally in focus. In the light microscope it is possible to build up a picture in depth from a series of optical sections obtained by alterations of focus, but this technique is not available to the electron microscopist. When more than one layer is visible it is often not possible to tell from single pictures which lies above which. Much of the valve, also, is completely opaque to electrons, and where this is so there is no information about differences in thickness from differences in transmission of electrons. Stereomicrographs accordingly provide much more information than single prints, as may be seen from the large number published by Helmcke and Krieger (1953, 1954, Helmcke *et al.*, 1961). These authors have applied stereogrammetric techniques to the study of their stereoscopic pairs and have produced models of the structure of a number of species, thus obtaining the maximal amount of information from the data recorded on the micrographs.

The possibility of being misled by photographs with inadequate resolution or of damaged specimens is best illustrated by particular examples. Hendey's (1959) list of the species examined with the electron microscope includes an indication of the ultrastructure of the valve. Both *Stauroneis anceps* Ehrenb. and *S. phoenicenteron* (Nitzsch) Ehrenb. are said to have laminar valves perforated by fully open holes. His information about *S. anceps* is derived from a picture published by Helmcke and Krieger (1953, plate 67) and that about *S. phoenicenteron* from three pictures published by Okuno (1949, plate 3, fig. 8, 1952, plate 19, fig. 4, 1955, plate 9, fig. 1). In both species, however, the striae consist of a series of elongated chambers with a membrane pierced by a slit on the outside and a membrane with fine pores in triangular tesselation on the inside. The outer membrane is visible with a lens on negatives taken at ×1000, but not easily so, whereas the inner membrane, in which the repeat distance of the pores is only about 170 Å, can only be seen on negatives taken at ×5000. Helmcke's and Krieger's and Okuno's pictures seem to have been taken at a much lower magnification than this and enlarged in reproduction. Recently Helmcke *et al.* (1961, plates 289–290) have published pictures of *S. phoenicenteron* showing the two membranes, but not all the detail described below (p. 401). The ultrastructure can also be damaged either by chemical cleaning or in fossilization. FIGURE 2 (p. 402) of a postpleistocene fossil specimen of *S. phoenicenteron*, which may be compared with the pictures of the species published by Helmcke *et al.*, shows an example of this.

When features are misinterpreted or imperfectly understood, and especially when, in consequence, like things are considered unlike or unlike things are grouped together, they will not provide satisfactory taxonomic characters. It is, therefore, necessary to base any taxonomic use of the ultrastructure of diatoms upon a proper understanding of that structure. Hendey (1959) has presented a classification of the types of ultrastructure in which the primary division is into laminar valves, consisting of one layer of siliceous substance, usually perforate, and locular valves, which are formed of a double layer of siliceous substance separated by vertical walls. In my opinion, however, such a distinction cannot be drawn. In most cases, at least, the diatom valves are pierced by chambers; these may occasionally be completely open on both sides, when they may properly be described as pores, but more usually they have a membrane, itself perforate, on one or both sides. In a number of cases, what were originally thought to be pores have been found, when more critically examined, to be closed by membranes on one or both sides. This makes it seem possible that such membranes will be found to be normal throughout the diatoms, and that only the mucilage pores that occur singly or in small numbers in some species will prove to be true pores. What Hendey classes as partially occluded perforations through a single-layered wall are exactly similar in structure to what he classes as loculi open on one side; the only difference lies in the closeness of their packing. His failure to realize this may be due in part to the difficulty of establishing relations in depth from single electron micrographs and his not recognizing in consequence that the membranes occluding the perforations were at the level of one or other surface of the valve. Accordingly, the classification of Helmcke *et al.* (1961), based entirely upon the structure of the individual chambers, is much more satisfactory. This sepa-

rates pores, open at both ends, from chambers, with a septum at one or both ends, and classifies these according to the position and type of perforation of the septum or septa.

The Use of Diatom Ultrastructure in Taxonomy

In spite of the large amount of information available about the ultrastructure of diatoms, it has until now been of little use in their taxonomy. Hustedt (1952, 1955), in the course of an interchange of opinion on the subject with Kolbe (1954, 1956), maintained that ultrastructure is more uniform than the features that can be seen with the light microscope, and that its variations show no correlation with the characters used to distinguish genera; ultrastructure, accordingly, cannot be regarded as having any taxonomic significance above the specific level (Hustedt, 1959, pp. V–VI). Hendey (1959) came to a similar conclusion, but added that when a large number of species have been examined it may be possible to subdivide the genus *Navicula* Bory. Views similar to Hustedt's are presented by Lund (1962) in his recent review of the criteria adopted in classifying algae.

It is probably not an unfair generalization to suggest that taxonomists are conservative in their outlook, especially in their views about which characters are important in classifying a particular group. They do not seize every opportunity of using a newly discovered set of characters to produce a new system supplanting the current one. They tend rather to keep alterations to a minimum, apart from the addition of numerous new species and taxa of lower rank. One of the most gratifying results of the study of diatom frustules with the electron microscope has been that it has brought to light nothing really surprising. Structure too fine to be resolved with the light microscope has been demonstrated, but this was only to be expected. Nothing which could be seen with the light microscope has been found to have a structure markedly different from that which it was thought to have. This represents a great tribute to the skill and acumen of those who used the light microscope at the limit of its potentialities to elucidate the structure of the diatom valve, especially O. Müller (1889, 1895, 1896*a*, *b*, 1898, 1899, 1900, 1901*a*, *b*, 1909) and Hustedt (1926*a*, *b*, 1928*a*, *b*, 1929*a*, *b*, 1935*a*, *b*). On the other hand it has meant that no revisions of the system have been forced upon diatom taxonomists, and in the absence of any such pressure they have not actively pursued the question of how far knowledge gained with the electron microscope could influence classification above the specific level.

Although conservatism has played its part in persuading diatomists that ultrastructure can only play a minor role in the taxonomy of the group, they have been helped to reach that conclusion by two other factors. Both of these have already been discussed; they are the inadequate number of species investigated with the electron microscope and the inadequate information about the ultrastructure of many of those examined. Thus, Hendey's (1959) list of the diatoms investigated with the electron microscope includes only 28 identified species of *Navicula*, out of at least 1000 at present known, and it is probable that the information about the structure of many of these is as inadequate as that which he gives about *Stauroneis anceps* and *S. phoenicenteron* (*cf.*, p. 397). For all other genera fewer species have been investigated, and

only in *Chaetoceros* Ehrenb. and *Pinnularia* of the larger genera is the proportion studied greater than in *Navicula*.

There are two parts of the system of classification of the diatoms in which the currently accepted taxonomy above the specific level is patently unsatisfactory: the families Naviculaceae and Biddulphiaceae. In both, species are grouped with others to which they seem only distantly related and separated from those which seem close to them. In a taxonomic investigation of a small group of species in the Naviculaceae on which I was recently engaged, I decided that electron stereomicrographs would be useful in elucidating a particular point about the structures connected with the central nodule. Through the kindness of K. Little of the Nuffield Orthopaedic Centre, Oxford, England, who is responsible for all the micrographs illustrating this paper, these were obtained. They showed not only the details of the central structure but also the ultrastructure of the perforations through the valve, and the correlations between these two suggested that ultrastructure might well form a guide to a revision of the limits of *Stauroneis* Ehrenb. and possibly certain other genera, and that the attitude of Hustedt (1959), Hendey (1959), and Lund (1962) to its use for this purpose was unduly defeatist. These observations are being extended, and much more needs to be done before any firm conclusions can be reached. This paper cannot, in consequence, be anything more than a report on progress to date, but its object will be fulfilled if it dissipates doubts about the value of ultrastructure as a source of taxonomic characters and stimulates others to work on similar lines.

Technique

This approach necessitates the accumulation of electron micrographs of a large proportion of the species in the group under investigation. Many species of diatom occur only as comparatively rare members of the assemblage contained in a particular gathering. To obtain the electron micrographs needed in a taxonomic investigation accordingly demands the use of a technique similar to that used in the making of selected slides of individual specimens for the light microscope. Reliance on serendipity, which has hitherto been the normal practice when choosing specimens for investigation with the electron microscope, will not suffice.

Each worker who makes selected slides of individual diatoms develops a technique which suits the resources of his own laboratory and his personal characteristics, in particular the steadiness of his hand. This account of the method I have used for selecting individual diatoms for study with the electron microscope, which is based upon that which I use when making selected slides for examination with the light microscope, should be taken only as a general guide and not as a model to be rigidly followed in all of its details.

One starts with a suspension in distilled water of chemically cleaned diatom frustules (for methods see Hustedt, 1927, 1958, Swatman, 1937, Hendey, 1938, 1951, Leboime, 1952, van der Werff, 1955, Barber, 1962) which is known to contain the diatom which it is desired to study. A few drops of this are allowed to evaporate, preferably on a mica surface, to which diatoms adhere less than they do to glass. Heat should not be used as convection currents cause the diatoms to clump together. Diatom frustules apparently adsorb some of the

chemicals used in cleaning and liberate these slowly into the water in which they are washed or stored. If these chemicals are present, they cause the diatoms to stick to the mica. It is, therefore, desirable to leave the diatoms in at least the last two washing waters for a period of 2 days or more, and to pour off the water in which they have been stored and replace with fresh distilled water immediately before the preparation of the strews from which specimens are to be selected.

The actual selecting is most conveniently done under a binocular dissecting microscope at a magnification of about $\times 100$. Except with the larger forms, it is not possible at this magnification to recognize the species to be selected with certainty. It is, therefore, necessary to locate them under an ordinary microscope and to note their position relative to prominent specimens that can act as markers. This process is facilitated if a grid is ruled on the back of the slip of mica with the point of a needle and the scratches filled with India ink. The mica slip can then be mounted with balsam on a microscope slide. It is usually more convenient to assemble specimens of each species to be investigated in separate groups near the edge of the mica slip before transferring them to the grids. When small diatoms are being dealt with, each group can then be examined under the ordinary microscope to see that all the specimens are of the correct species.

The necessary number of formvar-coated grids are attached to an ordinary microscope slide by tiny drops of gum arabic at their edge. They are held steady by this during mounting but can be readily detached for insertion in the microscope. A label can be placed at one end of the slide giving a numbered key to the grids. The diatoms can then be taken up individually on a bristle from the mica slip and placed on the formvar film over the spaces in the grid. This can usually be done without tearing the film. When the work is done in a dry atmosphere, the diatoms at times acquire an electrostatic charge, which causes them to fly off the grid when it is lifted off the slide. This trouble can be obviated by breathing gently on the grids after the diatoms have been transferred to them. After the thin film of water thus condensed on them has evaporated, they adhere sufficiently not to fall off when the grid is placed in the electron microscope, and will normally remain in position through a number of insertions into and removals from the instrument.

I find it possible to transfer the diatoms freehand, even specimens the major axis of which is between 10 and 15 μ in length. For this I use a bristle mounted on a cylindrical rod of wood about as thick as a pencil and sharpened like one to a point at one end. The bristle is stuck to this point with about 2 mm. protruding. Pelletan (1888) and Hustedt (1927) recommend a pig's eyelash as the most suitable bristle and I find one very satisfactory.

A number of types of mechanical fingers for the selection of diatoms have been developed, the most widely used probably being that designed by Meakin (1939). These could no doubt also be used for transferring diatoms to electron microscope grids. Stiffer bristles than those used for freehand mounting are, however, normally used in mechanical fingers and these would be more likely to tear the formvar films on the grids. When a mechanical finger is used to mount diatoms for the electron microscope it will probably be advantageous to replace its normal bristle by a more flexible one.

It has already been pointed out that stereomicrographs are much more informative than single ones. The techniques for obtaining these and mounting them for examination have been described by Little (1958, 1962). It is also important to ensure that the micrographs are taken at a magnification and with a resolution sufficient to show the true structure of the valve. Low power micrographs of the specimen, which will enable its identity to be checked, should also be taken.

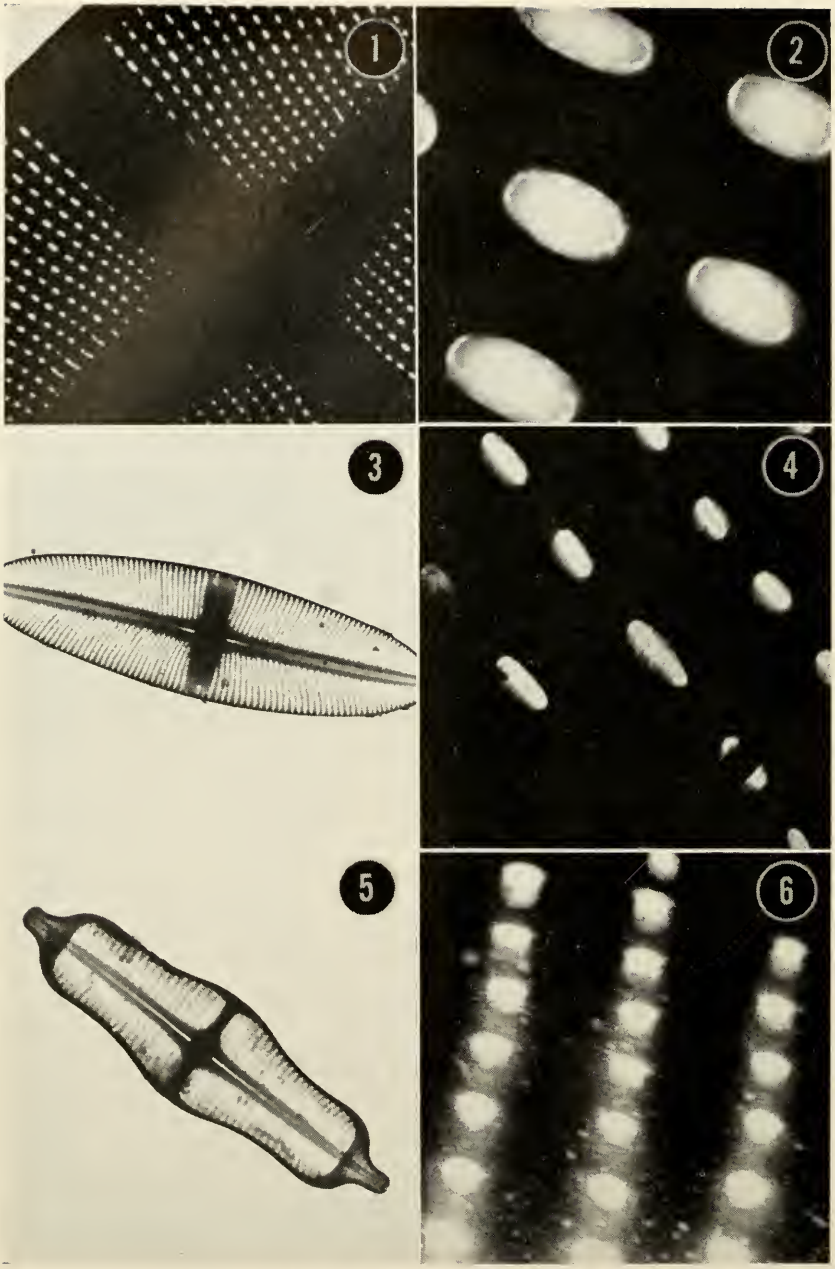
New Observations on Diatom Ultrastructure

The species originally described as *Schizostauron crucicula* Grun. ex Cleve and *S. karstenii* Zanon are currently placed in the genus *Stauroneis*, the structure associated with their central nodule being interpreted as a bifid stauros. Specimens of these two species were recently encountered in some gatherings from Lake Tanganyika, and in the same material two undescribed species which seemed related were also found. One of these was very similar to the two known species, but the other had the asymmetry characteristic of the genus *Amphora* Ehrenb., *i.e.*, both its apical and its pervalvar axes were curved. Although it was possible to be reasonably certain under the light microscope that the structures associated with the central nodule were not very similar to an ordinary stauros, details of their form could not be made out with certainty. There was also need to confirm that the asymmetric species differed from the others only in shape and not in any point of structure. Specimens of *Schizostauron crucicula*, *S. karstenii*, and the asymmetric form were therefore examined in the electron microscope and stereomicrographs of them were obtained. Specimens of the type species of *Stauroneis*, *S. phoenicenteron*, and of *S. anceps* and *S. smithii* Grun. were also examined for comparison. These observations, which are reported in detail by Ross (1963), confirmed that the species with a so-called "bifid stauros" were so different from *S. phoenicenteron* that they should be placed in a separate genus, for which the correct name is *Capartogramma* Kuff. Also, *S. phoenicenteron* and *S. anceps* were found to be very similar, but to differ greatly from *S. smithii*. The results may be summarized as follows.

(1) *Stauroneis phoenicenteron* (FIGURES 1 and 2) and *S. anceps* (FIGURES 3 and 4) have a stauros which is a wide but not very deep thickening of the valve. The chambers that form their striae are elongated along the direction of the stria, especially near the inner surface, where they are separated by a very narrow wall. These chambers are closed on their inner side by a membrane with fine pores in triangular tesselation and on the outer side by a membrane with a broad slit along the direction of the stria. The length of this slit is shorter than the length of the main part of the chamber.

(2) *Stauroneis smithii* (FIGURES 5 and 6) has a deep and narrow thickening across the valve. Its chambers are not close; they are approximately circular and are closed on the inner side by a membrane with fine pores in triangular tesselation and on the outer side by a membrane with a narrowly elliptical opening of which the major axis is across the direction of the stria and is longer than the diameter of the main part of the chamber.

(3) All three species of *Capartogramma* (for illustrations see Ross, 1963) have on either side of the central nodule two, or occasionally three, deep and very nar-



FIGURES 1–2. *Stauroneis phoenicenteron* (Nitzsch) Ehrenb. In FIGURE 2 are shown artifact structure caused by too rigourous cleaning (*cf.*, Helmcke *et al.*, 1961, plate 289 to 290) for true structure of this species. FIGURE 1, ×2500. FIGURE 2, ×40,000.

FIGURES 3–4. *Stauroneis anceps* Ehrenb. FIGURE 3, ×2000. FIGURE 4, ×40,000.

FIGURES 5–6. *Stauroneis smithii* Grun. These specimens are somewhat eroded but in FIGURE 6 it is shown that the slits in the outer membrane run across the striae. FIGURE 5, ×2500. FIGURE 6, ×40,000.

row flanges running from the central nodule to the valve margin, projecting at right angles to the valve surface but turned towards the apices at their free edges. Their chambers are not close; they are approximately circular and are closed on the inner side by a membrane with fine pores in triangular tesselation and on the outer side by a membrane with a broad slit that runs across the direction of the stria and is longer than the diameter of the main part of the chamber.

These observations not only confirmed that it is correct to separate the species with a "bifid stauros" from *Stauroneis* and to associate the species with amphoroid asymmetry and the symmetrical ones; they also suggested that other species now grouped in *Stauroneis* might belong to separate genera. To see whether examination of more species would provide evidence to confirm this, *S. acuta* W. Sm., *S. amphioxys* Greg., and *S. salina* W. Sm. were examined under the electron microscope, and more species will be as opportunity offers. The stauros of the first two species appears under the light microscope to be broad and narrow. The electron micrographs showed their structure to be as follows:

(4) *Stauroneis acuta* (FIGURES 7 and 8) has a broad and rather shallow stauros, as in *S. phoenicenteron* and *S. anceps*, and the ultrastructure of its striae is similar to that in those two species.

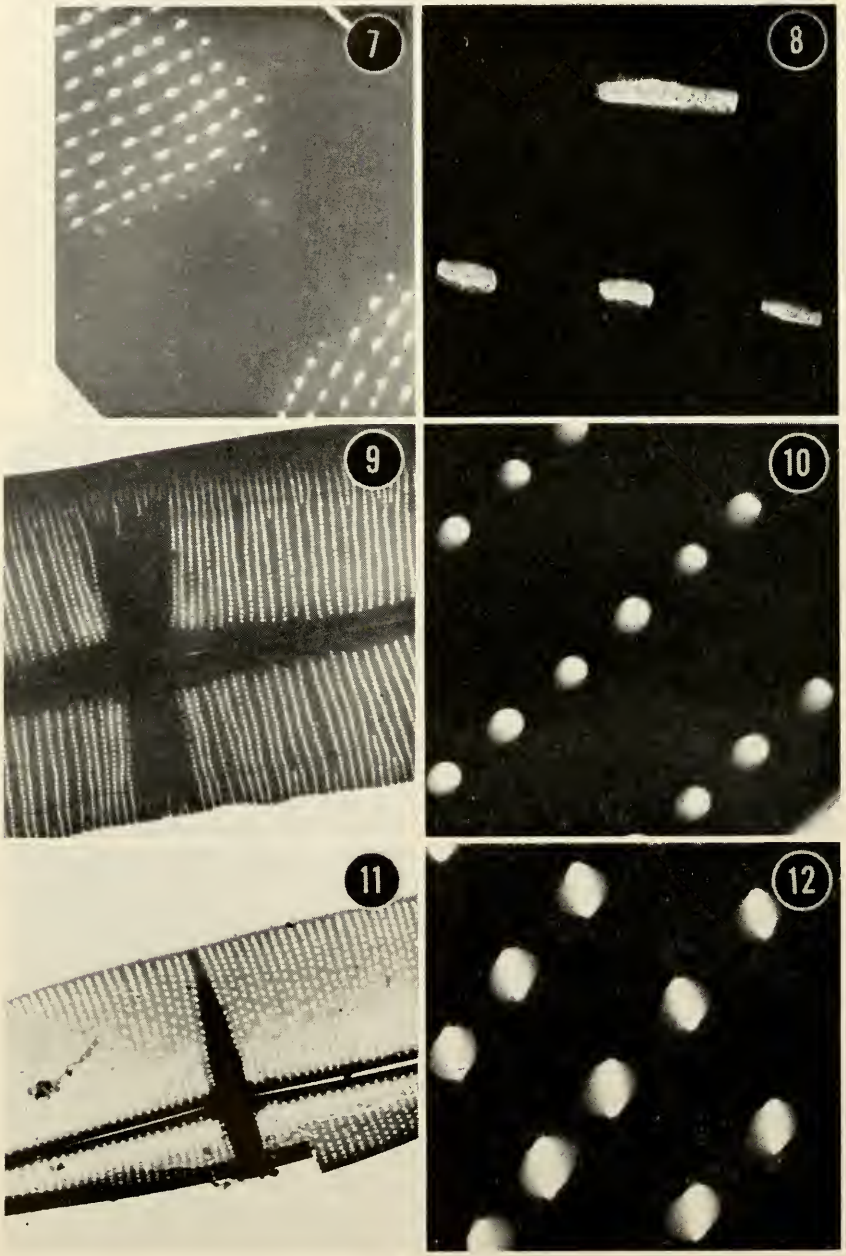
(5) *Stauroneis amphioxys* (FIGURES 9 and 10) has a broad and shallow stauros, which extends for less than two-thirds of the width of the valve. Its striae consist of distant circular chambers closed on the inner surface by a fine membrane with pores in triangular tesselation. The chambers taper outwards, *i.e.*, they have the shape of truncated cones, but they have no membrane on their outer side.

(6) *Stauroneis salina* (FIGURES 11 and 12) has a stauros that is rather deep at the center of the valve and becomes narrower and shallower toward the margin. The striae consist of distant circular chambers closed on the outer side by an oblique parallel-sided slit that is slightly longer than the diameter of the main part of the chamber and on the inner side by a fine membrane with pores in triangular tesselation. The valve surface is depressed between one-third and two-thirds of the distance from the raphe to the margin and throughout this area the chambers in the striae are more distant than elsewhere.

Taxonomic Implications

Attention is here drawn to some similarities and differences in ultrastructure that may have a taxonomic significance; not only the original observations recorded above but also published micrographs of various species of Naviculaceae are considered. The present state of our knowledge provides only a very tenuous basis for taxonomic speculations; the justification for indulging in these and putting them on record is that others may be stimulated to collect further data that will tend to confirm or refute them.

Stauroneis. Until recently the presence or absence of pseudosepta has been treated as a character distinguishing sections within this genus (Cleve-Euler, 1953). The close similarity which *S. acuta*, in which these are present, bears in all other respects to *S. phoenicenteron*, in which they are absent, confirms the view put forward by Hustedt (1959) that they are of little taxonomic significance. Also, Hustedt's (1959) contention that *S. amphioxys* (which he in-



FIGURES 7–8. *Stauroneis acuta* W.Sm. In FIGURE 7 is shown the extension of the chambers along the line of the striae, FIGURE 8 the inner membrane with fine pores in triangular tesselation and the broad slit along the line of the striae. FIGURE 7, ×5000. FIGURE 8, ×40,000.

FIGURES 9–10. *Stauroneis amphioxys* Greg. FIGURE 9, ×2500. FIGURE 10, ×40,000.

FIGURES 11–12. *Stauroneis salina* W.Sm. FIGURE 11, ×2000. FIGURE 12, ×40,000.

correctly calls *S. gregorii* Ralfs) and *S. salina* are quite distinct species is confirmed.

Ultrastructure confirms the view that *S. phoenicenteron*, *S. anceps*, and *S. acuta* should be placed in the same genus. *S. amphioxys*, *S. salina*, and *S. smithii* differ considerably from these and from one another. Mereschkowsky (1903*a*), on the basis of endochrome structure, removed *S. amphioxys* and *S. salina* from *Stauroneis* and created a new genus, *Staurophora*, for the two species. Although their ultrastructure indicates that they should perhaps be removed from *Stauroneis*, it provides no confirmation for grouping them together. Information about many more species is needed before any firm conclusions can be drawn about the correct position of these species. *S. smithii*, however, seems to be close to *Capartogramma* both in the structures associated with the central nodule and in the ultrastructure of the chambers, and *S. salina* bears some resemblance. It is noteworthy that *Frustulia rhomboides* var. *saxonica* (Rabenh.) De Toni (Helmcke *et al.*, 1961, plates 279 to 280) has an ultrastructure almost identical with that of *Capartogramma* and *S. smithii*, and so also has *Scoliopleura tumida* (Bréb.) Rabenh. (Helmcke and Krieger, 1954, plate 177), a species grouped by Cleve (1894) not with the other members of that genus but in his *Naviculae Microstigmaticae*, in which he also included *Stauroneis*. This ultrastructure has certain similarities to that found in most of the species of *Pleurosigma* and *Gyrosigma* examined. Whether the species that possess this type of ultrastructure in common form a group of genera more closely related to one another than to the rest of the Naviculaceae is a question that can only be determined as more knowledge is accumulated, but it seems that it is a possibility.

Amphora. As mentioned, there is a species which differs from the others placed in the genus *Capartogramma* only in shape of frustule; it has that characteristic of the genus *Amphora* although the other species of the genus are, like most Naviculaceae, symmetrical about the apical, transapical and pervalvar planes. Cleve, in 1896, (p. 99) made the suggestion that the species placed in the asymmetric genera *Amphora* and *Cymbella* Ag. were more closely related to symmetrical species of similar valve structure than they were to one another. The discovery of this new species of *Capartogramma* adds further evidence for the view that symmetry by itself is not a proper basis for delimiting genera. The only species of the large and variable genus *Amphora* the ultrastructure of which is known are *A. coffeiformis* (Ag.) Kütz. (Helmcke and Krieger, 1953, plate 76), *A. delicatissima* Krasske (Helmcke *et al.*, 1961, plate 294) and *A. ovalis* (Kütz.) Kütz. (Helmcke and Krieger, 1953, plate 77, 1954, plate 181). In *A. coffeiformis* and *A. ovalis* the ultrastructure resembles that found in *Anomoeoneis exilis* (Helmcke and Krieger, 1954, plate 169) and *A. serians* (Bréb.) Cleve (Helmcke and Krieger, 1953, plate 68), which may indicate relationship. *Amphora delicatissima* has a quite different structure.

Cymbella. This is another genus which, like *Amphora*, is distinguished from *Navicula* solely on the basis of asymmetry. Cleve (1894, p. 157) considered that its species were most closely related to those of *Navicula* subgen. *Navicula* (his *Naviculae Lineolatae*). As far as ultrastructure is concerned, this is true of *C. rabenhorstii* Ross (Kolbe and Golz, 1943, plate 1, fig. 3, Helmcke and Krieger, 1953, plate 75, as *C. gracilis* (Rabenh.) Cleve), *C. turgida* Greg. (Desika-

chary, 1952, figs. 17 and 18), and *C. ventricosa* Ag. (Desikachary, 1952, figs. 19 and 20, Helmcke and Krieger, 1953, plate 75) (*cf.*, *Navicula cryptocephala* Kütz., Helmcke and Krieger, 1953, plate 69, *N. digitoradiata* (Greg.) A. Schmidt, Helmcke *et al.*, 1961, plate 292 and 293, *N. radiosa* Kütz., Helmcke and Krieger, 1954, plate 172, and *N. viridula* (Kütz.) Kütz., Helmcke and Krieger, 1953, plate 73). *Cymbella delicatula* Kütz. (Helmcke and Krieger, 1954, plate 180) and *C. mexicana* (Ehrenb.) Cleve (Okuno, 1956, plate 21, fig. 2), however, each have an ultrastructure which is different from that of these species and from each other's. Electron micrographs of other species of the genus have been published but none give adequate pictures of the ultrastructure.

Mastogloia. The ultrastructure of *M. braunii* Grun. (Helmcke and Krieger, 1953, plates 57 and 58, 1954, plate 159) and *M. smithii* Thwaites ex. W. Sm. (Helmcke and Krieger, 1954, plate 160) is similar and resembles that of the only two species of *Navicula* subgen. *Lyraneis* Freng. of which adequate electron micrographs are available, *viz.*: *N. forcipata* Grev. (Helmcke *et al.*, 1961, plate 291) and *N. pygmaea* Kütz. (Helmcke and Krieger, 1953, plate 71). *Mastogloia angulata* Lewis (Okuno, 1957, plate 7, fig. 2) and *M. fimbriata* Cleve (Okuno, 1953, plate 1, fig. 3) resemble each other in their ultrastructure, but this is quite different from that of *M. braunii* and *M. smithii.*

Discussion

The principles of taxonomy have recently been much discussed, and from this discussion it has emerged that the amount of overall similarity is the only basis for a satisfactory taxonomic classification (Cain, 1962, Sneath, 1962). To accord overriding importance to a particular character, or to characters derived from a particular structure, even if there are *a priori* grounds for considering these of particular importance, results in an artificial and unsatisfactory system. Almost without exception, however, diatoms have been classified solely on the basis of the symmetry and structure of their siliceous frustule as seen under the light microscope: although this provides comparatively few characters, some of these, in particular symmetry, have been treated as having an importance overriding that of the others. This concentration of attention on the frustule has not been based upon any *a priori* reasoning but purely on convenience; in both fossil and recent material the valves are always present and recognizable, and provide sufficient information for identification at the specific level.

The current classification of the Naviculaceae rests on such a basis. The species are separated into genera on the common possession of a single character, or a combination of only two or three, all drawn from the structure of the frustule. Some of the genera so characterized are probably natural groups, *e.g.*, *Diploneis* Ehrenb., *Neidium* Pfitz., and *Pinnularia*; others contain very diverse elements, *e.g.*, *Amphora* and probably *Mastogloia* Thwaites ex. W. Sm. and *Stauroneis*. The species that do not possess any characteristic that has been seized on as a mark of generic distinction are left in the very large genus, *Navicula*, a hotchpotch of species of diverse affinity. The little that we already know of the ultrastructure of the Naviculaceae shows that it provides a series

of characters to some extent cutting across the present classification. Ultrastructure, however, provides few characters and a system based solely upon it would be as open to criticism as one based solely upon the structure of the valve as seen under the light microscope. All of the information about the frustule, whether obtainable with the light microscope or the electron microscope, must be taken into consideration with any that can be obtained about other characters.

A few authors have attempted to use characters from the cell contents, in particular the form of the chromatophores, for delimiting genera within the Naviculaceae (Pfitzer, 1871, Mereschkowsky, 1901*a*,*b*, 1902, 1903*a*,*b*) or subgenera within *Navicula* (Karsten, 1899). However, except where these groups could also be readily distinguished by characters of the valve, *e.g.*, *Anomoeoneis* Pfitz. and *Neidium*, they have not been adopted by subsequent authors. The principal reason that there has been no further work along these lines is a matter of technique. The greatest possible amount of detail in the structure of the valves of diatoms can be seen most easily under the light microscope if all of the organic matter is removed and the frustules mounted in a medium of high refractive index. Diatomists have rarely used any other method of making preparations and all collections of diatoms consist almost entirely of specimens treated in this way. They provide information perfectly adequate for identification, and hence workers on floristics and ecology have had no incentive to change their technique. These have been the chief fields of work of virtually all diatomists throughout this century and even when they have turned their attention to true taxonomy they have not altered their methods. It may be that it would not have been possible before the phase-contrast microscope was available to devise a technique which made both the fine detail of the valve structure and the cell contents visible in the same specimen. It would seem, however, that it was not attempted. The justification for ignoring the cell contents in taxonomic work has been the contention, also used in connection with ultrastructure, that a classification by chromatophore number, shape, and disposition within the cell runs counter to the currently accepted one (Peragallo, 1907). This criticism is valid insofar as it is directed against a classification in which characters of the chromatophore are accorded overriding importance, but it is not a reason for ignoring the cell contents completely.

It has been pointed out that the classification of the Naviculaceae is on a very unsatisfactory basis, at least above the specific level, and there is no reason for supposing that it is much better in other families of diatoms. Cell contents and ultrastructure provide characters of which the distribution does not, in places, accord with the current classification. There is no justification for arguing from this that variations in these features occur at random and have no taxonomic significance. To do so is to attach overriding importance to the particular characters of the frustule on which emphasis is placed in the current classification; not even *a priori* grounds have been advanced for this. Instead of arguing in this way from the lack of correspondence between the current classification and the distribution of types of cell contents and ultrastructure, this discrepancy should be regarded as an indication that there is a need for a new classification based upon the extent of overall resemblance with these features taken into account.

Future Developments

At present the data required to construct a classification by this method is not available. Progress in diatom taxonomy depends upon its being obtained. So far as ultrastructure is concerned, there are techniques for collecting the data (*cf.*, p. 399). The more difficult problem is to make it available. As can be seen when Helmcke and Krieger's (1953, 1954, Helmcke *et al.*, 1961) work is compared with other published electron micrographs of diatoms, the only method of reproduction that is really adequate is the making of photographic prints. The cost of publication of sufficient of these to cover most species of diatoms would be prohibitive. The most feasible method of building up files of micrographs will be by the exchange of duplicate prints between workers, or their institutions, in much the same way as herbarium specimens are now exchanged. It is to be hoped that diatomists who have the facilities for electron microscopy will enter into such a scheme. The desirability of stereomicrographs has already been stressed, and also the necessity for adequate resolution. A low magnification micrograph permitting verification of identity should accompany those showing the detail of the ultrastructure, and adequate documentation of the origin of the specimen is essential.

Collection of information about cell contents, on the other hand, depends upon the development of a technique of preparation that will enable details of both this and the valve structure to be seen in the same specimen. Now that the phase-contrast microscope is available, this should be possible. I plan to attempt it in the immediate future, but, in the words of the old proverb, two heads are better than one, and there is more likelihood of success if others also try to find a method. When such a technique is available, the same problem as with ultrastructure will arise: the examination of large numbers of species and the dissemination of the resulting information so that, as far as recent diatoms are concerned, a volume of knowledge about cell contents comparable to that about valve structure is available. Here again, the quantity involved is likely to make publication impossible and the most satisfactory alternative will probably be exchange of preparations.

Not until we know the ultrastructure and the cell contents of most of the species in a group will it be possible to consider whether, and if so in what way, the taxonomy of the group can be remodeled on sounder lines. At present all that is pertinent is to suggest that the methods of numerical taxonomy (Sneath and Sokal, 1962) are likely to be of great use at that stage. As Sneath (1962) has pointed out, at least 40 or 50 independent characters of each operational taxonomic unit (*e.g.*, individuals being classified into species or species being classified into higher groups) need to be taken into consideration when using the method of numerical taxonomy to construct a natural classification. If, as has been normal practice, we rely on intuition rather than calculation to evaluate overall resemblance, our judgments are likely to be sound only if we take note of a comparable number of characters. It is this which makes it essential that diatom taxonomists should no longer confine themselves to studying cleaned frustules under the light microscope, but should observe the cell contents and the ultrastructure and make use of the information these provide in their classifications.

Summary

Our present knowledge of the ultrastructure of diatoms covers only a very small proportion of the total number of species, and some of the published information is inadequate or misleading. The variations in types of ultrastructure found do not, in a number of cases, correspond with the current classification, which is based almost entirely upon characters of the valve as seen under the light microscope. On the other hand, the observations made with the light microscope have not been contradicted by work with the electron microscope. For these reasons it has been contended that ultrastructure does not provide information that can be used in diatom taxonomy. This view is criticized.

If the characters of the ultrastructure are to be used in diatom taxonomy, information about most species in a group is needed. As many species are often sparsely represented in gatherings, individual specimens need to be selected and mounted for examination in the electron microscope. A technique is described.

In a study just completed, electron microscopy has confirmed that a small group should be removed from *Stauroneis* and placed in a separate genus. A continuation of this work now in progress points to the need for further division of *Stauroneis*, and there are indications that ultrastructure may provide information that will assist in a revision of the present unsatisfactory generic classification of the Naviculaceae. In such a revision the characters of the frustule structure as seen under the light microscope, of the ultrastructure, and of the cell contents should all be given equal weight. It is, therefore, necessary to obtain information about the ultrastructure and cell contents of a large proportion of the species in the family: a prerequisite for this is the development of a technique for preparing specimens in such a way that both their cell contents and the structure of their frustules can be studied.

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MORPHOLOGY OF REPRESENTATIVE BLUE-GREEN ALGAE

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The blue-green algae, of the phylum Cyanophyta or Schizophyta, belong to the class designated variously as the Cyanophyceae, Schizophyceae, or Myxophyceae, and are plants of a gelatinous, stony, mealy, or leathery nature. They are firm or soft, extremely tough, and impregnated with salts or mucus and easily disrupted when the gelatinous material surrounding them is of slight viscosity. Their forms vary in size from micro- to macroscopic and in shape they occur as spheres, cushions, strata, or cylinders. The growth habit is frequently centripetal, and depending on the inherent characteristics of the plant and its environment, the adult plant can be a single cell and of less than 1 μ in diameter or a spreading plant of up to 1 m. in extent. They are cosmopolitan in nature and are found chiefly on soil and in water but also in a variety of other habitats wherever moisture, temperature, energy supply (sunlight), *p*H, biogenic salts, respiratory gases (CO_2 and O_2), and other conditions for growth and reproduction are favorable. They share with the bacteria a unique ability to survive, as well as reproduce, at the extreme limits of the natural environment.

The blue-green algae are considered to be an ancient group of plants extending back to the Archeozoic (Tilden, 1935) although the geological record is difficult to determine because they have few hard parts. The evidence of their presence is attributed frequently to calcareous and silicious strata and cushions and very ancient deposits of tufa, marl, travertine, and sinter developed by activities of mainly filamentous forms (Drouet, in press). The fossil remains that have been attributed to blue-green algae have not provided evidence as to their evolutionary sequence (Fritsch, 1942).

Characteristics of blue-green algae show that they resemble nonalgae as well as other algae. Because they resemble bacteria in some respects, *i.e.*, in having no organized nuclei or true cell walls and a similar mode of cell division, they have been classified as coordinate with the bacteria in the Schizophyceae (Breed *et al.*, 1958). It is also known that both groups contain members that produce spores, and some have demonstrated the ability to fix atmospheric nitrogen. Most contain α,α'-diaminopimelic acid, and their concurrence in similar ecological habitats and in cultures attests to similarities in certain physiological characteristics. Sufficient differences, however, are found for separation of bacteria from blue-green algae. Blue-green algae are rarely parasitic, pigmentation is not comparable with that of the bacteria, oxygen is evolved as a result of photosynthesis, movement is of a gliding or oscillating nature—there are no flagella—and the size range of cells and plants is much greater. Heterotrophic, colorless forms of blue-green algae usually can be attributed to bacteria or fungi which have been misinterpreted (Drouet, in press), unless one accepts an organism such as *Beggiatoa* as a colorless form (Pringsheim, 1949). Morphologically, the Myxophyceae show a greater structural complexity and diversity than bacteria, but less so than other algal

groups. It is recognized that the major taxa of algae may show little affinity with each other (Papenfuss, 1955), but they are still grouped on the basis of an "algal-type" of organization, the parallelism cited in the evolution of plant-body types, the morphology and physiology of the individual cells (Smith, 1950), photosynthates, and especially in regard to the principal protoplasmic pigments (Dougherty and Allen, 1960). Blue-green algae contain the phycobilins C-phycoerythrin and C-phycocyanin not found in other algae or bacteria although phycobilins are characteristic for red algae and have also been found in other groups, *e.g.*, green algae and cryptomonads (Eocha, 1960). Chlorophyll *a*, and β-carotene are shared in common with other algal groups, but certain carotenes and xanthophylls are unique to blue-green algae (Goodwin, 1960). By means of a fluorescence microscope, the pigments are found to show an orangish red, red, or reddish brown fluorescence in a darkened background. Photosynthates include polysaccharides and glycoproteins, and cell contents may become brown when treated with I-KI solution.

In some species cells form reproductive spores which are denoted from other cells by their larger size, thick walls, and more resistant nature. Colorless cells, or heterocysts, are also formed in some species. Their function and necessity are doubtful although they have been observed to germinate (Geitler, 1921), and they have been noted to anchor the trichome to the firm sheath (Bornet and Flahault, 1886). Endospores, undifferentiated reproductive cells, are formed by 1 family, the Chamaesiphonaceae, but for filamentous taxa, the random death of individual cells permits segments of trichomes, or hormogonia, to propagate the species when moisture is available. Cell division is by fission, *i.e.*, constriction into two parts, or by centripetal progression of a dividing membrane through the protoplast. Reproduction is frequently by fragmentation. Sexual reproduction, although recently reported for a strain of *Nostoc muscorum* (Lazaroff and Vishniac, 1961), is not considered characteristic for the group.

Cytologically, the cells are found to have the aforementioned pigments, protein granules, pseudovacuoles of a gaseous nature, and occasionally vacuoles, within a containing membrane. Pseudovacuoles are characteristic of planktonic "water-blooms"; they appear black in transmitted light, red in reflected light, and are dissolved when treated with detergent. Vacuoles occur in old or degenerated cells, particularly as the environment becomes anaerobic. The protoplast is said to be clearly divisible into two parts (Desikachary, 1959): the pigmented, peripheral chromoplasm and the central colorless centroplasm. It also may be recognized, however, that such a strict differentiation is superficial. Feulgen positive granules are found particularly in the centroplasm (Cassel and Hutchinson, 1954). Pigments are reported to be in grana-like lamellae of the chromoplasm according to electron microscope studies (Niklowitz and Drews, 1956). Few studies on nucleoproteins of blue-green algae have been undertaken although it has been reported that these are similar to those recorded for tissues of other organisms (Biswas, 1961).

Classification

The Myxophyceae have been classified in one or more orders. The classification followed here considers the blue-green algae to be in a single order,

the Chroococcales, and 8 families which diverge in morphological characteristics in a single evolutionary sequence (Drouet, in press). The coccoid families include the Chroococcaceae, Chamaesiphonaceae and Clastidiaceae. Filamentous families consist of the Stigonemataceae, Nostocaceae, Rivulariaceae, Scytonemataceae, and Oscillatoriaceae. Consideration of the first three families is given according to a recent comprehensive revision (Drouet and Daily, 1956), that of the other families follows the starting points according to the International Rules of Nomenclature (Gomont, 1892; Bornet and Flahault, 1886–1888*a* and *b*).

A representative member of each family is given (FIGURES 1 to 8). These members are not to be construed as "typical" because there can be wide variation inter- and intraspecifically in nature as well as in culture. However, *Anacystis montana* (FIGURE 1) is the most frequently collected of the coccoid species (Drouet, 1954). *Nostoc muscorum* (FIGURE 5) is of common occurrence on soil, and *Calothrix parietina* (FIGURE 6) is of wide distribution in moist habitats (Fan, 1956). *Scytonema hofmannii* (FIGURE 7) is also a frequently encountered species, and *Microcoleus vaginatus* (FIGURE 8) is an oscillatorioid member often found on soil as well as in aquatic habitats. These species have been recently described with others found in the United States north of the Rio Grande River (Drouet, 1959).

The Chroococcaceae consist of uni- or multicellular, micro- or macroscopic plants which are subaerial or aerial, free, as cushions or strata. The cells are spherical, discoid, ovoid, ellipsoid, cylindrical, or pyriform, in regular or irregular order, each cell dividing into 2 equal daughter cells which become separated from each other by the gelatinous matrix. Reproduction is by fragmentation as for most of the blue-green algae, but in some cases by cell division. Under most conditions, except for *Coccochloris*, cells are found in the process of division. Species of *Anacystis*, represented here by *A. montana* (FIGURE 1) have cells at first hemispherical, later becoming spherical. The cells then divide in 3 planes perpendicular to each other. *Coccochloris* resembles *Anacystis*, but has subspherical to long cylindrical cells and division at right angles to the long axis. Other genera include *Johannesbaptistia* which has a linear series of discoid cells within an elongate gelatinous matrix, and *Agmenellum*, *Microcrocis*, and *Gomphosphaeria* which have cells that divide successively in 2 planes perpendicular to each other. Plants of the first two genera are platelike, whereas those of the latter genus are unique in that the cells are frequently cordiform in division and the remains of individual sheaths form branched stalks radiating from the center of the plant.

The Chamaesiphonaceae contain one genus, represented here by *Entophysalis lemaniae* (FIGURE 2). Plants of this family are uni- or multicellular, aquatic, micro-, or macroscopic. The cells are at first solitary and affixed to the substratum, each dividing serially into first unequal then equal daughter cells which are not separated by gelatinous material. Subsequently, a stratum or cushion is developed above the substratum, and branched filaments grow downward from this into the substratum. Any cell is then capable of enlarging and dividing internally into a few or many endospores. Reproduction is by fragmentation as well as by endospores.

Plants of the Clastidiaceae are infrequently collected. The plants consist

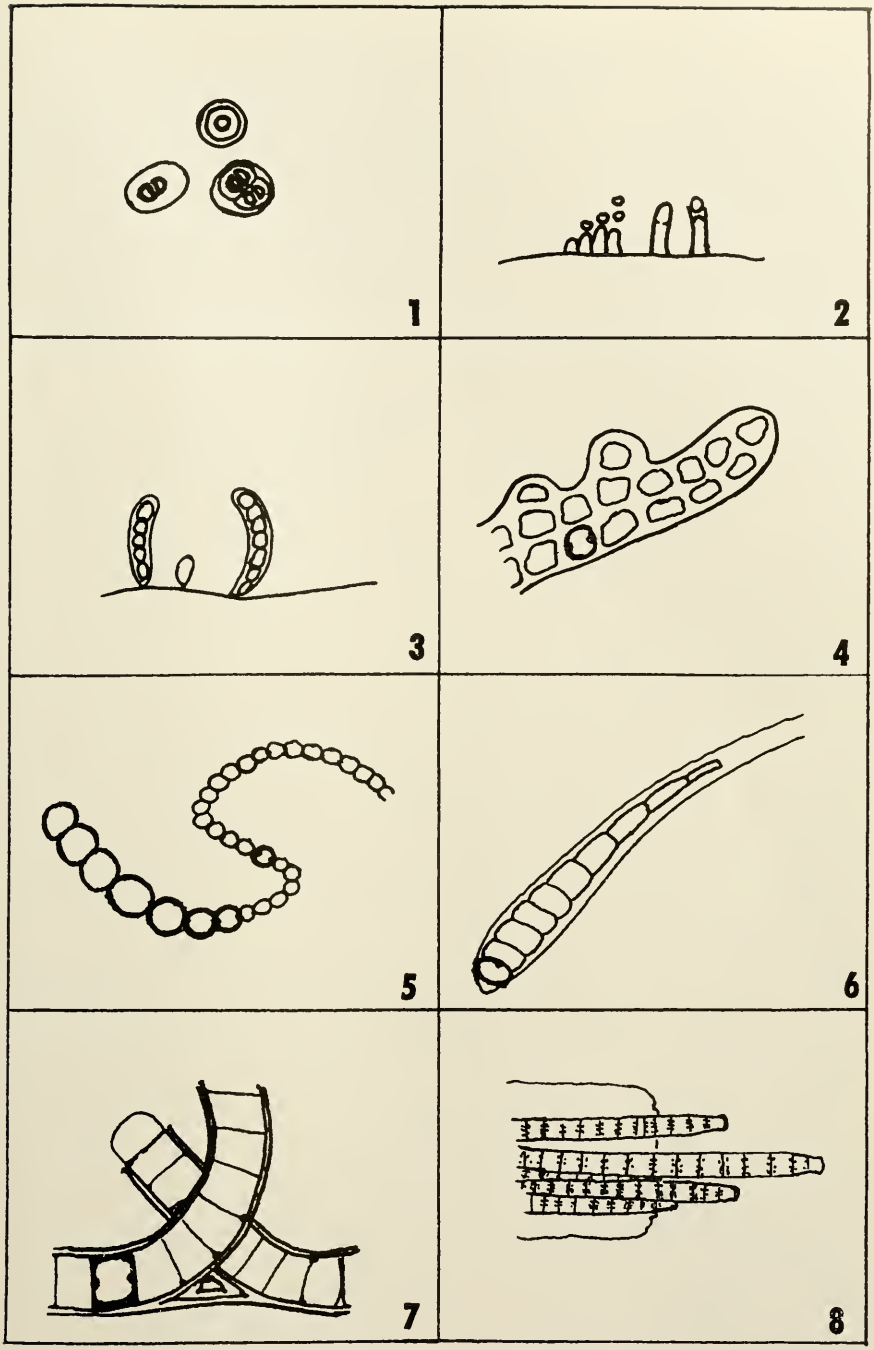

FIGURE 1. *Anacystis montana* (Lightfoot) Drouet & Daily.
FIGURE 2. *Entophysalis lemaniae* (Agardh) Drouet & Daily.
FIGURE 3. *Stichosiphon sansibaricus* (Hieronymus) Drouet & Daily.
FIGURE 4. *Stigonema paniforme* (Agardh) Bornet & Flahault.
FIGURE 5. *Nostoc muscorum* Agardh.
FIGURE 6. *Calothrix parietina* (Nägeli) Thuret.
FIGURE 7. *Scytonema hofmannii* Agardh.
FIGURE 8. *Microcoleus vaginatus* (Vaucher) Gomont.

of elongate, epiphytic unicells contained in thin gelatinous sheaths and attached to the substratum by basal developments of the sheath. The entire protoplast is found to divide into a uniseriate chain of rounded or compressed spherical cells which usually remain united by their membranes. As the cells enlarge, the trichome then bursts through the sheath of the mother cell, and the cells upon dissociation from each other then elongate into a new unicell and secrete new sheaths. The family is represented by two small genera, *Clastidium* and *Stichosiphon*, each containing one species. *S. sansibaricus* (FIGURE 3), has a smooth apex, whereas plants of *C. setigerum* terminate in a spinelike projection of the sheath at the apex.

Plants of the Stigonemataceae are floccose, feltlike, cushion-shaped, or spherical. The filaments are free or imbedded in a gelatinous matrix, the trichomes are branched, and the cells are uni- or multiseriate with division occurring in planes perpendicular to or parallel with the axis of the filament. Heterocyst formation is random, intercalary or terminal on short branches. Cell division in planes perpendicular to the axis of the trichome is followed by a growth in length of cells at filament apices which forms the resulting elongate and branched filaments. Cell division also occurs in planes parallel to the axis of older filaments with consequent increase in diameter and in the formation of subsequent branches. Reproduction is by fragmentation. The family is represented here by *Stigonema panniforme* (FIGURE 4). Members of this genus have filaments which soon develop multiseriate cells connected by protoplasmic strands. Other prominent genera include *Capsosira* which has upright and parallel filaments that form compact cushion-shaped plants, *Nostochopsis* which has radial filaments within a gelatinous matrix of coalesced sheaths and develops intercalary, pedicellate, or sessile heterocysts, and *Hapalosiphon* and *Fischerella* which contain trichomes of uniseriate cells except in the older basal portions of the plant. The latter two genera also exhibit scytonematoid branching.

The Nostocaceae contain aquatic or terrestrial plants which are free or attached to a substratum. The sheaths are mucous, gelatinous, membranaceous, or well hydrolyzed and absent. Trichomes are unbranched, frequently twisted and entangled; all of the cells divide at relatively the same time, and intercalary or terminal heterocysts are present. Reproduction is by fragmentation or by spores that are formed in most species. The trichomes of *Anabaena* are free or form a fragile layer; the matrix is composed of hyaline, hydrolyzed sheaths. Spores are variously situated in relation to the heterocysts. Trichomes of planktonic *Raphidiopsis* and *Aphanizomenon* resemble those of *Anabaena* except that the end cells are pointed in *Raphidiopsis* and colorless in *Aphanizomenon*. Trichomes of *Nostoc*, and *Wollea* develop within a gelatinous matrix of definite shape; all cells may apparently become spores or heterocysts. In species of *Nostoc*, *e.g.*, *N. muscorum* (FIGURE 5), the trichomes become much contorted, whereas in *Wollea* they are relatively straight. *Cylindrospermum* has comparatively short trichomes with terminal, solitary heterocysts and adjacent spores. Cells and spores of *Nodularia* are compressed or disciform in rather straight trichomes. *Hydrocoryne*, a rarely collected species, apparently forms no spores and has discrete although readily hydrolyzed cylindrical sheaths.

In the Rivulariaceae plants are aquatic or in moist habitats, spherical, cushion-shaped, crustaceous, velvety, feltlike, or brushlike. The filaments are branched or unbranched, radiate from the center of the plant outward, or are parallel and tuftlike. Trichomes are unbranched, thick at the base, tapering above, each ending in a colorless hair. Heterocysts are basal or intercalary, although absent in some species. Cell division is transverse and primarily in the middle of the trichome above the heterocyst. Reproduction is by fragmentation and spores. *Amphithrix* is a thin crustaceous plant, which lacks heterocysts and has terminal ephemeral hairs. Filaments of *Calothrix*, as represented by the most frequently collected species, *C. parietina* (FIGURE 6) (Fan, 1956) is usually unbranched, whereas the filaments of *Dichothrix* are more or less dichotomously branched, the bases of the branches included for a short distance within the parent sheath. *Rivularia* and *Gloeotrichia* have filaments of coalesced sheaths that develop radially to form spherical or cushion-shaped plants. No spores are formed in *Rivularia* but in *Gloeotrichia* they are thick walled and next to the basal heterocysts.

The Scytonemataceae contain irregularly cushion-shaped or matlike plants with branched filaments that are single or geminate. The sheaths are firm, tubular, at first colorless, but later yellow, or brown. Trichomes each consist of a single row of cells, one or more included in a sheath. Heterocysts and spores are variously disposed. Cell division primarily occurs behind the tip of the trichome, resulting in lateral perforation of the sheath by dividing and elongating cells which then give rise to single or geminate branches. Reproduction is usually by fragmentation of the trichome or filament, although one genus, *Aulosira*, is unique in that all vegetative cells are capable of forming thick walled cylindrical spores or heterocysts. Branching varies with the genera, depending upon its relation to the heterocyst. In species of *Scytonema*, *e.g.*, *S. hofmannii* (FIGURE 7), branches may be single and near a heterocyst, but commonly arise at a point somewhat remote from the heterocyst and are geminate. Branches in *Tolypothrix* are single and arise at the heterocysts. Branches of *Desmonema* are included within a common sheath. Filaments of *Fremyella* are short, uncommonly branched, and have basal heterocysts.

The Oscillatoriaceae is the largest family of the group. It is comprised of plants developing as layers or cushions and is differentiated from other families in that the trichomes do not form spores, heterocysts, or hairs. The cylindrical trichomes consist of 1 row of cells in branched or unbranched filaments; the broken ends or hormogonia regenerate in a mode characteristic for the various taxa. In many species, a terminal cell develops a thickened outer membrane. Cell division occurs throughout the entire trichome and at relatively the same time. Reproduction is by fragmentation. The current division of the genera is based largely upon the structure of the sheath (Gomont, 1892) and is in need of further study for clarification. The sheaths of *Oscillatoria*, *Arthrospira*, and *Spirulina* are seldom discernible even by application of various staining techniques. The sheaths of *Microcoleus*, *e.g.*, *M. vaginatus* (FIGURE 8), and *Schizothrix* contain one to many trichomes within diffluent or firm sheaths. Usually only one trichome is found in firm sheaths of *Plectonema*, *Lyngbya*, and *Porphyrosiphon*. Sheaths of the latter become red or purple; sheaths of *Lyngbya* may be hyaline or become yellowish-brown.

Plectonema may show scytonematoid branching. In *Symploca*, the sheaths are discrete and contain one trichome; adhering filaments form fascicles at the surface of the plant. The sheaths of *Phormidium* are thin, hyaline, and become diffluent.

General Ecology

Ecological studies on the Myxophyceae are quite limited. Most attention has been given to the collection of organisms from a variety of habitats and some information is available on their geographical distribution. In general, the blue-green algae occur in all parts of the world where light and water are available. Individual species may be distributed in the various climatic zones, but others are found at extreme limits of the environment, from cold regions such as the Antarctic or in the cryoconite of Greenland (Gerdel and Drouet, 1960), and from the low elevation of the Dead Sea to mountains over 14,000 feet in altitude. They are a part of the salt marsh flora (Chapman, 1960), occur in extremely saline Great Salt Lake (Flowers), hard and soft waters (Palmer, 1959) and hot, dry desert soils (Cameron, 1961; Killian and Fehér, 1939). Planktonic forms, frequently a single species, may grow prolifically in favorable seasons when nitrates and phosphates are high and in some cases release obnoxious toxins (Prescott, 1959). Aquatic species have also been found in the lower sublittoral zone where light intensity is low (Ruttner, 1953), and in hot springs where the temperature may reach 86° C. (Kaplan, 1956). Other aquatic habitats can include industrial wastes with a high content of metals and acids (Palmer, 1959). More exotic habitats include associations with animals such as sponges, corals, and snails. In barren, eroded soil, on wood, in sewage, on and under light transmitting rocks, and even in areas of comparatively recent volcanic activity (Treub, 1888), it has been found that blue-green algae are able to grow and survive. Furthermore, it has been determined that the Eh range of blue-green algae is from −0.200 to +0.700 volts and the *p*H from 1.5 to 11 (Baas Becking *et al.*, 1960). That they can resist desiccation for decades has been shown in the revival of species from old, stored soils (Bristol, 1919). Reproduction can be quite rapid, and oscillatorioid forms can develop macroscopic growth in a few hours on desert soil which has remained dry for a number of years. Prolonged resistance to desiccation has been found in a dried herbarium specimen of nonsporeforming *Nostoc commune* previously revived after 88 years of storage (Lipman, 1944), and later revived after an additional time period of 19 years (Cameron, in press). Resistance is also found to low temperatures. At −80° C., algae, in combination with fungi as lichens have been found to survive, and at −30° C. to even photosynthesize slowly (James, 1955). Parasitism of certain species of blue-green algae by fungi is not uncommon (Drouet, 1954), and where optimal conditions prevail for one of the organisms, the other is overwhelmed. The association between the alga and the fungus in forming and maintaining the lichen is exceedingly complex and although the alga excretes antibiotic substances, the fungus can have a lethal effect on the alga (Henriksson, 1961).

Environmental conditions which are most favorable for the entire group of blue-green algae are difficult to determine and correlate. Many species have been named as distinct on the basis of the kind of environment in which they

occur. Distinctions have also been made between plants which differ morphologically in some details but are actually only growth forms of the same species found in a slightly different environment. *Microcoleus*, for example, has been considered as a multitrichomatous organism occurring only on soils, and blue-green algae are said to be more abundant in cultivated than in noncultivated areas (Tiffany, 1951). Such restrictions have not been found valid upon further study. An exhaustive review of specimens and their subsequent enumeration on the basis of pertinent characteristics, as for the coccoid Myxophyceae (Drouet and Daily, 1956) is needed for the other blue-green algae. Culture studies, although valuable, are often confusing in that the cultured plant can lose its identity with more familiar forms occurring in the natural environment. Changes in any one of the environmental conditions can result in plants differing from the original organism in form and structure, as well as regeneration rate, cell division, size, shape, and contents. Pleomorphism among the blue-green algae will remain as a confusing factor until an extensive review has been made of all available material in herbaria and in other collections, and investigations performed on the growth of organisms in both natural and induced environments.

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LORICAE AND CYSTS IN THE CHRYSOPHYCEAE

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The unicellular algae, solitary or colonial, often have their cytoplasm enclosed within shells of various shapes and kinds called loricae or thecae. These thecae are found in numerous phyla of algae: Euglenales (Trachelomonas, Strombomonas), Volvocales (Phacotus, Coccomonas), Dinophyceae (Peridinium, Dinophysis, Exuviella), and in numerous Chrysophyceae and Craspedomonadinae.*

The Chrysophyceae may have, in addition, a phase of dormancy or of resistance in the form of siliceous cysts or statospores. These cysts always have an endogenous origin, and may arise from a simple encystment of a vegetative cell, or, on the contrary, of a zygote resulting from a autogamy or from an isogamic fusion.

The cysts of the Chrysophyceae are exclusively siliceous, and are of highly varied forms, but they exhibit a pore closed by a silicopectic plug. The formation of a siliceous cyst with a pore and plug is the basic characteristic which enables us to identify the whole Chrysophyceae group without any possibility of error.

Certain loricae of the Chrysophyceae (*Chrysococcus*, for example), are siliceous, and have very small pore openings. In the absence of a flagellum and the plug which closes the pore, one might easily confuse the cyst and the lorica. In fact, in the Chrysophyceae, the thecae are pierced with a pore opening from which the flagellum (or the flagella) or the pseudopodia emerge.

Loricae

If we take as an example of loricated Chrysophyceae, the genus *Dinobryon* (FIGURE 8) and the kindred genus *Hyalobryon*, we note that the morphology and the structure of the loricae vary with the species. In the two genera cited, the shell is in the form of a conical or cylindroconical horn, more or less flared out at the apex opening; the cellular body is bound to the lorica by a retractile cytoplasmic filament, the epipode. The shell is hyaline, of a cellulose-pectic nature, with a marked dominance of the cellulose. The outline of this lorica is either straight or undulating, according to the species The action of coloring agents (Congo Red) causes the appearance of a very fine helicoidal striation of the wall, accompanied at times by a spiral torsion indicated already by the undulating edge of the theca (*Dinobryon divergens* (FIGURE 1)). In all of the colonial *Dinobryon* which were studied, the basic helicoidal striation has the same direction of rotation (counter-clockwise), whereas the marginal undulations display a coiling in the opposite direction.

Dinobryon suecicum (FIGURE 1), a solitary species, free, with a smooth cellulose-pectic lorica, hyaline, with an helicoidal, projecting execresence, brown in color and of an unknown nature (calcareous substance impregnated with iron salt?) running throughout the greater part of its length.

An analogous feature is found in some *Pseudokephyrion*. The solitary fixed

* We will leave out the Silicoflagellates and the family of the Coccolithophoraceae, as these might constitute the subject of a special study.

Dinobryons: *Dinobryon utriculus* (FIGURE 1), have a lorica which is very rich in pectin, and made up of small elliptical scales, imbricated in helicoidal series. This structure presages the one which appears in the *Synura* and the *Mallomonas*.

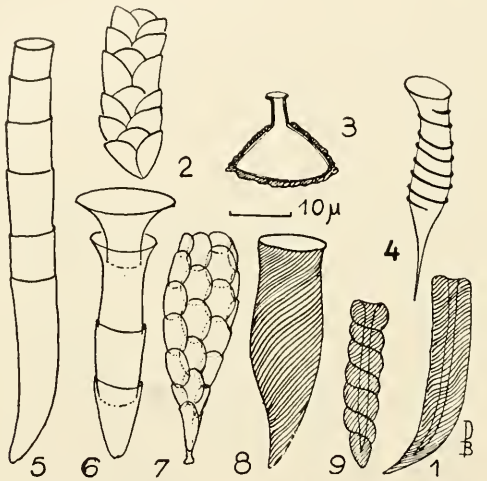

FIGURE 1. Loricae after Bourrelly, 1957. 1: *Dinobryon cylindricum* var. *palustre*; 2: *Dinobryon* sp.; 3: *Lagynion Janei*; 4: *Dinobryon suecicum*; 5: *Hyalobryon ramosum*; 6: *H. Borgei*; 7: *Dinobryon utriculus*; 8: *D. sertularia*; 9: *D. divergens* (1, 8, 9: after staining).

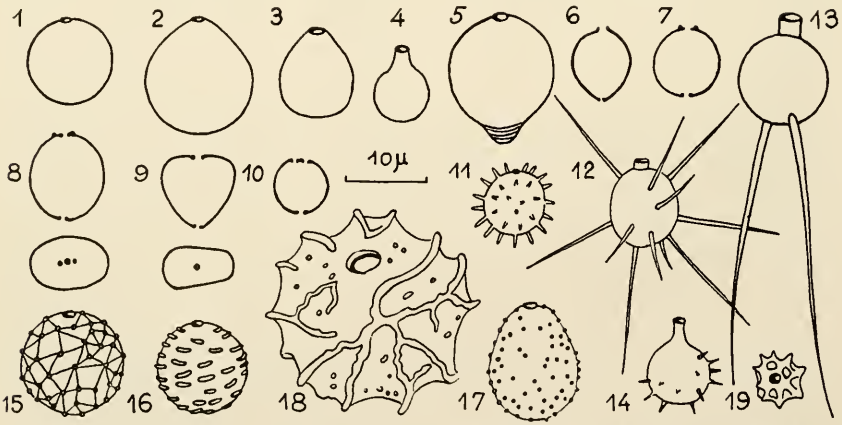

FIGURE 2. Loricae of *Chrysococcus* (after Bourrelly). 1: *Chrysococcus rufescens*; 2: *C. tesselatus*; 3: *C. ovoides*; 4: *C. elegans*; 5: *C. umbonatus*; 6: *C. porifer*; 7: *C. minutus*; 8: *C. rufescens* var. *compressa*; 9: *C. cordiformis*; 10: *C. rufescens* fo. *tripora*; 11: *C. dokidophorus*; 12: *C. radians*; 13: *C. bisetus*; 14: *C. spinosus*; 15: *C. klebsianus*; 16: *C. heverlensis*; 17: *C. ornatus*; 18: *C. areolatus*; 19: *C. sculptus*.

Finally, a genus very close to *Dinobryon: Hyalobryon* (FIGURE 1) is characterized by its very long lorica, cellulose-pectic, formed by pieces of encased cylindrical tubes, of unequal length, the widest one being the one at the base, and the narrowest one being at the top, presenting a flagellate opening.

With the genera *Chrysococcus* and *Pseudokephyrion*, we have loricae which are often very much embellished and are of a yellow-brown color. These loricae have a very fine pectic membrane entirely impregnated with calcareous sub-

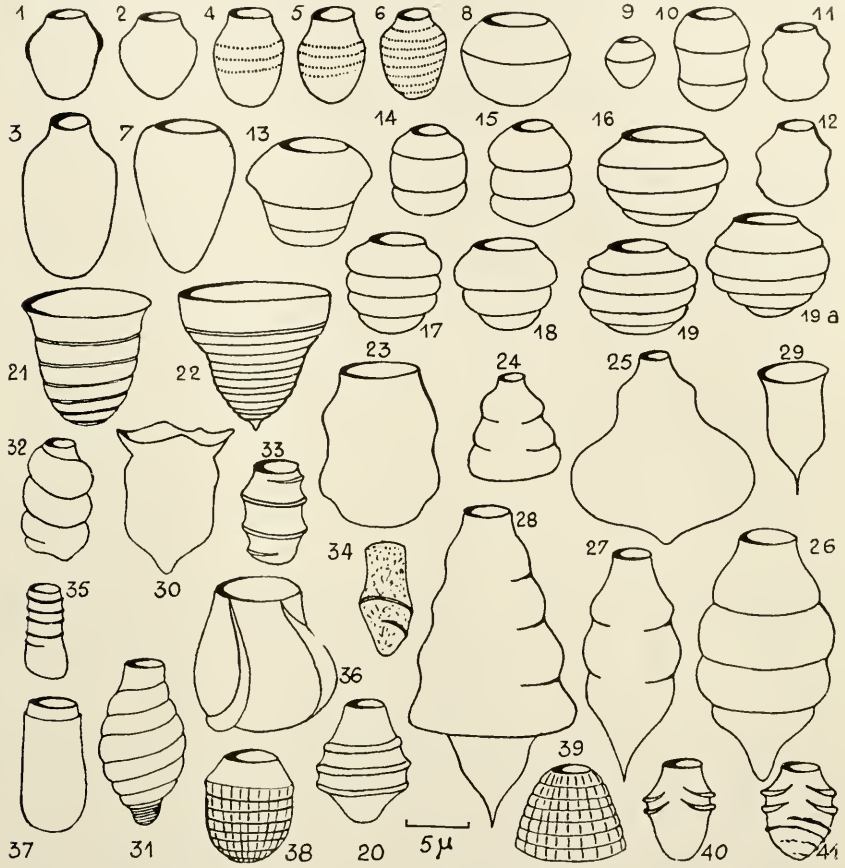

FIGURE 3. Loricae of *Pseudokephyrion* (after Bourrelly). 1–2: *conicum*; 3: *Entzii*; 4, 5, 6: *Entzii* fo. *granulata*; 7: *heverlensis*; 8: *poculum*; 9: *minutissimum*; 10: *Ruttneri*; 11–12: *cylindricum*; 13: *depressum*; 14: *cinctum*; 15: *obtusum*; 16–19a: *latum*; 20: *Skujae*; 21: *pilidum*; 22: *Schilleri*; 23: *urnula*; 24: *elegans*; 25: *ampullaceum*; 26: *undulatum*; 27: *acutum*; 28: *pulcherrimum*; 29: *tintinnabulum*; 30: *circumcisum*; 31: *undulatissimum*; 32: *spirale*; 33: *pseudospirale*; 34: *gallicum*; 35: *Klarnetii*; 36: *formosissimum*; 37: *ellipsoideum*; 38: *ovum*; 39: *ornatum*; 40–41: *circumvallatum*.

stance. Acetic acid dissolves the brown and brittle lorica quite well, and there remains a thin membrane which takes Ruthenium red color admirably.

Along with the numerous Chrysococci (FIGURE 2) with calcareous theca, two species embellished with spines or needles, have a siliceous wall. We note that the metabolism of the calcareous type and that of the siliceous type may co-exist in the same species. Also, some *Pseudokephyrion* with a calcareous shell produce siliceous cysts like the other Chrysophyceae.

With these calcareous or siliceous impregnations, the lorica becomes thick, and then presents a stable ornamentation in the same species, but quite variable from one species to another. Spines, bristles, warts, webs, dots, rings, and checks decorate the surface of the lorica.

The maximal diversity in ornamentation is obtained in the following two genera: *Pseudokephyrion* (FIGURE 3) and *Kephyrion*. Here we find forms with

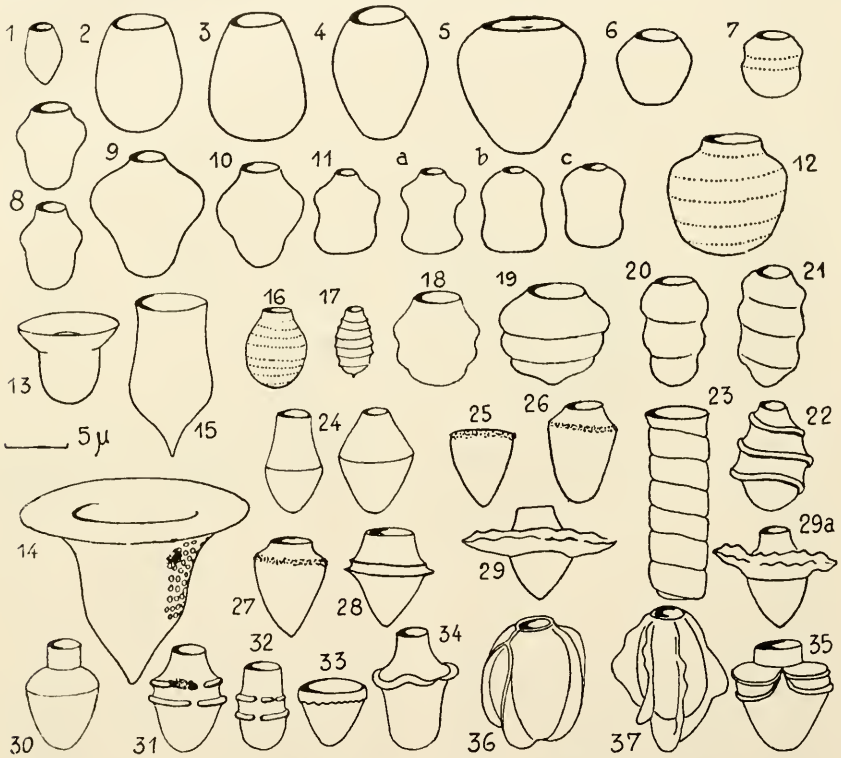

FIGURE 4. Loricae of *Kephyrion* (after Bourrelly). 1: *sitta*; 2: *doliolum*; 3–4: *mastigophorum*; 5: *cupuliforme*; 6: *littorale*; 7: *littorale* var. *constricta*; 8: *rubri-claustri*; 9: *rubri-claustri* var. *amphora*; 10: *impletum*; 11: *cylindricum*; 12: *hemisphaericum*; 13: *petasatum*; 14: *campanuliforme*; 15: *amphorula*; 16: *ovale*; 17: *cinctum*; 18: *Valkanovii*; 19: *globosum*; 20: *Starmachii*; 21: *mosquensis*; 22: *spirale*; 23: *bacilliforme*; 24: *densatum*; 25–29[a]: *asper*; 30: *Schmidii*; 31: *inconstans*; 32: *laticollis*; 33: *parvulum*; 34: *moniliferum*; 35: *circumvallatum*; 36: *prismaticum*; 37: *velatum*.

marked calcareous impregnation, and forms with little or no calcification. The small, more or less calcified cells, such as in *Pseudokephyrion undulatum* or *Pseudokephyrion latum* (FIGURE 3) recall the loricae of the Dinobryons in the undulating appearance of the edges, but they do not show the helicoidal torsion. But the forms with heavily colored, thick lime incrusted walls, have by contrast, a more varied ornamentation. One may recognize with them: (1) granulations or striations arranged in regular transversal circles: (*Pseudokephyrion Entzii* fo. *granulata*, *Ps. Skujae* (FIGURE 3)) or irregular ones (*Ps. circum-*

vallatum), (2) helicoidal protruding excrescence (*Ps. Klarnetii*, *Ps. pseudospirale* (FIGURE 3)), (3) regular cross checks (*Ps. ovum*, *Ps. ornatum* (FIGURE 3)), (4) longitudinally projecting sides (*Ps. formosissimum* (FIGURE 3)). The same remarks may be applied to the genus *Kephyrion* (FIGURE 4) in which we note the same diversity in the form of the small cells, but a smaller variety in the ornamentation of the walls.

In the genus *Lagynion* (FIGURE 5), the cells do not have flagella, but have more or less ramified pseudopoda issuing from the oral pole. This genus with

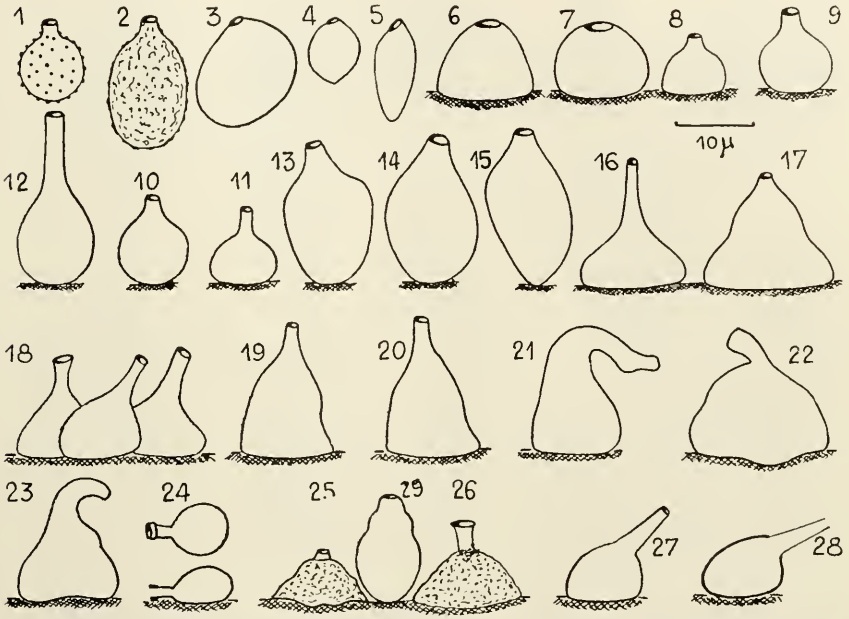

FIGURE 5. Loricae of *Lagynion* (after Bourrelly). 1: *fulvum*; 2: *oblongum*; 3: *arachne*; 4: *rhizopodicum*; 5: *notostomum*; 6–7: *reductum*; 8–11: *Scherffelii*; 12: *ampullaceum*; 13–15: *subovatum*; 16 and 18: *macrotrachelum*; 17: *triangularis*; 19 and 20: *triangularis* var. *pyramidatum*; 21–23: *reflexum*; 24: *sphagnicolum*; 25: *vasicola*; 26: *Janei*; 27–28: *cystodinii*; 29: *globosum* var. *undulatum*.

calcified pectic lorica does not show any characteristic ornamentation, the lorica is always brown or yellow, thickened, finely granulated. The forms are highly varied and the evolutive process comes to bear on the neck terminating the lorica. In some species (*Lagynion Janei* (FIGURE 5), for example), the wall of the theca is double, the inside is thin and hyaline, the outside brown, thick and calcified. This structure is found in the *Diploeca* series among the Craspedomonadines, a large group of collared flagellates related to the Chrysophyceae.

In the family of the *Stylococcaceae*, we note a large variation in the form of the loricae: along with sessile loricae, there are genera with pediculate shells (*Rhizaster*, *Stylococcus*). We also find genera in which the thecae show numer-

ous pores, from which issue the pseudopods (*Chrysocrinus*, *Stephanoporos*) for the sessile forms; *Porostylon* for the pedicular small cells.

The loricae held by a pedicel are found with other Chrysophyceae belonging to families very remote from the family of the Stylococcaeae: we cite only the

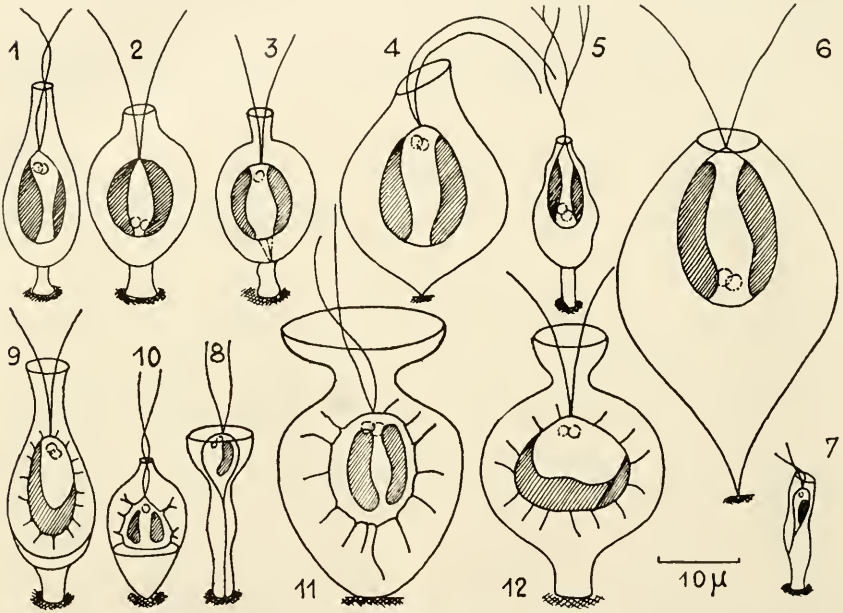

FIGURE 6. Loricae of *Derepyxis* (after Bourrelly). 1: *Derepyxis amphora*; 2–3: *ollula*; 4: *bulbosa*; 5: *anomala*; 6: *maxima*; 7: *tubulosa*; 8: *dilatata*; 9: *amphoroides*; 10: *dispar*; 11: *crater*; 12: *bacchanalis*.

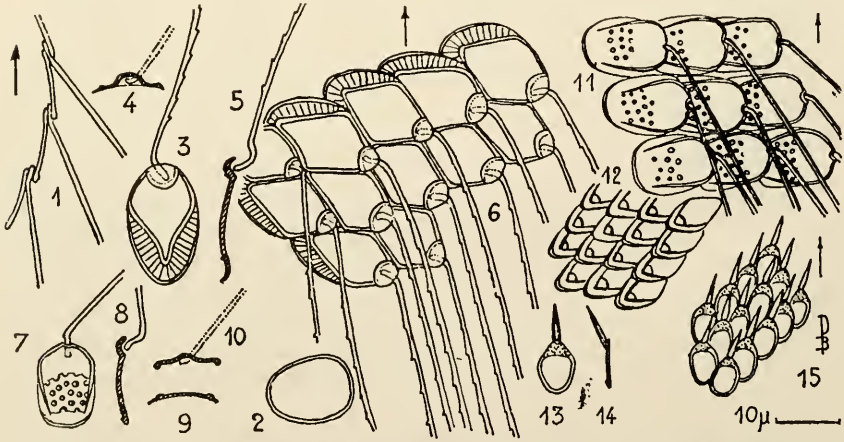

FIGURE 7. Scales of *Mallomonas* and *Synura* (after Bourrelly). 1–2: *Mallomonas fastigata* var. *Kriegeri*; 3–6: *Mallomonas Leboimei*; 7–11: *Mallomonas reginae*; 12: *Mallomonas tonsurata*; 13–15: *Synura Bioretii*.

Derepyxis (FIGURE 6), monads with two flagella and the *Lepochromulina* (FIGURE 8), with single flagellum. We mention also, the extraordinary *Chrysopyxis* of which the cellulose lorica, in the form of a saddle, attaches itself to the filamentary algae by a thin cellulose cord which completely entwines the supporting algae.

Alongside of the Chrysophyceae with loricae of homogeneous structure, we may place the species of the family of the *Synuraceae* in which the lorica is replaced by a covering of siliceous scales (FIGURE 7).

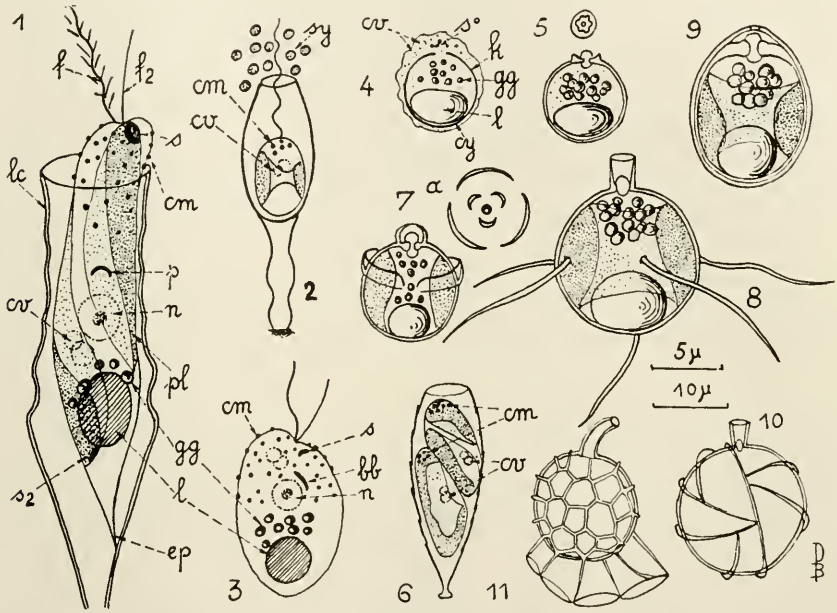

FIGURE 8. Loricae and cysts (after Bourrelly). 1: *Dinobryon divergens*, lorica and cytoplasm; 2: *Lepochromulina calyx*, lorica and cytoplasm; 3–5: *Heterochromulina vivipara* var. *minor*, building of cyst; 6: *Dinobryon utriculus*, lorica and division; 7: cyst of Chrysostomacea *Outesia*; 8: cyst of *Outesia*; 10: cyst of *Clericia*; 11: cyst of *Deflandreia* (?). *bb*: mouth-band; *Cm*: muciferous bodies; *cv-vc*: contractile vacuole; *cy*: cytoplasm; *ep*: epipode (contractile thread); *f-f2*: flagella; *gg*: oil-drop; *k*: membrane of cyst; *l*: leucosin; *lc*: cellulosic lorica; *n*: nucleus; *p*: parabasal body; *pl*: chromatophore; *s-s s2*: stigma; *sy*: symbionts.

The scales arranged in helicoidal series, such as these of the *Dinobryon utriculus* (FIGURE 1*), have been the subject of fine studies in electronic microscopy.

The systematization of the genus *Mallomonas* (about 100 species) of the genus *Synura* (12 species) is almost solely based upon the form of the scales and of the bristles which adorn them. The observation of a single siliceous scale is sometimes enough to permit the identification of the species. This is not the case with the true lorica, in which we have a convergence of form to such an extent that it is impossible in certain instances to decide from the study of an empty

* The scales of *Dinobryon utriculus* are not siliceous, but pecto-cellulosic.

lorica whether it is a Chrysophycean, Craspedomonadina, or even one of the colorless flagellates of the *Bicoeca* group.

Cysts

The same problem will arise for the Chrysophyceae cysts. We will have, at all times with the present forms, siliceous cysts with their pore and plug. But

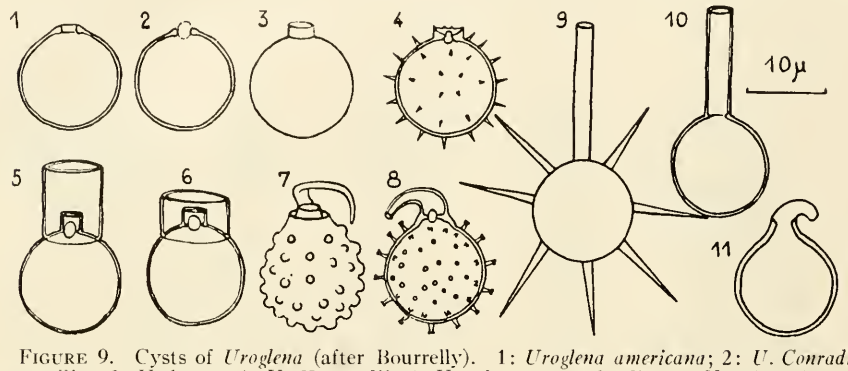

FIGURE 9. Cysts of *Uroglena* (after Bourrelly). 1: *Uroglena americana*; 2: *U. Conradi* var. *gallica*; 3: *U. botrys*; 4: *U. Nygaardii*; 5: *U. volvox* var. *uplandica*; 6: *U. volvox*; 7: *U. soniaca*; 8: *U. Lindii*; 9: *U. marina*; 10: *U. europaea*; 11: *U. notabilis*.

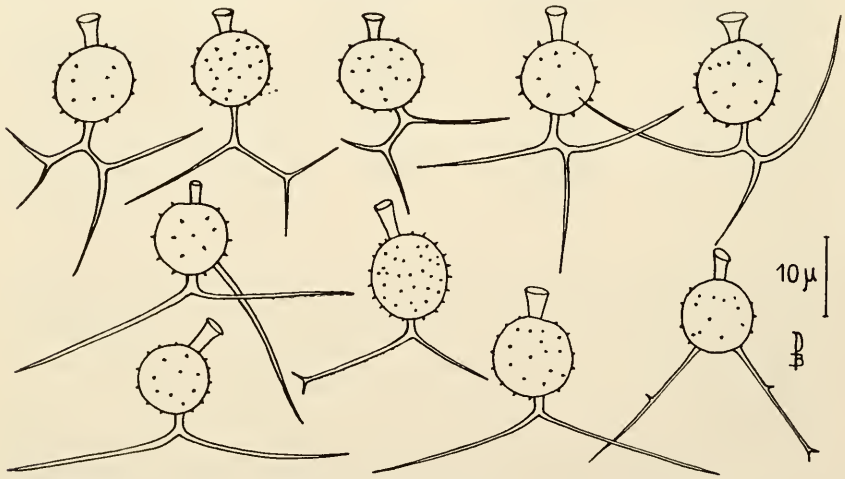

FIGURE 10. Cysts of *Chrysastrella furcata* (Chrysostomataceae): polymorphism (after Bourrelly).

within the same genus, the cysts have a highly varied ornamentation, and the identity of forms in the cysts does not seem at all related to the organism. The endogenous cyst is built within the cell, around the nucleus (FIGURE 8). The cytoplasmic parts left out of the cyst contribute to the external ornamentation of the cyst wall. The cyst is siliceous, but as was the case with the Diatomae, a pectic substance remains bound to the silica. The plug which closes the pore of the cyst is itself siliceous, but with a substantial pectic tendency.

The genus *Uroglena* (FIGURE 9) which shows a great structural and cytologic homogeneity, is an excellent example of the diversity form of the cysts, in fact, here knowledge of the cyst is indispensable for the determination of the species.

The cysts of many unicellular Chrysophyceae are still unknown. On the other hand, many cysts are known in which the free vegetative phase is unknown. This has led the protistologists and the micropaleontologists to give genus and species names to the cysts of which the vegetative phase is unknown. It is a convenient method, but these are not true species, only provisional names without classification value.

The fresh water cysts, both fossils and recent have been placed in the pseudofamily of the *Chrysostomataceae*, whereas the fossil marine cysts make up the *Archaemonadaceae*.

The *Chrysostomataceae* (FIGURES 8 and 10) are abundant in the present and fossil peat bogs, and in the Diatomae lacustrine deposits. More than 200 forms have been observed from the Tertiary period to the present time. The fossil marine forms of the *Archaemonadaceae* are found in association with Diatomae from the Cretaceous and Tertiary periods (less than 100 fossil forms are known).

The fossilization of the cysts is often perfect (the pore plug usually being missing, however) whereas that of the loricae of Chrysophyceae seems much more difficult, and observations of fossil loricae have been very rare (2 or 3 observations only).

In closing, it must be noted that although the present Chrysophyceae are well known in fresh waters, the forms of marine nanoplankton are very scant because their study has been much neglected. There is a vast domain in which investigation has only begun, and the rare current projects in this field have already yielded a harvest of interesting and novel facts.

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MORPHOLOGICAL TRENDS AMONG FOSSIL ALGAE

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The algae may be considered as a vast subkingdom of primitive plants that exhibit an enormous range in structure, reproduction, and life history. Structurally at the base are unicellular forms, often motile, that are indistinguishable from similar unicellular animals except for the presence in the cell of color spots or chromatophores, which contain photosynthetic pigments. At the other extreme are tree-sized multicellular plants in which there is some differentiation of tissue for different functions.

For convenience in study and classification the algae are divided into a number of major groups. These groups have been considered as classes by the older authors but the tendency today is to think of them as phyla. They are named on the basis of the pigmentation, for example, the Chlorophyta or green algae; the Rhodophyta or red algae. Nine such groups are recognized in most classifications, 11 in others.

Before considering the morphological trends among fossil algae it will be desirable to review two things regarding recent algae. (1) The structural trends, and (2) structural parallelism among the major groups of algae.

Algal Morphology

General. The algae show a great range in form, size, and structural development. At the bottom are the microscopical unicellular forms. These occur in all but two of the major groups and in a number of them no higher structural types have ever developed. A majority of the unicellular forms are motile flagellate types or at least in their life cycle pass through a flagellate stage.

Structural evolution seems to have followed the steps shown in TABLE 1, with the first three, either 4 or 6, and 5, forming an evolutionary series.

Parallelism. One of the most striking facts facing a student of algal morphology is the evidence of parallel evolution and development among the members of the various groups (TABLES 2 and 3).

Marked structural complexity of the plant occurs only in two groups, the Rhodophyceae and the Phaeophyceae, with some of the green algae (Chlorophyceae) reaching a high medium of complexity. It should be emphasized however, that even in these three groups a majority of the known species have simple types of structure. The highest structural features have developed among the brown algae, with some of the reds not far behind. The green algae probably show the greatest diversity of structural types with, however, the highest types missing.*

Fossil Algae

General. A review of the structural types and evolutionary trends among Recent algae, as briefly summarized in the previous section, and a study of

* This has been explained by numerous writers on the basis that the highest types moved ashore and gave rise to the land plants.

fossil algae, bring out 2 basic facts. (1) The beginnings of the algae are to be found in very remote ages of geological time, at or very close to, the origins of life upon Earth. They were among the earliest forms of life to appear and the evidence available suggests that each of the major groups started independently,

TABLE 1
STRUCTURAL TYPES

Simple types

Unicell
Palmelloid and dendritic
Coccoid habit
Filamentous habit
Heterotrichous habit { a creeping basal portion / an upright portion above
Siphoneous habit

Advanced types

Heterotrichous filaments
Discoid
Crusts or cushions
Elaborately erect type
Compact (uniaxial)
Compact (multiaxial)
Foliose
Tubular

TABLE 2
PARALLELISM IN DEVELOPMENT OF SIMPLER TYPES OF GROWTH FORMS

Type of algal structure	Algal group										
	Chlorophyceae	Xanthophyceae	Chrysophyceae	Bacillariophyceae	Cryptophyceae	Dinophyceae	Chloromonadineae	Euglenineae	Phaeophyceae	Rhodophyceae	Myxophyceae
Motile holophytic unicell	X	X	X		X	X	X	X			
Motile colorless unicell	X	X	X		X	X	X	X			
Encapsuled unicell	X		X					X			
Motile colony	X		X			X					X
Dendroid colony	X	X	X	X				X			
Palmelloid colony	X	X	X		X	X					X
Coccoid (zoosporic)	X	X	X		X	X					
Coccoid (azoosporic)	X	X	X			X					
Simple filament	X	X	X			X					
Heterotrichous filament	X		X			X			X	X	X
Siphoneous type	X	X									
Holophytic amoeboid type		X	X			X					
Holozoic amoeboid type	X		X			X		X			
Plasmoidial type	X	X	X			X					

probably at approximately the same time, and have developed along more or less parallel courses since. (2) By the beginning of the Paleozoic Era (earliest Cambrian time) roughly 500 million years ago, the algae had developed to the point where the algal population was probably equal to that found today, with

all the major groups present, and even some of the existing orders and families already present and showing their characteristic features.

Our knowledge of fossil algae is limited and very spotty with many vacant spaces both in time, and in algal groups. The study is still in its early childhood. There are a number of reasons for this. (1) Geologists and paleontologists have only recently become interested in fossil algae, and to begin to search for and to study them. (2) The nature of the fossils, (TABLE 4); and (3) the difficulties in accurately identifying and classifying the fossils will be discussed later.

Thanks to the fact that the oil companies have discovered that algal limestones make good reservoir rocks, petroleum geologists and paleontologists are becoming interested in fossil algae. However, as yet, very few have the knowledge and experience to use them. I seriously doubt if there are 10 people in the world with a good working knowledge of the subject. In the Western Hemisphere there are only 3 people working full time in the field, and 2 of these

TABLE 3
PARALLELISM IN DEVELOPMENT OF ADVANCED FORMS

Type of growth form	Group of algae		
	Chlorophyceae	Phaeophyceae	Rhodophyceae
Heterotrichous filament	X	X	X
Discoid type	X	X	X
Crusts or cushions	X	X	X
Elaborate erect type	X	X	X
Compact (uniaxial)	X		X
Compact multiaxial	X	X	X
Foliose	X	X	X
Tubular	X	X	

are interested only in certain groups. However, there are a number who are learning, and are studying either certain groups or the fossil algae present during certain geological periods.

From the very nature of the majority of the algae their chances of being preserved as fossils are very slight.

A tiny drop of jelly surrounded by a thin wall of organic material will only be preserved under very exceptional conditions, and even then the chances of it being found are very slight. Normally only those microscopical forms which are encased in a covering of silica or other mineral material are likely to be preserved, as in the case of diatoms and silicoflagellata. Among the larger forms it is also true that the chances of the bodies of such soft organisms being preserved are almost nil. The only common exceptions are those higher types which have developed the habit of secreting or depositing calcium carbonate within or around the plant tissues, and the microscopical forms which are enveloped in a siliceous or calcareous covering, or have a hardened encysted stage.

Groups with known fossil representatives (TABLE 5). This program deals with the Protobiota, so emphasis is placed on the microscopical forms. However, other speakers are giving detailed papers on the diatoms, dinoflagellates, and

other types of the chrysophyceae and the silicoflagellates, and because my work has been largely with the megascopic limestone building forms, I cannot refrain from discussing them briefly. (From the point of view of evolutionary trends

Table 4

Methods of Preservation of Fossil Algae

Type	Information given by fossil	Value for accurate classification
Impressions	Give a general idea of size and shape, some surface markings. No internal structure	Very little
Molds and casts	Same as above	Very little
Preservation in chert	Variable. Some remarkable preservation of microorganisms and small megafossils	Often very good. Probably the best
Carbon films	Size, shape, and surface features beautifully preserved, sometimes. Rarely traces of internal structure	Fair
In coal or peat	At times remarkable preservation of microfossils, and internal structure of larger ones	Good to very good
Calcareous algae. Original material or calcified or silicified	Good internal structure. Even the size, shape, and arrangement of cells in the tissue in case of coralline algae	Good to very good

Table 5

Groups with Fossil Representatives

Group	Representatives	Size	Age range
Chlorophyceae	Codiaceae (some genera)	Mega.	Cambrian–recent
	Dasycladaceae	Mega.	Cambrian–recent
Chrysophyceae	Silicoflagellata	Micro.	Miocene–recent
Bacillariophyceae	Diatoms	Micro.	Jurassic ?–recent (possibly older)
Dinophyceae	Dinoflagellata	Micro.	Ordovician–recent
Rhodophyceae	Solenoporaceae	Mega.	Cambrian–cretaceous
	Corallinaceae	Mega.	Jurassic–recent
	Gymnocodiaceae	Mega.	Permian–cretaceous
Myxophyceae (Cyanophyceae)	Stromatolites	Mega.	Precambrian–recent

these are of interest as they have made much of their development since Cambrian time with a fair fossil record to document the development.)

Megascopic fossil algae. The remains of multicellular algae, as well as microscopical ones, may be preserved in a number of ways. The nature of the fossils, resulting from the way in which they were formed and preserved, is of great

importance as it controls the amount of information, especially with regard to structure (TABLE 4).

The most common and the most useful algal fossils are those of calcareous algae. If not recrystallized these commonly show not only the external form and surface features but at least some details of the microstructure. In the case of the coralline algae they actually show the size, shape, and arrangement of the cells in the tissue and details of the reproductive organs permitting definite, accurate classification.

Identification and classification of the fossils. This is the most important and frequently the most difficult part of the study of fossil algae. The remarkable parallelism in structural development and growth form in several of the major groups and numerous orders and families gives a perplexing choice of possible assignments for the fossils, which can only definitely be decided on the basis of internal structure and reproductive organs. As just pointed out, very few of the fossils can give this information except the calcareous algae.

This means that the calcareous algae are the only groups of megascopic fossil algae for which we have enough solidly based information to be able to discuss the evolutionary morphological trends.

Morphological trends. Among the green algae two families, the Dasycladaceae and the Codiaceae have a long fossil record. Both appear in the record during the Cambrian and continue down to the present.

Dasycladaceae. The general form for most members of this family suggests a test tube brush, consisting of a central stem from which develop more or less regularly spaced whorls of primary branches. From the tips of the latter may arise tufts of secondary branches, which in some genera may produce tertiary branches. In the earlier, primitive forms the primary branches are not collected in regular whorls, but may be irregularly spaced, or develop in more or less regular rows which spiral upward around the central stem. However, genera with regular whorls of primary branches are definitely present during the Silurian period and characterize most of the genera thereafter. From Silurian times on the general trend is toward greater structural complexity, involving greater numbers of whorls, the development of secondary, and tertiary, rarely even quaternary branches, and the differentiation of the branches into whorls of purely vegetative branches, and whorls of fertile sporangia bearing branches, with, in some cases, the modification of certain branches into elaborate holders of sporangia or spores. This trend toward greater elaboration of structure reaches its climax during the Jurassic period, after which a tendency toward simplification begins. This has continued to the present.

Codiaceae. The early Paleozoic record of this family is meager but sufficient to show that by Ordovician times some members had reached a high structural level quite close to that of present day types, like *Halimeda* which they closely resemble. Since then "increased structural complexity suggesting evolutionary changes, such as are seen in the Dasycladaceae, can scarcely be recognized among the Codiaceae. This fact suggests that, as a consequence of vegetative differentiation and evolution from primitive plants sometime during the Precambrian, the family was already well established," (Konishi, 1961, p. 233). Actually, from the Mississippian up into the Lower Cretaceous various mem-

bers of this family are probably numerically the most common fossil algae. They changed in detail, but the general morphology and structure changed but little.

Red algae. Calcareous red algae were relatively rare throughout the Paleozoic. From the Cambrian to the Pennsylvanian, all found to date appear to belong to two genera of the family Solenoporaceae. During that time they show little morphological change. Then, during the Pennsylvanian, several quite different types of red algae appear. One of these, the genus Archaeolithophyllum, has much higher structural features with the tissue differentiated into a well developed hypothallus and perithallus, and definite conceptacles. Also the Pennsylvanian genera show a much greater variety in growth form. Whether this rapid rise of new types represents an evolutionary surge, or for some reason long established groups of plants acquired the calcareous habit and begin to be preserved as fossils, we do not know.

During the Permian another family of calcified red algae, the Gymnocodiaceae, appear and in the Late Permian become abundant and widespread, adding additional morphological types.

The record of Triassic red algae is scanty, but during the upper half of the Jurassic the group undergoes a strong evolutionary push. Within the family Solenoporaceae many developments and new growth forms appear, and representatives of the family Corallinaceae begin to emerge. The first recorded articulated corallines appear during the Middle Jurassic, and the earliest known crustose corallines during the Late Jurassic. By the end of the Cretaceous, almost all of the common genera of the coralline algae had appeared. They were well established by the middle Eocene and had developed essentially all the morphological features known today.

Myxophyceae (*Cyanophyceae*). The only other important type of calcareous algae are the stromatolites. These are calcareous masses of distinctive form and surface markings, commonly showing thin arched laminae, built largely or entirely by the activity of certain types of glue-green algae. They have been reported from rocks as old as the late Archaeozoic, and are faily abundant in the Huronian of few areas. They were the limestone building organisms of the Proterozoic and Early Cambrian. With the appearance of limestone building animals in the Cambrian and Ordovician their importance decreases greatly, but they have continued in considerable numbers down to the present day. However, in morphology and structure, they show practically no change after Late Cambrian times, consisting of mats or felts of tiny algal filaments which often trapped some silt or organic debris and was encased in a mold of fine calcareous dust precipitated by the algae. Commonly they developed colonies of a consistent shape, show growth laminae, but little or no microstructure.

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PALEOECOLOGICAL CONSIDERATIONS OF GROWTH AND FORM OF FOSSIL PROTISTS

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Protists known as fossils range from bacteria (0.5 μ in size) to foraminifers and tintinnids (from 10 to 1000 μ or greater in size). Some protists, for example, bacteria and lime secreting algae, are first recorded as fossils in rocks of Pre-Cambrian age; others, including radiolarians, hystrichosphaerids,* and foraminifers, apparently make their first appearance in Paleozoic time. Finally, Mesozoic rocks bear the first record of a dominant element of the living phytoplankton, namely, the diatoms, as well as other protists, such as, the coccolithophorids, silicoflagellates, tintinnids, and the Chrysomonadina.

There are numerous studies by protozoologists on variations in size and form, as well as structure and physiological characteristics of protozoans. They have found it necessary to distinguish races, varieties, and strains within a given species to italicize the observed variation.[2] By contrast, studies on the skeleton of various protists in which the differential morphology, as well as causative factors, is considered, are relatively few.

Thompson[3] approached protist skeletal morphology and factors influencing it from a different point of view. With mathematical-physical considerations, he reached some insightful conclusions. One need but regard the minute mass of protoplasm that is involved in secreting a protist skeleton as a fluid drop and subject to all the physical forces known to affect such a drop to explain its form by the laws of surface tension. It is apparent that many protists tend to have skeletons of spherical configuration. The sphere, of course, offers the least surface area for a given volume. Because a chain of such drops is possible, and any individual drop can be acted on by gravity, the observed variation in protist skeletal morphology can be simply explained.

Ecology and Paleoecology of Protists

There is a very extensive literature on ecological factors that influence growth of living phytoplankton.[4-8] Nitrogen and phosphorus are primary nutrient factors.[5] Other elements of importance include: silicon,[9-11] iron,[12,13] and possibly manganese.[5] A sudden increase in vitamin B_{12} may be the stimulus for certain phytoplankton blooms.[14] Among physical factors, temperature and salinity are effective "selective agents" on the species level. The species specific salinity response has recently been attributed to "special requirements for the concentration of sodium ions in the medium."[15] Radiation is obviously of primary importance affecting as it does, latitudinal and seasonal variations in phytoplankton production. In addition, the photic zone must be replenished by nutrients from deeper waters. This redistribution is attained in coastal waters by vertical circulation.[5,7]

* Affinities to dinoflagellates are indicated for several, but not all, hystrichosphaerids.[1] Tasch (in press) has found undoubted dinoflagellates in the Permian of Kansas. These were associated with hystrichosphaerids.

It seems valuable to reiterate, with fossil protists in mind, that "we may assume that during the period for which we have good fossil evidence, the sea has remained very much the same in overall chemical composition."[16] Certainly, this is applicable to the Tertiary and Mesozoic. By extrapolation, for protists such as the radiolarians, it may be referred back to the older Palcozoic. Ecological studies of living marine biotas suggest "dim outlines of food chains that must have had links similar to those of the present day"[7] in the geological past.

In thinking about assemblages of fossil protists, their growth and form in ancient seas, coastal and inland waters, one can refer to the same or equivalent physical-chemical factors known to influence living phytoplankton.

The Diatom Frustule and Dinoflagellate Armor

Certain physical realities of the environment have to be satisfied to ensure survival for various protists including pelagic diatoms and dinoflagellates. We may speak of these as "fence" or limiting conditions. These restrictions influence not only distribution but growth and form as well. The first "fence" is the specific weight of living protoplasm, which is 1.02 to 1.06, and hence heavier than pure water.[4] There will be a tendency to sink if the added increment of a skeleton (test or armor) is superimposed on this naked weight. Whether the protist is a passive floater like the pelagic diatoms, or capable of feeble flagellar locomotion like the dinoflagellates, the fence condition will apply. The second "fence" is established by the requirements of photosynthesis. Pelagic protists need to be physically positioned, or located in a specific zone of the sea, the photic zone, or both.

Given these fence conditions, a selective advantage will favor individual pelagic diatoms and dinoflagellates with slight variations in skeletal morphology that tend to retard the rate of sinking. Natural selection would then become effective within the available band of skeletal variation characterizing a given population.

Projecting spines, chains of cells, disc-shaped tests or needle and hair types, curvature of cells, bevelled ends of tests, are all structural adaptations to resist the gravitational force. Spines, for example, aid flotation, as do spiral or flattened chains of cells. This last feature produces more surface area and hence greater frictional resistance.[17] It should be emphasized that test shape and modification of the ends of tests do not prevent sinking. Rather, these features either facilitate a return to the horizontal from a vertical position, or expand the path of passive descent from a straight line to a zigzag path or a widely circular one. In this way, removal from the photic zone is slowed down or delayed.[4,18,18a]

Weight and spination of diatom frustules have been observed to vary according to species, season, and habitat. Generally, pelagic species tend to be thin shelled, whereas bottom and littoral forms are not. Viscosity, which varies inversely as the temperature, is a factor in flotation of pelagic protists. Heavier frustules tend to sink under reduced viscosity. It follows then, that cold water or winter forms will have heavier shells.[4,7,17] In all such instances, silicon metabolism and the supply of silicon are also involved.[10,11]

The shapes of some nonmarine diatoms can be influenced by other ecological factors. Individuals of the genus *Desmogonium* were long and had barely capitate ends in fast flowing water but were short and had broadly capitate ends in standing water.[17]

Similar considerations also apply to the morphology of armored dinoflagellates.[4,15,21-26] Kofoid studied skeletal development (*i.e.*, an armor of loosely cemented cellulose plates) in the genus *Gonyaulax*.[21] He found that all modifications in this genus were variants of the spherical configuration (*cf.*, Lejeune-Carpentier[27] for fossil *Gonyaulax*). In turn, this ensures least surface area, and hence confers an advantage on protists leading a pelagic existence.

Braarud[15] stresses that form variation is observed in every species. Study of Schiller's work on dinoflagellates brings this out clearly. *Dinophysis hastata*,[21] for example, shows a whole spectrum of variation from ovate to subovate and subelliptical configurations, and corresponding size and shape variations in epithecal and hornlike structures. Braarud[17] noted that in some instances, form variation appeared to be "phenotypic" and "tentatively related" to a whole series of ecological factors such as salinity, temperature, nutrient salt concentration, and day length. An excellent example of such infraspecific form variation is found in the fossil record of the dinoflagellate *Nannoceratopsis* recovered from beds of Jurassic age.[28]

Twenty specimens of *N. deflandrei* show variations in form from ovate to subovate hanging drop configurations. These may bear weakly defined antapical horns or lack them. Other forms are broadly and acutely subtriangular with the base faintly or markedly concave between short horns. This strikingly contrasts with the long horn type, *N. pellucida*.[29]

It is apparent from our previous discussion that these variants are adaptive modifications for flotation. Something similar to pelagic diatom adaptation in thickness of test is found in the armored dinoflagellates. Thus, in colder waters of the South Equatorial Currents in which viscosity was greater, the horns of *Ceriatia* were found to be longer than those of equivalents taken in the warm water of the Guinea stream.[4]

The short horn, *N. deflandrei*, may be related to warmer waters, whereas *N. pellucida*, the long horn species, would then indicate colder waters. A third type tentatively assigned to *Nannoceratopsis* has been found in the Permian. This form is flask-shaped and bears stubs in place of horns (Tasch, in press).

Other structural modifications that have adaptive value in armored dinoflagellates, include a variety of specializations to ensure suspension or flotation when the flagella are at rest or swimming is feeble.[4,21,25,26] In the genus *Triposolenia*, the ends of the antapical horns are deflected. The significance of this asymmetry has engendered speculation. Kofoid[19] thought that the asymmetry must bear a "profound relationship" to the survival of the forms in which it appears. It occurs in more than one species and in the genus *Amphisolenia* also.[26] Still other genera have analogous structures. After a descent of about 10 times the body length, the asymmetrical horns serve to orient the long axis horizontally, *i.e.*, the position of greatest resistance to downward pull.[4,19]

A few of the morphological variants in armored dinoflagellates include: round and egg-shaped skeletons (*Glenodinium*) sometimes bearing spines on the

hypotheca; hanging-drop configurations to subspherical forms with a tapered hypotheca and bearing or lacking antapical horns (*Peridinium*), an eccentric expression of the same configuration with partly deflected apex and horns (*Heterodinium*); bizarre, multihorned *Ceratium* in which horns may deflect at all angles and in all directions.[21,26]

Nine varieties of *Ceratium hirundella* were found in various European waters.[2,21] Size variants have been reported from different Swiss lakes: 92 μ in Lake Como, to 707 μ in Lake Schwendi.[24] Sampling several ponds in the vicinity of Darmstadt, Germany at 2-week intervals over a 5-year period, List[29a] found that population density fluctuated with rise and fall of temperature. He noted seasonal variation in both horn length and horn number in *C. hirundella*. In the summer four-horned type, for example, horns were shorter during very hot summers than they were during cooler ones. Apparently, in fresh, as well as marine waters, horn development is an adaptive modification to resist sinking below optimal food and sunlit levels.

Flaring, sail-like, structures from the girdle, inverted umbrella and parachute type membranes as in *Ornithoceras*, *Dinophysis*, and other forms[4,26] all tend to increase the surface area of the anterior over the posterior. In turn, this helps to orient properly the given protist.

A third group of protists of polyphyletic origin are the hystrichosphaerids. Many forms classified under this group are apparently dinoflagellate cysts.[1,30] Fossils often show dinoflagellate plates although many forms lack a distinguishable plate system. Configuration of the central body is often globular but all variations are known from subround and ovate to subelliptical. Arising from the central body are spines or tubular processes, or both, with flattened or bifurcate terminations. It has become clear that these tubular processes were originally connected to a circumscribing membrane. A recently found globular hystrichosphaerid from the Kansas Cretaceous bears a short, tubular process that terminates in two fine flagella-like extensions within the body of the enclosing membrane.[31]

For those hystrichosphaerids which are definitely dinoflagellate cysts, morphology was determined by encystment. *Hystrichosphaera furcata* and *H. speciosa* bear an equatorial girdle and dinoflagellate plates, and are good examples. Generally, hystrichosphaerid form is a variation of the spherical configuration. Why this is so can best be understood if one observes a cyst inside a subtriangular-to-bullet-shaped dinoflagellate like *Deflandrea phosphoritica*.[32] The spherical shape is the most efficient configuration that can be enclosed in the volume available.

Radiolarian Scleratoma and Tintinnid Lorica

Radiolarians and tintinnids occur together in the Mesozoic fossil record in the Mediterranean area[33] and hence it seems desirable to discuss them together. Both groups have living representatives which occur in great abundance. Radiolarians found in the fossil record are almost invariably "upper-zone pelagic types"[35] although abyssal forms are known.

Although radiolarians are incapable of horizontal locomotion, tintinnids can swim rapidly by the aid of bristles and cilia.[34] Both forms had to solve the problem of resisting passive sinking below optimal levels of the sea.

The form of radiolarian species seems to be adaptive to environmental conditions[35] although experimental study of factors influencing shell morphology are wanting.[34] Thompson,[3] as noted previously, provided some useful insights into radiolarian morphology.

Free floating radiolarians, among both fossil and living assemblages, tend to be spherical and elliptical, with a foamy or spongy appearance. Such forms occur in the Spumellina, Nasselina, and Acantharina. The shells are delicate, small, and bear various structures such as, numerous slender apophyses, large pores, thin bars between pores, and varied spinose development. Inhabitants of deeper layers (Phaeodarina and some Nassellina) are heavier, more massive, and tend to bilateral symmetry. They are infrequently burrlike. Structures found in such forms include: short apophyses and small pores with thick trabeculae.[34,35]

In some forms (Semantidae, etc.)[34] one can observe configurations not too different from those of the silicoflagellates.[36] The shell in the Challengeridae bears a fine hexagonal mesh resembling similar structures of the diatom frustule.[34] Some configurations of radiolarians are analogous to those of armored dinoflagellates, for example, *Coelodecas*.[34] *Hexaspyris papilio*[38] is reminiscent of the bizarre spinose development in the dinoflagellate *Ceratium*.

It is generally agreed that variations in scleratoma configuration and in skeletal structures found in radiolarians reflect adaptations to retard sinking below certain depth levels of the sea. Within a given species of course, variations of shape and structure are merely those of a normal population spread.

The gelatinous or pseudochitinous cuplike or elongate lorica of tintinnids is frequently agglutinated. Foreign particles encrusted or included in the delicate membranous wall may consist of fine mineral grains, coccoliths, diatoms, and organic debris.[33,38,39] Shape of skeleton in both fossil and living tintinnids is extremely diverse.[33] Surface markings of the lorica include: ribs, ridges, plications, flutings, shelves, reticulations, fenestrae, and lacunae. Among aboral structures are apophyses, pedicel, knob, lance, and skirt.[38]

Because tintinnids move like squids with oral end directed backwards, streamline configuration of the aboral tip would offer less frictional resistance to forward movement. It is also likely that the lorica may aid flotation.[38] The total effect of such configurations is to check descent below optimal levels.

Modification of Shape and Form in Foraminifera

Work on living foraminiferal distribution and ecology has clearly established characteristic faunal suites in distinct brackish and marine environments.[40-42] Although the majority of foraminifers are vagrant benthos, planktonic forms that float at or near the surface such as the Hantkennidae, Orbulinidae, and Globorotalidae have been more closely studied in the past decade.[40,43]

Bandy[44] found a striking correlation in form, structure, and environment in benthonic foraminiferal assemblages in modern seas. Among the variations he observed are overall size, shape, and size of chambers, chamberlets, coiled and uncoiled forms, spinosity, surface sculpture of the test (costa, striae). These were found to vary with bathymetry (bay, shelf, and bathyal zones). Phleger[40] believes that the influence of temperature may have been overstated

in the literature and suggests a whole spectrum of ecological factors that may have been involved.

In both modern tropical and subtropical waters, spindle-shaped tests seem to characterize definite depth zones (20 to 80 meters). By extrapolation, Bandy[44] ascribed equivalent depth zones to fusulinids—an extinct Paleozoic family—and to the Cretaceous spindle-shaped *Loftusia*. Similarly, he noted that deeper water assemblages seem to show a size increase and coarser surface features.

The planktonic foraminifers show a variety of morphological and structural adaptations for their floating existence.[43] The variations are ascribed to temperature and salinity. Thin walled shells, for example, characterize surface *Orbulina universa* and *Globigerina* in contrast to thick walled shells for individuals living at greater depths. Reduction in the specific gravity of the planktonic test is also affected by increase in pore size, aperture enlargement, or the development of supplementary apertures.

Resistance to sinking which is the critical problem facing all pelagic protist inhabitants, is attained in planktonic foraminifers as follows: flattening of the body accompanied by a radial test, and elongate or clavate chambers. In the Orbulinidae and Hantkennidae spinose projections develop. Other adaptations include: globose chambers that increase in size as added, large primary apertures, and in such forms as *Globigerinoides*, development of many secondary openings.

Although all of the above named variations may be related to genetical events and the operation of natural selection, there are other nongenetical factors known to influence foraminiferal morphology. On the Argentine shelf, a depauperate foraminiferal fauna was found to be characterized by its small size, partial or complete loss of ornamentation, a tendency toward asymmetry, and growth retardation. Spectrographical study of trace elements in the shells revealed the presence of lead in depauperate, as compared to, normal faunas in which it was absent.[45,46] Study of *Allogromia laticollaris* in culture revealed occasional populations with a large number of flattened discoidal individuals. In this instance, the flattening was directly attributed to "downward pressure exerted by rapidly multiplying algal filaments."[47] Dwarfed foraminifers are reported from poorly ventilated basins.[42]

An unusual example of a testate protozoan, *Difflugia oblonga*, can be cited here although it belongs to a different order than the foraminifers. A small pond (10 x 6 meters) in the environs of Prague, Czechoslovakia, contained numerous individuals of this species. They exhibited an astonishing morphological variation. Every variant was observed from a globose bowl with a smooth base, to elongate figures with tapered basal projections variously curved. Some specimens took on the configuration of an Erlenmeyer flask with knoblike projections from each basal edge. The heavy discharge of industrial waste gas (CO_2) in the environs was thought to be the causative factor.[48]

Classes Chrysophyceae, Coccolithophorida, and Silicoflagellata

The several flagellates cited in the subtitle of this section, with the diatoms discussed earlier, constitute the phylum Chrysophyta. Members of the order

Chrysomonadina are either solitary or colonial. They are widespread in both fresh[2,49] and marine waters[15] and have fossil representatives in the family Archaemonadaceae Deflandre.[50]

Formation of siliceous resting spores or cysts is a "most characteristic feature of the order."[2,18,51] Such cysts have a funnel-shaped opening or neck and resemble a stoppered or plugged spherical jar. The plug is formed of cytoplasm retracted from outside the cyst wall.

In the cyst of *Microglena*,[18] Conrad has distinguished "numerous minute lens-shaped masses of silica" embedded in an outer layer of pectic substances. A delicate, inner smooth layer of cellulose underlies this outer layer. This genus with other Chromulineae is closely related to the coccolithophorids in cell structure although it differs from the latter in flagellation and composition of its cyst.

Cysts are usually spherical but variants from this configuration occur. *Archeomonadopsis*, which is flask-shaped, is such a variant. Surficial ornamentation finds diverse expression: ridges that may form a reticular network; encircling equatorial flanges; spine and knob structures on ridges, and peripheral spines. The size range of cysts is 10 to 25 μ.

Although little is known about the marine Chrysomonadina, it is apparent that the morphology and small size of the cyst, together with the cytoplasmic plug, would favor both resistance to sinking below the pelagic zone and widespread passive distribution. Fossil cysts also indicate a broad geographical spread.[50] The same types of adaptive modification found in living representatives occur in fossils.

A third large group of planktonic algae are the Coccolithophoridae.[15,18a,52a,53,54] They are typically open sea biflagellates although in places like the Oslo fjord, they may occasionally occur in such densities, that the water looks like milk.[4] Fresh water forms like *Hymenomonas* are also known.[18]

One may study a form like *Coccochrysis*,[51] *Discosphaera*,[2] or *Syracosphaera* and *Coccolithus*[18] and observe a subovate configuration in the first and third and a more spherical form in the second and fourth. Lohman[52] figures several different species of *Pontosphaera*, *Calyptrosphaera*, and *Coccolithophora*, as well as species of the second and third genera named above. All of these species show the same trend in configuration. Generally, therefore, the shape of coccolithophorids are modifications of a sphere.

The formation of the coccolithophorid skeleton is gradually achieved. At fairly equal intervals, numerous, minute, variously shaped, calcareous discs (coccoliths) are "imbedded in an investing membrane."[51] This envelope of variable thickness is gelatinous initially. The coccoliths become "rigidly united when the mucilage calcifies in older individuals."[18] Coccoliths have a central perforation or are imperforate.[2] Although living biflagellates commonly range from 5 to 20 μ,[4] sizes can attain 50 μ.[52] Coccoliths found in sediments range between 2 and 30 μ.[55]

Several coccoliths bear anteriorly and medially spinelike processes. Of interest, is the successive formation of new coccoliths within the old as the old are gradually dislodged[18] and contribute to oceanic bottom deposits. Although today coccoliths are but a "minor part" of oceanic carbonate muds, in Miocene and Oligocene time, for example, they formed "coccolith ooze."[56]

Braarud[15] and others have experimented on variation in salinity and its influence on the growth of the coccolithophorids, *Hymenocaras carterae* and *Coccolithus huxleyi*. For the first species, salinity was excluded as an important environmental influence on growth. This corresponded to the littoral habitat in which it is most abundant and in which salinities are quite variable. The second species, *C. huxleyi*, is distributed worldwide in oceanic waters (35 per thousand)[57] and in northern European coastal waters (15 to 20 per thousand). Experiments have shown that between these ranges of salinity there was good growth.

Salinity apparently does act as an ecologic fence in excluding *C. huxleyi* from brackish waters. A vertical size distribution of coccolithophorids at equatorial stations has been reported.[57] Small forms were abundant in the upper 50 meters. Near surface temperatures are also a probable factor in distribution. It is thought that variety, large size, and abundance of Eocene coccolithophorids indicate "warmer seas."[55]

The life cycle of coccolithophorids has recently been shown to be more complex than previously thought.[18] A motile stage and a cyst stage have been experimentally demonstrated for *Coccolithus pelagicus*.[15]

From these data, shape, size, and encystment seem to be adaptations similar to those in the closely related siliceous Chrysomonadina. Coccolith formation, shape, their even spacing in the membrane, and spinelike processes arising from some coccoliths, are all adaptive devices to aid flotation. Abnormal amounts of calcite in some Tertiary coccoliths are thought to reflect calcium carbonate rich waters and not a diagenetic effect.[54] Conceivably, this abnormal deposition may have served to aid buoyancy or to adjust specific gravity.

One can confidently transfer the general interpretation given to Tertiary coccolithophorids.

The silicoflagellates have a siliceous skeleton which is covered by a delicate layer of cytoplasm containing chromatophores. This occurs in early development when the skeleton is internal, whereas in the adult individual it is external.[2,18] The skeleton ranging in size from 10 to 150 μ is essentially a "latticework case of hollow siliceous bars."[50] *Distephanus* (=*Dictyocha*) *speculum* with 6 radial spines may be taken as an example of the group. In most silicoflagellates, the spines give the skeleton a stellate appearance. There may also be accessory and basal spines. The basal body ring may be from 3 to 10-sided with as many radial spines. Radial spines issue from the point of intersection of any 2 sides. The basal body ring of some fossil forms like *Mesocena* and *Corbisema*[58] is 3-sided with a small spine at each angle. Others, like *Dictyocha crux* are 4-sided and have longer spines. *D. speculum* is 6-sided, and *D. octonaria* is 8-sided.[36]

The siliceous skeleton is most often a complex of 2 rings or polygons joined by a series of rods.[18] *Dictyocha speculum* is a good example of this construction. The basal body ring of *Mesocena* forms an ellipse, and in *Corbisema*, it forms a triangle.

Silicoflagellates are exclusively marine plankton[18a] and are found in colder seas. Frequently they occur associated with diatoms and radiolarians in ancient and modern sediments.[36] Although they are not uncommon in food

vacuoles of tintinnids, quantitatively they are a minor contributor to the food economy of the sea.[4]

Thompson's explanation[3] of the basket-shaped skeletal units of *Dictyocha* envisioned 4 or more vesicles side by side in one plane and separated by a "polar furrow." The radial spines normal to the main basket or lattice work were interpreted to be uncompleted portions of a larger basket. This last interpretation seems unacceptable in light of the work of K. Gemeinhardt.[18] He demonstrated that adult individuals had a smaller skeleton fitted into the larger one. In this instance, the inner set of radial spines were not the beginnings of a still larger skeleton, but rather parts of the skeleton of a daughter cell, and its appearance preceded division. Hovasse confirmed this finding, in 1932,[18] and noted that the new skeleton was a mirror-image of the old one.

Thus, the opaline silica lattice work may be envisioned as derived by secretion on a tiny sphere of protoplasm that had a vesicular surface. Open space, ovate, elliptical, and polygonal skeletal configurations can then be readily explained. The radial spines which confer a stellate appearance are most likely adaptive modifications to sustain flotation when the single flagellum is at rest. All other accessory spines and ornamentation, such as beads and pits on the discs,[58] may constitute minor adjustments of specific gravity of the skeleton that had selective value.

In the evolution of the silicoflagellates there is a tendency to increase the number of radial spines from 3 or 4, to 6, 8, and 10. That trend clearly denotes the adaptive value of particular skeletal modification.

Miscellaneous Protists

In this section, bacteria and lime secreting algae will be considered from the special point of view of our discussion. Despite the frequency of pleomorphism, there are three common or fundamental forms of true bacteria; spherical or ovoid (*coccus*), rod-shaped (*bacillus*), and spiral (*spirallum*). Spherical forms may grow in pairs, in fours, or in chains. Rods vary in configuration from cylindrical to ellipsoidal with rounded-to-flattened ends. In young cultures and favorable media, bacteria tend to "exhibit characteristic morphology," whereas in senescence, there are a decrease in size and considerable form variation. Other factors influencing shape are: temperature and age of culture, concentration of substrate, and composition of medium.[59] A barophilic property (pressure-dependence) has also been reported. Near their threshold of pressure-tolerance, cells of many bacteria grow into long filaments and mutations are promoted.[60,61]

Bacteria are commonly about 0.5 μ in size but range to 10 μ. Fossil bacteria are generally identifiable by size, shape, and arrangement alone.[62] However, viable bacteria of Permian age have since been reported from the United States[63] and from Germany (Dombrowski, 1960). In such instances, physiological activities which distinguish modern bacterial species can also be studied in ancient populations.

The descriptive literature on lime secreting algae known as fossils is very extensive.[64-66] A good review of recent stromatolites and their ancient analogues is given by Ginsburg.[67] Types of stromatolite configurations include:

laminated algal-mats such as can be observed forming today at Turner and Price Falls, Oklahoma, or equivalent forms described by Black from the mudflats of Andros Island, Bahama; domes, heads, and more extensive digitate masses. Onkolites (unattached forms) can have the shape of the nucleus or be variously shaped biscuits or flattened discs.[67]

Factors such as a slight increase in iron above tolerance amounts have long been known to retard growth, affect size, and ultimately, the shape of several nonmarine, nonlime secreting, filamentous algae.[46] Cyclicity in occurrence of the Cambrian form *Cryptozoon undulatum* was attributed to inhibition of growth due to increasing turbidity caused by transported sediments.[68] Pre-Cambrian bioherms of Northwestern Montana show different forms—columns, domes, sheets—which were "apparently" determined by physical conditions such as water movements.[69] The ultimate external form of Recent algal biscuits is credited to two determinants: stability of the surface on which the biscuits grow and the strength of attachment to it.[67]

Work in progress (Tasch, unpublished) on newly discovered algal reefs and onkolites in the Kansas Permian provide some evidence on controls of ultimate form. An influx of mud over the growing algal mat (stromatolite) inhibited growth in certain directions only. Turbidity, of course, can exclude or diminish light penetration and hence interfere with photosynthesis. If, however, sediment influx is negligible (4 to 5 mm.), filamentous algae can "move up through the sediment and reestablish themselves on the surface."[67] The topography of the substrate on which the original filamentous algal mat spread, also can be a partial determinant of shape of a stromatolite.

Sporadic circular to elliptical perforations of algal blades in the fossil genus *Eugenophyllum* appear to represent adaptive modifications. Although these forms lived below normal wave agitation, the perforations would help to dissipate even gentle current action against the upright blades which are several inches in height.[70]

Among factors influencing growth of stromatolites and onkolites are: substrate, turbidity, amount of light penetration, depth of water, wave and current action. Influence of metallic cations can also be inferred.

Terrestrial Microproblematica

Microproblematica are apparent fossils observed in sections of rock sufficiently thin to transmit light. They are primarily of Mesozoic age, but are also known from the Paleozoic and Pre-Cambrian. Distinctive structure and form characterize them. However, they cannot confidently be assigned to any known taxa. Occasionally, additional study and collection permits ultimate resolution of assignment.[71,72]

The microfossil *Nannoconus kamptner*, 1931, is a good example. The object ranges from 5 to 50 μ in length, with an average of 15 to 20 μ; width varies from 5 to 15 μ. It is definitely an "organized object." There is a distinctive wall composition (spirally arranged calcite wedges, 1 μ in thickness). In longitudinal section, it is either conical, spherical, barrel-, or pear-shaped, or cylindrical U-shaped. There is an axial canal or a basal cavity, or both, and 2 apertures opposite each other. Through time, it shows apparent speciation.[73]

Nannoconus is widespread in distribution, having been reported from the

Mesozoic (U. Jurassic-Lower Cretaceous) pelagic deposits in the Mediterranean area, Rumania, Cuba, and Mexico.[39] It is always associated with the pelagic facies containing radiolarians and tintinnids, and occasionally, with smooth ammonites.

The following affinities have been suggested: (1) the object represents an embryonic stage of the flask-shaped foraminifer *Lagena*; (2) it is a unicellular chlorophyllous alga; (3) it is of inorganic origin having formed from calcite crystals in a highly saturated medium; (4) it belongs to the oögonia of certain Charophyta; and (5) it represents a little known coccolithophorid.[39,73]

There are then a whole set of constants and some variables to explain. Constant factors include: distinctive wall composition and structure, 50 μ or less in length; persistence of faunal associations; and occurrence in pelagic facies intermittently deposited over a span of tens of millions of years in different parts of the globe. Variable factors include: nine species of *Nannoconus* based upon variations in axial canal and basal cavity, overall shape and size; three distinct *Nannoconus* faunas in as many zones of the Lower Cretaceous.[73]

In light of our previous discussion on form in many protists, the likelihood is that configurations in *Nannoconus* are variants of a sphere.[73] Thus, selective modification of the sphere gives an elongate type or a cylindrical type. The basal cavity of circular types have no axial canal when seen in thin section. Circular types were spherical in life. Elongate, conical, and subovate types do have an axial canal. The size and configuration of axial canal and basal cavity could be a function of compression of an original sphere. Although this can account for the variation in morphology and inner spatial relationships of the object, it is unclear whether mechanical compaction or genetics was the active agent.

Although we assume the first of these possibilities, the list of constant features still remains to be explained. Colum[39] notes that *Nannoconus* at times appears in great numbers in pure limestone lithotopes. Population density is thus another variable.

What is the likelihood that inorganic precipitation of calcium carbonate and mechanical distortion alone can account for *Nannoconus*? The nearest approach to a regular type of inorganic carbonate deposit is the example of oolites. These may be radiate in internal structure or bear concentric bands around a nucleus. In size range, oolites are also restricted wherever they are found. Mineralogy of the bands tends to be relatively uniform although alterations are known. There is a definite spherical-to-elliptical configuration. When compressed, flat, pelletiferous shapes result. Why cannot *Nannoconus* be an object of this type?

The best argument against an inorganic origin is the persistent crystallization of minute calcite wedges, all of which are perpendicular to, and form a band about a hollow basal cavity or axial canal. Inorganic origin cannot account for the discrete thickness of the wall in this case as it can for the successive bands of oolites. If the calcite wedges were invested in an organic membrane that surrounded a cavity or canal, both wall thickness and mineral orientation could be readily explained.

Once the conclusion is reached that *Nannoconus* is of organic origin, the other

array of factors readily supports the interpretation that it represents a pelagic protist of uncertain affinities.

Among other organized micro-objects of uncertain position are *Favreina*, *Globochaeta*, *Eothrix*, *Lombardia*,[73] *Pithonella*,[39] and objects described by Elliott.[71,72]

Discoaster, an object 3 to 15 μ in diameter, is represented by calcareous, stellate, or rosette-shaped plates. In many species the central area bears a stem. These objects are abundant in pelagic sediments of Tertiary age. The sediments containing discoasters also bear coccoliths, *Globigerina*, and other pelagic foraminifers.[54,55] These objects are now thought to be the skeletal remains of nannoplanktonic organisms of uncertain affinities.

One ecological observation has been made about discoasterids. Across the Eocene-Oligocene boundary, Riedel found not merely a change of radiolarian fauna, but "surprisingly," a change in discoasterid assemblage. This is thought to reflect "some change in surface waters."[55] The active factor here might be surface temperature.

A whole series of related forms of uncertain position among the calcareous nannoplankton include: *Clathrolithus*, *Discoasteroides*, *Fasciculithus*, *Heliolithus*, *Tsthmolithus*, *Polycladolithus*, *Sphenolithus*, and *Rhomboaster*.[54] Even though *Rhomboaster* "is suggestive of some unusual habit of inorganic calcite growth," three considerations refer it to the nannoplankton: specimens occur in abundance; they are found only with coccolithophorids; their occurrence in time is restricted.[54]

Numerous reports of minute sporelike and other types of bodies and "meshwork filaments" from Pre-Cambrian algal stromatolites are now at hand from Russia, Scandinavia, France, West Africa, and the United States (Gunflint formation of Northern Michigan). The biological organization of Barghoorn's material "is supported by geochemical evidence" (*i.e.*, the quantity of C^{13} per mil).[74] There is no equivalent verification of pyrite spherules thought to have replaced microorganisms.[74]

Summary

Pelagic protists tend to configurations of least surface area. The sphere and its modifications is a recurrent shape. The many shapes and structures (spinosity, for example) of the scleratoma of radiolarians, the lorica in tintinnids, the frustule of pelagic diatoms, the armored skeleton of dinoflagellates, the test of planktonic foraminifers, the siliceous and calcareous skeleton of chrysophytes, appear to be adaptations to resist sinking below optimal food and photic levels of the sea.

Examples of nongenetic factors affecting differential morphology of protists include: variable oceanic temperature, salinity, depth, and turbidity; presence of lead, excess iron and copper as well as carbon dioxide; condition of encystment; nature of substrate, barophilic property, nutrient salt concentration, and amount of light penetration. Some of these factors apply only to specific protists.

Fossil microproblematica of Mesozoic age seem to be nannoplankton of uncertain affinities among the protists (for example, *Nannoconus*). Microobjects of Pre-Cambrian age may represent spores and algae.

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FOSSIL ORGANISMS FROM PRECAMBRIAN SEDIMENTS

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In widely scattered outcrops of the Gunflint Iron Formation, Lake Superior region, Ontario, Canada, dense, black, nonferruginous, fossiliferous cherts occur as thin units in the sedimentary sequence of black shales, argillites, and dolomites. In its geological setting, the Gunflint Iron Formation is generally regarded as comprising the middle unit of the Animikie Series (Middle Huronian equivolent) of the Lake Superior region. Absolute age of the Gunflint Formation has been determined by P. W. Hurley by measurement of the potassium-argon ratios in authigenic minerals which occur in direct association with the cherts and interbedded in the Gunflint sedimentary sequence. Replicate determinations have yielded consistent values of 1900 M years (1.9×10^9 years).

The cherts have been studied with the use of thin sections, acid maceration, and a variety of chemical techniques. Thin sections of the chert, when viewed in transmitted light, reveal that its black color, as seen *en masse*, is caused by the abundance of finely disseminated organic matter that appears light amber to dark brown in color in sections 50 μ or less in thickness. In this respect the chert behaves petrographically much as a typical bituminous coal, which in thin section exhibits a range in color of the petrographical components from light yellow through amber to dark orange red to opaque. In the Gunflint chert a large fraction of the organic constituents reveal a distinct morphological organization consisting of filaments, septate and nonseptate, spheroidal or spherical bodies, and more complex asymmetrical structures. The discrete entities are all microscopical in size and present an appearance analogous to masses of anastomosing algal filaments in which are enmeshed other microorganisms. The chert matrix in which the organisms are embedded varies from clear and hyaline to granular and crystalline. In polarized light the chert is microcrystalline. Crystals of pyrite, calcite, and apatite vary in abundance, but in no case are more than minor petrographical constituents.

The biological affinities of the organisms preserved in the Gunflint chert present a curious paleontological problem inasmuch as a number of the distinct entities or "types" possess a morphology that is quite unlike that in existing microscopical organisms, either plant or animal. In this connection it should be emphasized that the organic structures are 3-dimensionally preserved and not flattened or unilaterally distorted. They are hence amenable to morphological and histological study.

The most abundant organisms in the assemblage are filaments ranging in diameter from 0.6 to 6.0 μ. In the most favorably preserved state these are found to be both septate and nonseptate. The septate types exhibit a form indistinguishable from that of filamentous blue green algal (*vis.*, *Oscillatoria*, *Lyngbya*, etc.). The nonseptate types are more difficult to interpret in terms of biological affinities. With exceedingly few exceptions they are unbranched

and visibly devoid of internal structures or inclusions. Whether these represent coenocytic algae or fungi is not possible to determine, although the general form and undulating outline of the filaments is more characteristic of algae than of aquatic fungi. Among the larger nonseptate filaments very occasionally forms have been observed in which the lumen of the filament contains numerous spherical sporelike bodies. In living organisms a somewhat comparable morphology may be found among certain of the iron bacteria (*Crenothrix polyspora*).

The sporelike bodies which are ubiquitous and irregularly distributed throughout thin sections of the chert vary in size between 1.0 to 16.0 μ in diameter (measured along the long axis if ellipsoidal). They are predominantly spheroidal and are not appendaged. The range in size, thickness of wall, and variation in the sculpture pattern of the wall residues indicates that they comprise an assemblage of forms the morphology of which gives little clue to phylogenetic affinity.

A very common and distinct organism in certain facies of the Gunflint chert is an entity whose closest morphological comparison among living organisms can be found in certain groups of the phylum Coelenterata. Rather than to accept the existence of coelenterate animals in an assemblage of such geological age as the Gunflint sediments exhaustive efforts have been made to compare these structures with algae, various of the larger colonial bacteria, and protozoa. It has not been possible, however, to find morphologically comparable structures in these diverse groups and the authors have been forced to conclude, on the grounds of morphology, that the organisms most probably represent metazoons, the closest structural affinity of which is among the Coelenterata. A detailed description of these organisms and other microstructures occurring in the chert will be made in a forthcoming paper dealing with the detailed geology and paleontology of the Gunflint chert.

The organic fraction of the darker and more organic samples of the Gunflint chert varies between 0.2 to 0.6 per cent by dry weight. As previously noted SiO_2 comprises the major mineral component and constitutes more than 99 per cent of the dry weight of much of those chert samples that exhibit the best preservation of organic structures. The organic residues yield small amounts of benzol-acetone-methanol soluble substances, probably hydrocarbons of molecular weights C_{20} or above. These extracts fluoresce strongly in ultraviolet light. Upon destructive distillation at 400° C. the insoluble organic residues yield small amounts of aliphatic hydrocarbons, chiefly methane (87 ppm), ethane (4 ppm), and propane (0.7 ppm) and traces of aromatic hydrocarbons (benzene, 0.34 ppm; toluene, 0.15 ppm; xylenes, 0.45 ppm). Degassification of the chert at room temperature yields methane (6.0 ppm) and butane (0.2 ppm). The chemical data, although limited, are entirely consistent with the paleontological interpretation that the black chert represents the silicified remains of a biocoenose of microscopical organisms the organic matter of which is partially retained, although highly modified through time by very low thermal and metamorphical alteration. For these reasons the Gunflint chert is unique among earlier Precambrian sediments in exhibiting the morphological organization of an assemblage of very ancient and primitive organisms, some of which have counterparts among existing primitive groups.

BACTERIA FROM PALEOZOIC SALT DEPOSITS

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Stimulated by the bacteriological findings in the mineral springs of Bad Nauheim, which carry salts from Permian deposits, I investigated from a bacteriological point of view the Zechstein salts, obtained by means of mining and drilling. Müller and Schwartz (1953), Rippel (1945), and Strong (1956) only achieved the isolation of dead bacteria from Zechstein salts. Reiser and Tasch (1960) reported the living isolation of a diplococcus from Permian salts. We now succeeded in isolating living bacteria. Yet, this achievement seemed rather improbable; for if we had actually extracted living bacteria from Zechstein salts, then we have to assume that we found creatures of the highest individual age ever registered.

The following is a description of the isolating technique we used.

In bacteriological work it is obviously very easy to get unwanted secondary infection. To be sure that this secondary effect would not spoil our results, we used extraordinary precautions. (1) We chose a small research laboratory in which an ultraviolet sterilization lamp was kept burning for four days before the experiment. No one entered this room during these four days. (2) The two researchers entered the laboratory in sterile clothes and sterile rubber gloves after thorough disinfection of their hands and arms. (3) The table and necessary tripods were covered with sterile towels. (4) All necessary instruments, glassware, and apparatus were thoroughly sterilized. (5) The research material, *i.e.*, the piece of salt under consideration, was suspended on thin, sterilized wire from the tripod. (6) This suspended piece of salt was then flamed for one minute with a hot bunsen flame. (7) Immediately afterwards a glass with a culture solution was brought under the piece of salt, so that it was suspended in the solution. (8) The supporting wire was then cut and the glass was closed after sterilizing the rim and the stopper also with the bunsen flame. (9) The cultivation was carried out at a temperature of 40° C. (10) As soon as the culture began to grow, the elaboration to the pure culture proceeded in the usual bacteriological manner.

To working procedure 6, I must add that the necessary time for the surface treatment of the salt with the bunsen flame was ascertained in preliminary experiments. Salt-pieces, which were brought into a fresh suspension of living *Pyocyaneus*—about 80,000 per cm.^{3}—could be sterilized in 45 seconds.

Because salt is a poor heat conductor, the temperature fell rapidly toward the center of the crystal. We heated the surface for 45 seconds. Then 3 cm. from the surface, the temperature rose only by 6.2° C. Thus, we achieved a sterility of the surface and regions close to the surface without producing sterilizing temperatures in deeper layers. Of course, the crystals must be large enough; they must have a diameter of at least 6 cm. Such specimens have a weight of about 250 to 300 gm. A crystal this large saturates about 1 liter of culture solution; a saturated solution is necessary for the cultivation of halophil and halotolerant organisms.

For the duration of this work we set up culture plates on which germs in the air could germinate, which in most cases did not happen. If the germs of the air did germinate, however, they were brought into saline solutions to prove their tolerance to salt. This test always showed an intolerance to salt, so that there was no identity to the bacteria that came from the salt specimens.

In counter-checks we sterilized salt crystals for 4 hours at 200° C., before investigating them bacteriologically in the prementioned manner. These crystals proved to be sterile. We also examined crystals coming up from a depth of more than 4300 m.; in the Mesozoic era these salts lay about 1000 meters deeper than today. At this depth the temperature is at least 160° C., and as expected these salt specimens also showed no sign of life.

Now, how can we find an explanation for the conservation of life over such an extended period of time, that is for over 180 million years? There are two possibilities. First, one is reminded of the method for conserving bacteria that is practiced today, *i.e.*, dehydration at low temperatures. If one extracts almost all the water from the protein of micro-organisms, it is possible to preserve them for years without changing any of their particular characteristics, although there is no metabolic activity whatsoever. We know of certain germs, which lived for more than 30 years, although their metabolism was totally inhibited. Starke and Harrington (1931) consider the vitality of dried bacteria as unlimited. If this is correct, then the hypothesis of finding living organisms in Paleozoic layers could not have received better support, and we would then have found a way of understanding the survival of these organisms over such long periods of time. Second, there is the possibility of reversibly denaturing protein by salification. This method can also be used on higher organisms with good results. For instance, the protein from the eggs of sea urchins can be denaturized in a saturated solution of ammonium sulfate. After months, this process is reversible by simply removing the salts. The eggs retain the ability to be fertilized. Perhaps in our specific case both methods, that of dehydration and that of salification, were in effect.

If this interpretation was true, then the method should be reproducible in a laboratory experiment. For this experimental reproduction we used *Pseudomonas halocrenaea*, which were isolated from Zechstein salts. This bacterium does not bear spores.

If the nutrient solution in which it started growing is slowly dehydrated, the bacterium will die. This will not happen if one slowly saturates the solution by adding 1 gm. of salt per week. This substratum is now slowly dehydrated, until all salts are completely dry and crystalline. In this dry state it can be kept for long periods of time. When bringing these salts into a fresh nutrient solution again, the original vitality of the bacterium can be re-established.

I would like to point out a further peculiarity: the optimal temperature for many of the germs that we found lies between +45 and +55° C., which is astonishingly high. But, elucidating enough, this temperature corresponds exactly to that temperature which, geologists say, was present when the Zechstein sea was slowly drying up.

I believe that this correspondence of temperatures is certainly not accidental. Because the bacteria were embedded in the crystals, they were assured against

destruction by mechanical pressure. After considering the depth of our findings, we can estimate a maximum of 1400 m. With the normal geothermic gradient, which gives the temperature at a certain level, we get a maximal value of +42° C., which the germs were exposed to during their long latent life. This temperature in no way prevents the preservation of life.

The question of which geological specimen is to be examined is of foremost importance. At first I used all sorts of Zechstein salts, while trying out the bacteriological working procedure. But later, I carefully selected the specimens to be investigated. All specimens, which came from questionable regions, such as near faults or the upper salt level, were discarded. Specimens showing signs of recrystallization were also discarded. We used only pieces which definitely showed signs of being primary Zechstein salts, and of these only those which came from perfectly undisturbed points in the middle of larger successions of rock salt, the layers of which were formed normal-hypidiomorphic to allotriomorphic. Their grain size lies in the order of millimeters. But even with this careful selection of specimens, only about every second culture showed results.

Because it is very probable that the organisms are of primary genesis, we can undertake an estimation of the age of these isolated living bacteria. Because pollen grains were isolated, which served as characteristic fossils, it was relatively easy to establish the age of the bacteria.

We also centered our attention on another aspect of the problem: in undisturbed geological layers the rock salt has practically no pores, if we disregard the lye enclosures. If the salt is taken out from its natural environment, it will not be subject to the pressure of the overlaying strata anymore. It relaxes and thus increases in volume by a few per cent. Due to this loosening, pores begin to form and air can automatically enter the salt. This would make possible the entering of bacterial contamination from the outside. To prove that this was not happening, we prepared petrographic thin sections of the salt. In examining these, we found the bacteria to be embedded in the crystalline structure of the salt and not in the capillary crevices (FIGURE 1).

Contrary to the previously shown Paleozoic microorganisms, this form (FIGURE 2) is a direct decendent of the Paleozoic germ, which was obtained by cultivation, and identified as *Bacillus circulans*. I found this form in three different Zechstein formations. It is a very rare specimen, which has been described only eight times since 1890. A comparison of the Paleozoic and the Recent representatives of this group is of special interest. When the Recent germs are compared from an evolutionary point of view they are neither older nor younger than the Paleozoic ones, but the Recent type has gone through completely different stages of development. They were not preserved in a latent stage of life, but have probably gone through an immensely great number of cell divisions. If it were not for the phenomenon of circular migration, which is peculiar to both the Paleozoic and the Recent type, it would be very difficult to find a relationship between the two.

Comparing them biochemically, we find very distinct differences. Our 3 Paleozoic strains show almost identical biochemical properties. The strain found by Kienholz lost all its saccharolytic characteristics, which its Paleozoic relatives had. The only new characteristic is their ability to liquefy gelatine.

Beyond this fact, a comparison over such long periods of time gives the following results: (1) The paleozoic strains of the *Bacillus circulans* have quite a lot more biochemical characteristics than those described in the preceding 70

FIGURE 1 (Top). Bacterium in the center of a thin section of a thickness of 15 μ, enlargement 3600:1.
FIGURE 2 (Bottom). *Bacillus circulans* from the Zechstein salt, enlargement 950:1.

years. (2) It seems that the long, latent life of about 180 million years has brought about no loss of characteristics for the Paleozoic species. (3) A loss of characteristics was proved, however, for the Recent representatives of *Bacillus circulans*, which have gone through a vast number of cell divisions. (4) Although the differences in biochemical behavior are very distinct, there is an

absolute accord in the morphological characteristics between the Paleozoic and the Recent representatives of the *Bacillus circulans*. (5) This leads us to believe that the genes responsible for the morphological differentiation are much more stable than those leading to the biochemical characteristics of a species. There is no doubt that this goes for other species as well, but at the moment we are only considering *Bacillus circulans*.

We could not have made these statements, if this species did not have the characteristic of migration. Relying only on the peripherally whipped bacterium and its micromorphology, as with *Bacillus circulans*, any definite determination would have been impossible. Even biochemical investigations and comparisons would lead nowhere, because there are great doubts concerning the question of whether or not characteristics of the Paleozoic germs came to a

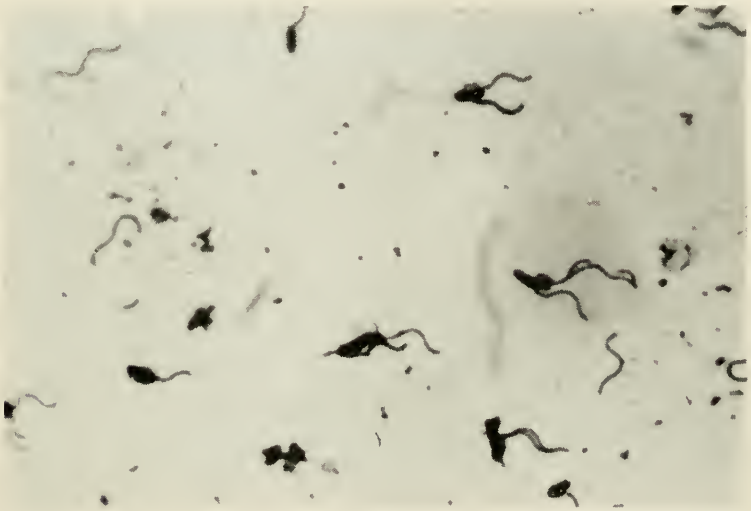

FIGURE 3. Bacterial strain VIII/D from the Middle-Devonian, enlargement 1200:1.

further development in Recent types. Therefore, it should be very difficult to show the identity of other types of bacteria, isolated in mineral salts, with Recent species beyond the probable affinity to a species.

If all of these considerations were true, then it should be possible to cultivate bacteria from salts of even older origin than those of the Permian age, provided that these salts come from regions where no tectonic movement had occurred since their original formation. These experiments had positive results. In FIGURE 3 are shown bacteria from Middle-Devonian salts from Saskatchewan. All in all we achieved the isolation of six different species from Middle-Devonian salts. We were also fortunate to be able to isolate three different species from Silurian salts, coming from Meyers, New York (FIGURE 4).

Because it was possible to cultivate 2 bacterial species out of Precambrian salt specimens from Irkutsk, we have reached a sort of absolute level of research. It is highly improbable that scientists will find even older individual life than Precambrian, already approximately 650 million years old.

In FIGURE 5 is shown a bacterium from the Precambrian salt after silver

impregnation by the method of Zettnow. Both bacteria found in the Precambrian seem to be closely related to each other.

A list of biochemical data of the isolated germs from paleozoic salts is given in TABLE 1.

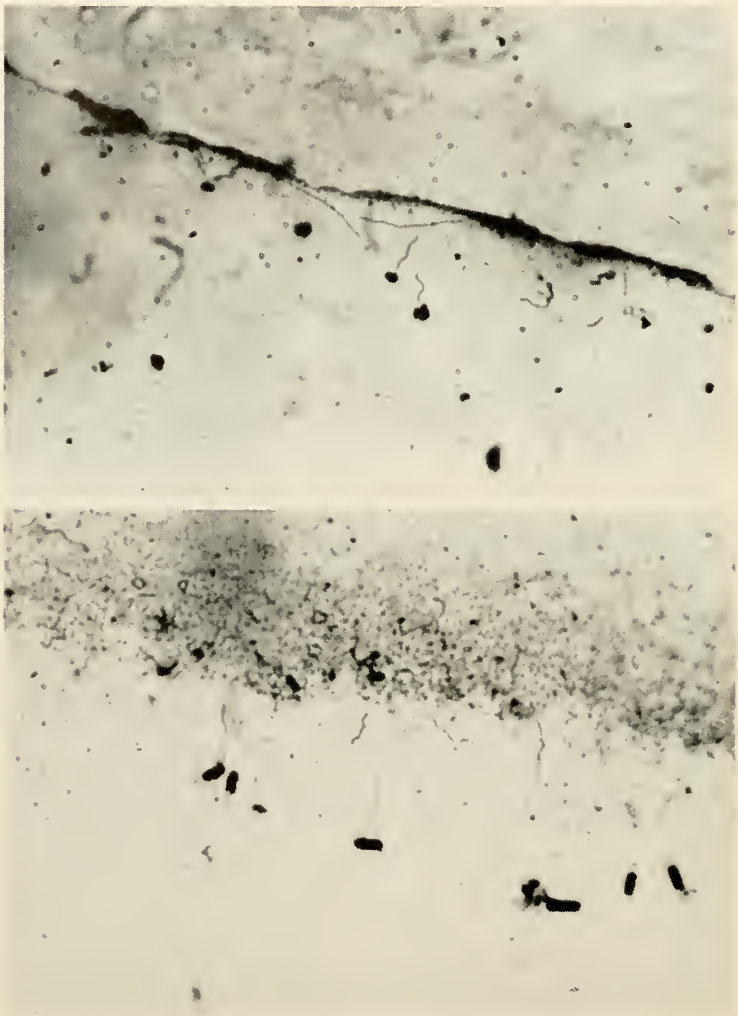

FIGURE 4 (Top). Bacterium from the Silurian, strain XV/1, enlargement 1200:1.
FIGURE 5 (Bottom). Bacterium from the Precambrian salt, strain XXX/1, enlargement 1200:1. (The pictured bacteria are probably the oldest known living organisms with their approximate age of 650 million years.)

I have not yet examined salts from the Carboniferous. The bacteria from the Precambrian, Silurian, and some from the Devonian show only few biochemical properties. The "younger" these germs are, the more they are able to perform biochemically, only to lose this ability in later life, as shown in the comparison

TABLE 1

MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERS OF PALEOZOIC BACTERIA

Origin (age)	Pre-Cambrian		Silurian			Midde-Devonian						Permian (Zechstein)						
No. of strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Morphology																		
Spore forming	+	+	+	+	+	−	−	+	+	+	+	+	+	−	+	+	+	+
Motile with flagella	+	+	+	−	−	−	+	+	+	+	+	+	+	+	+	+	+	−
Gram	+	+	+	+	+	−	+	±	±	±	−	+	−	−	±	±	±	±
Physiology																		
Starch hydrolysis	+	+	+	+	+	+	−	+	+	+	−	−	+	−	+	+	+	+
Nitrate reduction	(+)	−	−	−	−	−	+	−	+	+	+	+	+	−*	−*	−	−	−
Indol production	−	−	−	−	−	−	−	−	+	−	−	−	−	+	−	−	−	−
Pigment production	−	+	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−
Gelatin liquefaction	+	+	+	+	+	(+)	(+)	−	+	(+)	(+)	−	−	+	−	−	(+)	(+)
H_2S production	+	+	+	+	+	+	+	−	+	+	+	+	+	+	(+)	+	+	+
Salt tolerance	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Methyl red test	−	−	+	+	+	−	−	−	−	−	+	−	−	−	(+)	(+)	+	−
Voges-Proskauer test	+	+	−	−	−	−	−	−	+	+	−	−	−	+	−	−	−	−
Hemolysis:	+	+	+	+	+	−	−	−	−	−	−	−	+	+	−	−	−	(+)
	α	α	β	β	β								β	β				β
Acid from:																		
Glucose	±	±	+	+	±	−	−	−	+	−	±	±	+	−	+	+	+	+
Laevulose	−	−	−	(±)	+	−	−	−	(±)	±	±	±	+	−	+	+	+	+
Sucrose	−	−	−	−	−	−	−	−	+	−	+	−	−	−	±	+	+	±
Maltose	−	−	−	−	−	−	−	±	−	+	+	−	−	−	+	−	+	+
Lactose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±	+	−	−
Raffinose-hydrate	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±	±	−	−
Rhamnose-hydrate	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
l-arabinose	−	−	−	−	−	(±)	−	−	(±)	(±)	±	−	+	−	+	−	+	+
Salicin	−	−	+	+	+	−	−	−	±	+	±	−	+	−	−	+	+	+
Inulin	−	−	−	−	−	−	−	−	±	±	−	−	−	−	−	−	±	±
Xylose	−	−	−	−	−	(±)	(±)	(±)	(±)	±	(±)	−	−	−	±	±	−	−
Trehalose	−	−	(±)	−	−	−	−	−	±	±	+	−	+	−	−	−	+	±
Dulcitol	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Inositol	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Mannitol	−	−	−	−	−	−	−	−	+	+	+	−	+	−	−	−	(±)	±

* Gas.

with *Bacillus circulans*. A final proof of my findings is now in preparation. Because it is now possible to find the bacteria in thin sections of the salts, I want to isolate each bacterium individually with a micromanipulator and let it grow in a microculture. During this process it will be kept under constant observation until it shows the germination of spores, or until it starts the first cell division after being dormant for more than 650 million years. I hope soon to be able to show this exciting moment in a motion picture film.

Other institutes are now doing research on the coenzymes and proteins of these Paleozoic bacteria.

Summary

For the first time it became possible to isolate and cultivate bacteria from Permian deposits. The methods of isolation are described in detail and the arguments, which lead to the assumption that the discovered microbes are living representatives of the oldest known individual ages, are summarized. (1) Only such salt deposits were investigated, which showed indications of being of primary genesis. (2) From these salt specimens pollen grains were isolated, which served as characteristic fossils for establishing the age of the deposit. (3) None of the geological prerequisites, such as tectonics, orogenesis, and geothermic gradients, proved to be contrary to the findings. (4) The method of isolation, as well as the precautionary measures and the controlling experiments, are discussed in detail. (5) The results of dehydration at low temperatures and the reversible method of denaturation by salification are pointed out. (6) The embedded bacteria are shown optically in thin sections of the examined salts.

Studies on other salt deposits were made, and living bacteria were isolated from salt deposits from the Middle-Devonian, the Silurian, and the Precambrian. A comparison of the biological characteristics of the Paleozoic germs with Recent bacteria was carried out.

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FOSSIL PROTOBIONTA AND THEIR OCCURRENCE

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The body of paleontological research consists essentially of knowledge of organisms having a preservable skeleton. Therefore, one cannot expect that the oldest organisms will have been preserved. Amino acids, but not the organisms themselves, have been found in lower Precambrian limestone. The earliest phase—origin of the basic building blocks, their development into primitive organisms, as well as the primary evolutionary forms—is beyond the scope of present paleontological basic research.

By the Cambrian (600 million years ago) many highly diversified skeleton-forming organisms had developed. Therefore these organisms are within the focus of paleontological research. At that time life had already attained a considerable level of evolution, with almost all the invertebrate families present. In spite of this fact, our knowledge of fossil protobionta is still incomplete; it is quite possible that a whole array of different organisms is still unknown. However, the following groups may be classified: (1) bacteria; (2) protobionta with a preservable outer skeleton of organic material; (3) protobionta with an outer skeleton of inorganic material; and (4) sporomorpha and spores.

(1) The presence of fossil bacteria has been revealed by different techniques. Since 1960 H. Dombrowsky's observations regarding bacteria from paleozoic salt deposits have shown, however, that paleobacteriology is still in its infancy. The role of anaerobic or sulfate-reducing bacteria in the development of life lies outside the field of paleontology.

(2) In the group of protobionta with preservable outer skeleton those composed of organic material are believed to be the older species. In this group only the already relatively complicated structures are known, among them such Dinoflagellate forms as Chitinozoa and Hystrichosphaeridae. Fossil Hystrichosphaeridae with shells of material resembling cutin, which developed in the Cambrian, cannot be readily distinguished from later forms. In fact, their close resemblance to spores of fungi (Zygospores) is noteworthy. Skeletons of fossil protobionta show remarkable resistance under suitable conditions of fossilization.

(3) Microorganisms of simple structure, such as Archaemonadidae, Silicoflagellatae, Diatoms, and Radiolaria, deposit silica in their shells. Foraminifers built shells by cementing stone particles with calcite, or occasionally of calcite alone. In rare instances they employed chitin-like substances. In contrast, Dinoflagellata *sensu lato*, undoubtedly represent a later phase in evolution and offer a vast amount of material for research. Coccolithophoridean skeletons show small calcite particles which may form rock strata under favorable conditions of fossilization.

(4) The terms sporomorpha and spores imply a state of reproduction considerably different from the fully developed organism. Their resistant outer layer (exine) composed of sporopollinin ensures preservation under favorable conditions. Sporomorpha and spores result from reduction division, which

may take place in a limited number of ways of which the following are known: (a) tetrahedral—resulting in trilete spores; (b) abortive—same as (a) except that only one spore develops fully; and (c) rhomboidal—resulting in monolet spores.

The most common form, tetrahedral meiotic division, necessarily results in trilate spores with three-sided symmetry. Some of the organized elements from carbonaceous chondrites described by G. Claus and B. Nagy appear to resemble such trilete spores. It should be emphasized that in *Circulina sp.* of the upper Trias—although similar to the above-mentioned organized elements—the tetradic (trilete) mark is not evident.

The existence of truly multicellular organisms is allied to the function of reduction division; otherwise, polyploidy would result. We know, however, that in terrestrial conditions tetraploidy may cause sterility.

A so-called *Dauerstadium* is usually linked with the formation of spores or sporomorpha. Protecting the plasma is a strong hull which consists of the exine or the sporoderm and the intine. The sporoderm is made up of sporopollinin, a terpene derivative, which can become soluble in the presence of oxygen but is very resistant and capable of fossilization in the absence of oxygen when minerals are present. It can bind iodine, bromine, and chlorine. During coal and peat formation, where bacterial activity is reduced because of the acidic environment, spore preservation is enhanced. Under suitable conditions rich deposits of sporoderms may occur (fimminit).

The *Dauerstadium* allows the organisms to live through highly unfavorable periods—an especially important consideration if they are subjected to wide variations in environment, such as extremes of cold or drought.

The majority of skeleton-forming fossil protobionta lived in the oceans of primeval times, although sporomorpha and spores form in marine, limnetic, and terrestrial biotopes. Adequate preservation of all residues of organisms depends upon the particular fossilization process. Skeleton-forming protobionta have been described mainly in marine sediments. Sporomorpha and spores occur in both marine and limnetic deposits and very exceptionally in terrestrial deposits. Quick embedding in all instances is favorable to the preservation of fossils. Concentration of residues depends upon the following: (1) mass of the organisms; (2) mass of the inorganic material involved in the sedimentation process; (3) resistance of the organic substance; and (4) destructive factors before and during fossilization (diagenesis). Granting factors 1 and 3 even relatively small organisms may affect the mineral composition of rocks, *e.g.*, enrichment of Coccolithophoridae will affect the lime content of marl.

The rule for concentration of fossil spores or pollen is: 20,000 to 40,000 exines per gram represents the accumulation of normal flora in a given sediment. A larger number per gram is positive proof of autochthonous flora. However, the occurrence of fewer and scattered exines points to contamination.

Minute fossilia, except for spores and pollen, are found principally in the marine biotope. An aquatic medium is usually necessary for preservation and fossilization of such organisms.

Theories of extraterrestrial life are based on existing conditions on earth. Each organism, wherever it occurs, must fulfill certain regular functions in line with a given physical law in order to remain alive. The most simple organism

is a single cell whose plasma is protected by a resistant cell wall. The stronger the wall, the more likely it is to have perforations (pores or marks) which allow the plasma to come in contact with the surrounding environment. Only the most primitive organisms reproduce by simple cell division. All higher forms of life depend upon sporomorpha to survive hostile periods and to reproduce. According to G. Erdtmann, sporomorpha in the broadest sense are spores whose position in the system in unknown. Although they do not always exhibit trilete markings, their three-sided symmetry may indicate that reduction division has taken place. One of the criteria of survival is that during the *Dauerstadium* substances needed to maintain life be reduced to a minimum.

The basic importance of reduction division (meiosis) to genetic propagation has already been mentioned. It should also be noted that tetrahedral meiotic division results in spores with three-sided symmetry. However, three-sided symmetry is the rule with the widespread trilete spores and the exception with protobionta and, in fact, with the total animal and plant kingdom. From the above definition of sporomorpha, it is reasonable to apply this term to the organized elements of extraterrestrial life having three-sided symmetry, *i.e.*, the triporate or trilete forms. This does not specify their position in the system, nor does it suggest that an organism similar to an organized element is equivalent to it. The possibility that organized elements with three-sided symmetry result from reduction division may not be excluded in the case of extraterrestrial life. The function of such division is also a possibility in an extraterrestrial environment.

Residues of extraterrestrial organisms could not be preserved at all except for a process which may be called fossilization. The following rule holds in all circumstances: the more residues, the more favorable were the conditions of fossilization. This requirement is undoubtedly best fulfilled on earth in the aquatic medium which offers conditions for suitable embedding.

The organized elements with resistant exines or organic material must have depended on the functions of protein molecules. In this event the extraterrestrial temperature range of the organized elements' environment would have to be similar to that on the earth.

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STUDIES IN EXPERIMENTAL ORGANIC COSMOCHEMISTRY

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The four most abundant elements in the universe, with the exception of the noble gases, are hydrogen, oxygen, carbon and nitrogen,[1-3] which are also precisely the four major constituent elements of organic compounds and of living matter. Indeed, as has recently been said, "the composition of living matter turns out to be a better sample of the universe than the dead earth."[4]

These four elements exist mainly as atoms and diatomical combinations, such as CN, CH, C_2, CO, NH, and OH, in the atmospheres of relatively cool stars,[1,5] including the sun,[6] and in interstellar or circumstellar space.[5,7,8] They also exist as di- and polyatomic combinations in planets,[9-11] comets,[12-14] and meteorites.[15-25] Thus, simple and complex compounds of carbon are found widely distributed in the universe. In principle, these compounds will exist wherever the prevailing temperatures are compatible with the stability of the bonds between carbon and the other elements. If the carbon containing diatomic combinations, CN, CH, C_2, and CO, are considered, it is observed that their thermal stability ranges from the low temperatures of interstellar space to the relatively high temperatures prevailing above the surface of stars. In fact, such diatomical combinations have been detected in the atmospheres of supergiant carbon stars at temperatures of the order of 6000° K. at which some of the most thermally stable oxides, namely titanium and zirconium oxides, are dissociated into their metallic ions.[1]

Observations bearing on the distribution of simple and complex compounds of carbon in cosmic bodies and on the natural formation of these compounds, form part of a space science which may be called "organic cosmochemistry."

Because of the limited observational data so far obtained and the importance of the fundamental problems involved,[26-28] attempts have been made to follow an experimental approach in this study. As a result of the initial experiments of Garrison *et al.*,[29] Miller,[30,31] and the more recent ones carried out in this[32] and other laboratories,[33] it has become apparent that processes of organic synthesis which may have occurred in the primitive Earth's atmosphere, or may be occurring in certain cosmic bodies such as comets, can be partially reproduced in the laboratory. These experiments have opened a field of investigation for which the name "experimental organic cosmochemistry" is proposed.

Models for Organic Synthesis

Any experimental approach to duplicate an incompletely known natural process requires the formulation of assumptions about the experimental model to be used. It is recognized that it would be difficult to determine with certainty all the conditions applying to a cosmic model for organic synthesis. However, if it is understood that organic reactions pathways are determined by rather general laws, then it becomes possible to obtain significant knowledge about natural organic synthetic processes even with only partially complete models.

We have focused our attention on a cometary model[32] primarily because

comets are supposed to contain large amounts of reactive carbon compounds and because it is considered that their composition reflects approximately the composition of the primordial solar nebula and protoplanets.[34] Indeed, a recent model for the protoplanets of the solar system,[35] as suggested by Fowler, is almost identical to a cometary model proposed some time ago by Whipple[36] and recently revised by the same author.[37] On the basis of this physical and chemical similarity it is reasonable to assume that the chemical processes which occur in comets by the action of solar radiation, when these bodies are at distances of less than 3 A.U. from the sun may have also occurred, but in a much larger scale in the Earth protoplanet.

Furthermore, it is possible that the conditions for organic synthesis were quite favorable during the transformation of the gravitationally undifferentiated protoplanet into the primitive planet. This would result from the mixing of the reactive precursors of organic compounds with inorganic particles, such as silicate and metallic grains, which could have acted as surface catalysts. Due to the low density of the synthesized organic compounds, these compounds would migrate toward the exterior of the planet during the process of gravitational differentiation. The nonvolatile combinations, ionic or high molecular weight compounds, would accumulate on the surface of Earth, whereas the gases and the compounds volatile at the prevailing temperatures, would be evaporated into the outer region of the solar system where comets originate presently.[38] The difficult problem of the escape of gases and volatile compounds from primitive planetary atmospheres has been discussed mainly by Suess[39] and Urey.[9,40]

With regard to the composition of the model, it is known that the spectra of comets show fluorescence emission bands corresponding to the molecules or radicals CN, CH, CH_2, C_2, C_3, NH, NH_2, and OH, to the radical ions CH^+, OH^+, CO^+, N_2^+, and CO_2^+ and to the atoms of Fe, Ni, Cr, and other elements.[12-14,40,41] These emission bands are observed in the heads or in the tails of comets when these bodies are at less than 3 A.U. from the sun. The band corresponding to the CN radical is generally the first emission band to appear on the tails of comets during the travel of these bodies toward the sun, and it is also the band with the largest degree of extension into the comets' heads followed in intensity by the C_2 (Swan) and C_3 bands.

The above compounds exist in the nuclei of comets either as frozen free radicals,[42-44] or as "ices"[36,37] (or crystalline clathrate type hydrates[45]) of molecules, which are vaporized and dissociated into radicals by the solar radiation. In general, it is considered that the parent molecules of CN, NH_2, and OH are hydrogen, cyanide or cyanogen, ammonia, and water, respectively. The parent molecules of the carbon radicals are supposed to be methane, acetylene, and other hydrocarbons. Therefore, a simplified experimental model could be made of hydrogen cyanide, ammonia, and water. A slightly more complex model could contain, in addition, cyanogen, acetylene, carbon monoxide, carbon suboxide, and other compounds. There are certain relations between this model and the 2 atmospheric models which have been studied previously, namely, the "primitive planetary atmosphere" model,[28] and the "volcanic atmosphere" model.[46,47] These models should not be considered as providing

alternative, but rather complementary, approaches to the study of the formation of organic compounds on the abiotic earth.[48,49] In fact, they represent progressive stages in the development of the earth. An important condition which is common to all of these models is that they are essentially reducing or at least nonoxidizing in character, of which we have cosmochemical[50,51] and geochemical[52] evidence. Additional evidence for the reducing conditions of the atmosphere of magmatic origin is provided by the fact that the terrestrial rate of oxygen production by photolysis of water is less than the rate of volcanic carbon monoxide production.[53]

Energy Sources

Several sources of energy were available for the synthesis of organic compounds during the transformation of the Earth from protoplanet into planet. The main source was, of course, the sun providing ultraviolet light and ionizing radiation at a rate 10^7 times as high as that observed at the present time.[35] A second source was the earth itself with its natural radioactivity[47,54] and the heat derived from gravitational compression and radioactivity.[47]

However, I wish to emphasize that if, as indicated above, some of the primordial constituents of the earth protoplanet were radicals or reactive chemical compounds, then organic synthesis could have occurred spontaneously at relatively low temperatures during the melting of the protoplanetary ices in the absence of highly activating forms of energy. It is surmised that these spontaneous syntheses were responsible for the formation of substantial amounts of organic and biochemical compounds. Furthermore, due to the relatively low prevailing temperatures and the reducing conditions of the protoplanetary environment, the compounds thus formed would have been preserved for very long times.

During the further stages of geological development additional sources of energy were available on the surface and atmosphere of the earth. It is likely that in addition to ultraviolet light and ionizing radiation, electric discharges and the heat from plutonic processes contributed also to the formation of organic compounds.

Synthesis of Amino Acids and Hydroxy Acids

The synthesis of amino acids and hydroxy acids under possible primitive Earth conditions has been accomplished by several investigators who used electrical discharges, ultraviolet light, and ionizing radiation. Moreover, when some of the reactive carbon compounds detected in comets were used, the formation of amino acids and hydroxy acids was observed to occur spontaneously at moderate temperatures.

(*1*) *By electric discharges.* In particular, Loeb,[55] Miller,[56-58] Hough and Rogers,[59] Abelson,[60] Heyns *et al.*,[61] Pavlovskaya and Pasynskii,[62] Franck,[63] and Oró and Engberg,[64] applied silent and spark discharges to aqueous mixtures of totally reduced (CH_4, NH_3) or partially oxidized carbon and nitrogen compounds. The products obtained include the amino acids glycine, alanine, β-alanine, sarcosine, α-amino-n-butyric acid, α-aminoisobutyric acid, glutamic acid, aspartic acid, valine, and leucines, and the hydroxy acids glycolic, lactic, succinic and hydroxybutyric.

The yield of total amino acids in these experiments was usually less than 5 per cent of the theoretical and the relative yield of each individual amino acid was approximately inversely proportional to the number of carbon atoms in the molecule. When methane was used the amino acids formed contained almost exclusively from 2 to 4 carbon atoms. When methane was replaced partially by ethane or higher hydrocarbons, valine and leucines were formed in addition to the other amino acids.[64] Aside from these and other small variations, the overall qualitative composition of amino acids obtained in different experiments by several investigators is very similar, if not identical.

Although the mechanisms of synthesis have not been studied in detail, it seems that the first phase of one of the possible mechanisms involves the formation of radicals which recombine to form many compounds including hydrogen cyanide, aldehydes, amines, nitriles, and aliphatic hydrocarbons. The primary formation of methyl radicals has been suggested by the experiments of Franck,[63] with either isooctane or methanol in the presence of ammonia and water. When methanol was used, the observed amino acid yield was increased more than 50 per cent as compared to that obtained from methane. This is in line with the fact that 20 per cent less energy is required to form a methyl radical from methanol than from methane.[65] That methyl radicals are formed can also be deduced from a study of the products formed by the action of electrical discharges upon methane,[66] and upon mixtures of methane and ammonia.[67] Because of the high thermal stability of the triple bonded radical C_2H derived from acetylene[68] one would expect that this radical should act as a trap for other radicals giving rise to the formation of methyl, ethyl, vinyl, and ethynyl derivatives of acetylene. In fact, these compounds were precisely the products identified in the aforementioned experiments.[66] In a similar manner the nitrile analogues of the above compounds, namely, acetonitrile, propionitrile, acrylonitrile, and cyanogen should also be expected to be formed from the thermally stable triple bonded CN radical derived from hydrogen cyanide. And in fact some of these compounds were detected by Sagan and Miller[67] in model experiments with Jovian atmospheres.

The second phase of this mechanism of amino acid synthesis does not seem to occur in the gas phase, but rather in aqueous solution. It involves a Strecker condensation of aldehydes with hydrogen cyanide in the presence of ammonia.[56,57] The resulting α-amino acid nitriles which can be detected during the first hours[64] are progressively hydrolyzed into the corresponding amides and acids.

In addition to α-amino nitriles, β-aminonitriles have also been detected in the reaction product. In particular, β-aminopropionitrile which is a precursor of β-alanine and of pyrimidines has been detected by paper chromatography.[64] This nitrile gives a characteristic green derivative when it reacts with ninhydrin.

An alternative mechanism for the formation of amino acids in the experiments with electrical discharges is suggested by the presence in the reaction product of polymers of hydrogen cyanide which are known to be converted into amino acids (section (4)).

(*2*) *By ultraviolet light.* Studies on the photochemical synthesis of amino acids in aqueous systems were reported some time ago by several investigators. Baudisch[69] claimed the formation of amino acids from potassium nitrite, carbon

monoxide, and ferric chloride. Dhar and Mukherjee observed the formation of glycine from glycol, and of arginine from glucose. Nitrates were used as a source of nitrogen and titanium dioxide or ferrous sulfate as catalyst. More recently, Bahadur *et al.*,[71-73] also with the use of nitrates and ferric chloride have observed the formation of serine, aspartic acid, and asparagine from paraformaldehyde. Other amino acids formed in these experiments as detected by paper chromatography (without previous separation from other ninhydrin positive compounds by ion exchange) were glycine, alanine, and threonine and in particular C_5 and C_6 amino acids which are formed with difficulty in the experiments with electric discharges. These include valine, ornithine, arginine, proline, glutamic acid, histidine, leucine, isoleucine, and lysine. The above amino acids comprise essentially all the building blocks of proteins with the exception of the aromatic and sulfur containing amino acids.

It would be difficult to visualize the presence of nitrates in a primitive Earth environment or in a cosmic body. However, the nitrate ion *per se* should not be considered as the immediate precursor of the amino group of amino acids. It is clear that the nitrates must be reduced at the expense of the oxidation of part of the carbon compounds, such as formaldehyde, which are always present in a large excess in these experiments. In fact it is known that in the presence of metallic ions and partially reduced carbon compounds, nitrates,[74] and nitrites[75] are rapidly reduced by the action of light to some nitrogen compound of a lower oxidation level.

Hydroxylamine was suggested by Oró *et al.*,[76] as one of the nitrogen compounds which may be involved more directly in the formation of amino acids. In fact, this could also be deduced from the synthesis of amino acids from formhydroxamic acid and formaldehyde by Baly *et al.*[77] The preferred participation of hydroxylamine in the comparative photochemical synthesis of amino acids from formaldehyde and either nitrates, nitrites, hydroxylamine hydrochloride, or ammonium chloride has been confirmed in our laboratory.[78] The same conclusion has been arrived at by Ferrari[79,80] from similar comparative photochemical experiments but with more complex carbon compounds instead of formaldehyde.

From a conceptual point of view, ammonia and ammonium chloride are perhaps the most logical precursors of the amino group of amino acids in a primitive Earth environment. Experiments carried out by Miller,[58] and by Groth and von Weyssenhoff,[81,82] have given evidence that the amino acids glycine and alanine can be synthesized by irradiating with short wave ultraviolet light (Krypton 1165, 1235 Å, Xenon 1295, 1470 Å, and mercury vapor 1850 Å), aqueous mixtures containing ammonia as the nitrogen source and either methane or ethane as the carbon source. A higher amino acid yield was obtained when ethane was used instead of methane. On exposing a mixture of methane, ammonia, carbon monoxide, and water to the radiation of a hydrogen lamp through a thin LiF window, Terenin[83] observed the formation of the alanines and of several other amino acids.

On the basis of the experimental quantum yields obtained by Groth and recent theories of solar evolution, Sagan[84] has calculated that the synthesized organic compounds in the contemporary atmospheres of the Jovian planets,

and in the primitive reducing atmospheres of the terrestrial planets is of the order of 1000 g. per cm.^{2} of planetary surface.

Experiments carried out by Pavlovskaya and Pasynskii[62] and also in this laboratory,[78] have shown that several amino acids can be synthesized by irradiation with ultraviolet light of aqueous mixtures containing formaldehyde and ammonium salts. The synthesized amino acids, which were separated by ion exchange resins and detected by paper chromatography, include glycine, serine, alanine, and glutamic acid. The Russian investigators found also valine, isoleucine, phenylalanine, and basic amino acids.

With regard to the mechanism of photochemical synthesis of amino acids it has been pointed out previously, that the amino group may be derived from either ammonia or hydroxylamine. However, very little is known about the mechanism of formation of the hydrocarbon chain. Perhaps monosaccharides of 2 to 6 carbons are first formed photochemically and then transformed by redox processes into α-keto acids which upon transamination are converted into amino acids.

That hexoses and hydroxy acids or their lactides are formed by the irradiation of formaldehyde solutions with ultraviolet light was shown by Baly[85] and Irvine and Francis.[86] Moreover, when the syrupy product, thus obtained, was heated with a trace of acid at 100° C. it was found to resinify into a polymeric material. This suggested the additional presence in the reaction product of furfuryl alcohols or polyhydroxyphenols. If phenolic compounds were formed from formaldehyde these compounds may be the precursors of the aromatic amino acids.

That hydroxy acids and also keto acids and dicarboxylic acids react photochemically with ammonia, ammonium salts, or other nitrogen compounds to produce amino acids has been shown by Deschreider[87] and by Cultrera and Ferrari.[88,89] Nonphotochemical transamination reactions are also well known.

The synthesis of amino acids containing straight chains with 5 or 6 carbon atoms could be explained by the intermediate formation of C_5 or C_6 monosaccharides, respectively. These compounds become stabilized by the formation of furanose and pyranose cyclic structures, stopping the growth of the monosaccharide chain by preventing the condensation of additional formaldehyde molecules. Therefore, essentially no monosaccharides and amino acids with a linear chain of more than 6 carbon atoms are formed. Branched chain amino acids could be derived from branched chain monosaccharides such as dendroketose.

It is of interest that the same maximal amino acid chain length is observed in these photochemical experiments as in the experiments with electric discharges. Whereas in the present case the maximal chain length may be determined by the stability of cyclic structures, in the experiments with electrical discharges it may be the result of the decreased probability of formation of long chains by processes of methyl radical recombination.

(3) By ionizing radiations. The synthesis of organic compounds by ionizing radiation was reviewed by Swallow.[54] After the pioneering investigations in this area by Garrison *et al.*,[29] the formation of amino acids by the action of ionizing radiations has been studied by several investigators. Hasselstrom *et al.*,[90]

obtained glycine, aspartic acid and possibly diaminosuccinic by irradiating with β-rays an aqueous solution of ammonium acetate. Paschke *et al.*,[91] irradiated solid ammonium carbonate with the γ-rays from a cobalt-60 source and obtained glycine, 2 other ninhydrin-positive compounds, 1 of which was tentatively identified as alanine, and ammonium formate.

It is known that formic acid and simple aldehydes are formed by the action of ionizing radiation over aqueous solutions of carbonic acid.[29,92] It is also known the glycolic acid is produced by the irradiation of formic acid.[93] Therefore, it is conceivable that glycine and other amino acids could also be obtained by the irradiation of aqueous solutions of ammonium carbonate.

Although from the above experiments it is evident that amino acids can be synthesized from partially oxidized compounds such as ammonium carbonate, it would seem more logical, on the basis of theoretical considerations,[31] to study the irradiation of aqueous mixtures of reduced carbon and nitrogen compounds, such as methane and ammonia. This has been done by Dose *et al.*,[94,95] and a larger number of amino acids and bases have thus been obtained. More recently, Calvin[96] and Palm and Calvin[97] have irradiated mixtures containing C^{14}-methane, ammonia and water, among other compounds, with 5 MeV electrons and have obtained a number of amino acids including glycine, alanine, and aspartic acid. Radiochemical and nonradiochemical mechanisms of synthesis may be involved in this case because hydrogen cyanide, which is known to condense into products which yield amino acids, was also formed in substantial amounts in these experiments.

Apart from these amino acid syntheses, it may be added that the γ-irradiation of mixtures of carbon dioxide and ethylene at room temperature yields significant amounts of long chain carboxylic acids containing as many as 40 carbon atoms.[98] Also, high energy proton or electron irradiation of methane, ammonia, and water at 77° K., in a simulated cometary model, yields a number of organic compounds.[99]

(*4*) *From reactive precursors.* As pointed out earlier it is known from astronomical observations that in the atmospheres of carbon stars, very reactive diatomic combinations of carbon, nitrogen, oxygen and hydrogen are formed. These combinations are presumed to diffuse out and eventually become part of interstellar matter, cosmic bodies and protoplanets, being converted in the process into simple but reactive compounds. These may include hydrogen cyanide, acetylene, carbon monoxide, formaldehyde, acetaldehyde, ammonia, hydrazine, and hydroxylamine among others. Some of these compounds have also been produced in the laboratory from aqueous ammonia-methane mixtures.

Thus, it was considered of interest to discover whether some of these compounds are sufficiently reactive to yield amino acids, and other biochemical compounds in the absence of electrical discharges, ultraviolet light, or ionizing radiation.

It was first shown in our laboratory[76] that aqueous mixtures of formaldehyde and hydroxylamine hydrochloride at moderate temperatures and under slightly acidic conditions yield large amounts of glycine and smaller amounts of alanine, β-alanine, serine, threonine, and aspartic acid, the last 3 having been only identified by paper chromatography. Amino acid amides, glycinamide in

particular, were found as intermediates, and formic, lactic, and glycolic acids as side products.

It was found[76] that the mechanism of synthesis involves the initial formation of formaldoxime and its dehydration into hydrogen cyanide. Strecker and cyanohydrin condensations yield nitriles which are hydrolyzed first into amides and then into acids. Condensation of formaldehyde with glycinamide is presumed to yield serinamide which can be converted into serine and alanine.[100] A similar formation of serine and threonine involving aldol type condensations of formaldehyde and acetaldehyde with methylene-activated glycine derivatives, such as glycine chelates or polyglycine, was also shown by Akabori *et al.*[101-103] It may be added here that when the formaldehyde-hydroxylamine hydrochloride mixtures were made slightly basic, pyridines were also formed in addition to amino acids.

A subsequent study in our laboratory of the products formed by refluxing aqueous mixtures of formaldehyde and hydrazine revealed the formation of glycine, valine, and lysine as detected by paper chromatography.[104] The mechanism of lysine formation is thought to involve the intermediate formation of hexoses and their reduction-oxidation by hydrazine. It is well known that hexoses are formed from formaldehyde by base catalysis, that hydrazine is formed by the action of electric discharges on ammonia,[105] and that hydrazines can be both reducing and oxidizing reactants.

As mentioned earlier, 3 of the major compounds which are supposed to exist in comets are hydrogen cyanide, ammonia, and water. For this reason, a study of the products formed with mixtures of these 3 compounds was subsequently undertaken in our laboratory. It was observed that the amino acids glycine, alanine, and aspartic acid, and other biochemical compounds were formed spontaneously at moderate temperatures in these mixtures.[106] Oligomers of hydrogen cyanide are presumed to be the intermediates of the amino acids. In fact, tetrameric hydrogen cyanide was observed to be one of the first products formed in the above mixtures,[107] and it is known that tetrameric hydrogen cyanide can be hydrolytically degraded into glycine.[108,109] Two possible degradation mechanisms of tetrameric hydrogen cyanide into glycine have been suggested by Loquin[110] and Ruske.[111] Other mechanisms involving processes of reductive deamination can be postulated for the formation of alanine and aspartic acid.

The formation of amino acids in the hydrogen cyanide-ammonia-water mixtures has been confirmed and extended by Lowe *et al.*[112] In addition to the above 3 amino acids, Lowe and co-workers have also detected the presence of β-alanine, α,β-diaminopropionic, α-aminoisobutyric, glutamic acid, arginine, leucine, and isoleucine in the reaction product. The formation of hydroxy amino acids could conceivably take place in these mixtures if aldehydes were present, because it is known that formaldehyde and acetaldehyde condense with methyleneaminoacetonitrile to form serine and threonine, respectively.[113]

It can thus be seen that, with the exception of the aromatic and sulfur containing amino acids, most of the building blocks of proteins can be synthesized nonenzymatically in aqueous sytems from very simple precursors in the absence of highly activating forms of energy.

With regard to the formation of sulfur containing amino acids, simple nonenzymatic pathways can also be visualized. Cysteine could be formed in a similar manner as serine by condensation of thioformaldehyde[114] with a methylene-activated glycine derivative, such as glycine nitrile, glycinamide, polyglycine or a metal chelate of glycine. Methionine could be formed by the addition of methyl mercaptan to acrolein, followed by the condensation of the resulting methional[115] with hydrogen cyanide and subsequent hydrolysis of the nitrile. One of the possible pathways for the synthesis of aromatic amino acids could be through monosaccharides or similar compounds obtained from formaldehyde.[86]

Synthesis of Monosaccharides

Since the early studies of Butlerow,[116] Loew,[117] and Fischer[118] it has been known that formaldehyde in aqueous solutions condenses into sugars by the action of basic catalysts. As a result of the work of Fischer[118] and others,[119,120] fructose, sorbose, xylulose, and glycolaldehyde were identified among other compounds in the formaldehyde reaction product.

Relatively recently, Mariani and Torraca[121] analyzed by two-dimensional paper chromatography the product of the base catalyzed condensation of formaldehyde and confirmed and extended the previous results. They detected the presence of the hexoses galactose, glucose, mannose, fructose and sorbose, and the pentoses arabinose, ribose, ribulose, xylose, xylulose, and lyxose in addition to 10 more unidentified monosaccharides. More recent studies by Mayer and Jäschke[122] and by Pfeil and Ruckert[123] have shown the formation of glycolaldehyde, glyceraldehyde, dihydroxyacetone and tetroses in addition to pentoses and hexoses. Dendroketose was also obtained as the product of the condensation of two moles of dihydroxyacetone.

The reaction is supposed to be initiated by the condensation of two moles of formaldehyde into glycolaldehyde which occurs at a very slow rate (induction phase).[124] This is followed by aldol condensations which lead to the formation of trioses, tetroses, pentoses, and hexoses and use up all the formaldehyde in a very short time (autocatalytic phase).[123] The overall reaction is catalyzed by calcium carbonate, calcium oxide, and other bases.

Because no attempts had been reported on the synthesis of 2-deoxypentoses, in particular 2-deoxyribose, we undertook the synthesis of this compound,[125] which is known to be one of the essential building blocks of deoxyribonucleic acid. This deoxypentose and its isomer, 2-deoxyxylose, were obtained in yields of about 5 per cent by the condensation of acetaldehyde with glyceraldehyde in aqueous systems. The reaction occurs very rapidly at room temperature when catalyzed by calcium, magnesium and other divalent metallic oxides. Results from our laboratory have shown that the reaction is also catalyzed by ammonia and other simple nitrogen bases which may have been the predominant bases in the primitive Earth's environment. In contrast to the fast reaction which divalent metallic oxides catalyze, the reaction occurs in a slow and controllable manner when ammonium hydroxide is used as catalyst. In fact, the continuous synthesis of this compound was observed for an uninterrupted period of more than 2 months. 2-Deoxyribose was also obtained

in smaller yields from aqueous solutions of formaldehyde and acetaldehyde in the presence of calcium oxide.[125]

Synthesis of Purines and Purine Intermediates

The formation of purines on the primitive Earth or in cosmic bodies poses *a priori* a difficult conceptual problem because it requires the formation of two fused heterocyclic structures, an imidazole and a pyrimidine.

In principle, there are, however, two relatively simple mechanisms or pathways which can be visualized for the formation of the purine ring. One involves condensation of a 3-carbon compound with a 1-carbon reactant to form a 4,5-disubstituted imidazole and the other involves condensation of a C_3 compound with a C_1 reactant to form a 4,5-disubstituted pyrimidine. The reaction terminates by cyclization of either the disubstituted imidazole or the disubstituted pyrimidine with another mole of the C_1 reactant.

It is known that the formation of purines in living organisms occurs by a pathway involving 4,5-disubstituted imidazole derivatives,[126] and it has also been observed that the acid degradation of adenine yields 4-aminoimidazole-5-carboxamidine as an intermediate.[127] On one hand we have the very mild conditions of enzymatic synthesis and on the other hand the very drastic conditions of acid hydrolysis, yet in both cases a 4,5-disubstituted imidazole shows as an intermediate. Shortly after these observations were made it became apparent to the author that if a nonenzymatic synthesis of purines under possible primitive Earth conditions was discovered, it may likely proceed through the imidazole pathway. The first demonstration of the spontaneous synthesis of adenine from hydrogen cyanide under conditions presumed to have existed on the primitive Earth was made relatively recently in our laboratory,[128] and in line with the above reasoning 4,5-disubstituted imidazoles were found in the reaction product as intermediates.

Adenine was synthesized in substantial amounts by heating a solution of hydrogen cyanide (1 to 15 M) in aqueous ammonia for 1 or several days at moderate temperatures (27 to 100°). The insoluble black polymer of hydrogen cyanide was removed by centrifugation and adenine was isolated from the red-brown supernatant solution by chromatographic methods. The main ultraviolet absorbing compound of the reaction product was identified as adenine by a number of different procedures including ultraviolet spectrophotometry and the melting point of its picrate derivative. The synthesis was found linear with time at room temperature, and in a typical experiment at the end of 4 days more than 100 mg. of adenine per liter of reaction mixture were obtained.[129]

Since adenine is an essential building block of nucleic acids and of the most important coenzymes, and since hydrogen cyanide, ammonia, and water are presumed to be common natural constituents of the solar system, these findings were considered to be of special significance in relation to the problem of the origin of life.

In addition to adenine several purine precursors, namely 4-aminoimidazole-5-carboxamide (AICA), 4-aminoimidazole-5-carboxamidine (AICAI), formamide, and formamidine were also found in the reaction product.[130,131] The

mechanism of adenine synthesis is supposed to be initiated by the base catalyzed polymerization of hydrogen cyanide into nitriles.[132] The role played by ammonia in this synthesis is 2-fold. It acts as a basic catalyst and it causes the ammonolysis of hydrogen cyanide into formamidine and of nitriles into amidines. One of the resulting nitriles, possibly aminomalonodinitrile, condenses either directly or after transformation to its mono- or diamidine with formamidine to form AICAI. In the last step, AICAI condenses with another mole of formamidine to yield adenine. This last step has been confirmed in a separate experiment in our laboratory.[133]

The other purines were postulated to be formed from 4-aminoimidazole-5-carboxamide.[131] Recent experiments in our laboratory have confirmed this assumption.[134] It has been observed that AICA and guanidine condense in aqueous ammonia systems to yield guanine. Moreover, when AICA is allowed to react with urea under similar conditions, guanine and xanthine are formed.[134] The formation of the 1-carbon reactants, guanidine and urea, in the absence of free oxygen, poses no special problem because compounds of this oxidation level, such as urea, were detected by Miller,[58] Berger,[99] and Palm and Calvin,[97] in their respective experiments with electric discharges, high energy protons, and high energy electrons, which were carried under reducing conditions. Other workers have also observed the formation of guanidine[112] and urea[112,135,136] from cyanides, cyanogen, or cyanates.

The above experiments on the synthesis of adenine from mixtures of hydrogen cyanide, ammonia, and water have been confirmed by Lowe *et al.*[112] who have found an additional purine, hypoxanthine, among the reaction products. A significant extension of these experiments has been carried out recently by Calvin,[96] and Palm and Calvin,[97] who have observed the formation of adenine by irradiating with 5 MeV electrons a mixture containing methane, ammonia, and water among other reduced compounds. In summary, it seems to be well established that the 4 major biological purines can be synthesized, from very simple precursors, in aqueous systems under possible primitive Earth conditions.

From a historical point of view, it should be said that at the turn of the last century, cyanogen[137] and hydrocyanic acid[138,139] were thought to be involved in the synthesis of proteins and purines in living organisms. These have since been found to be erroneous concepts. Nevertheless, it is of interest that such early ideas may apply to the abiogenic formation of these compounds. Studies on the polymerization of hydrocyanic acid were initially carried out more than 150 years ago,[132] and, therefore, it is highly probable that purines, purine intermediates, and other compounds of biological significance were synthesized in the laboratory many times since then, yet have remained unidentified until the present time. Interesting observations bearing on the synthesis of purines from hydrogen cyanide were made by Gautier,[138] Fischer,[140] Salomone,[141] and Johnson and Nicolet,[142] and they are discussed in some detail in a recent paper from our laboratory.[131] Aside from these early unsuccessful attempts on the synthesis of purines from hydrogen cyanide, it should be added that uric acid was synthesized from glycine and urea by Horbaczewski,[143] and purine from formamide and other simple compounds by Bredereck *et al.*[144,145] However, none of the biochemical purines found in nucleic acids was isolated or identified in these experiments.

Synthesis of Pyrimidines

With regard to the formation of pyrimidines it was proposed recently[32] that derivatives from the C_3 molecular species found in comets could be the source of these heterocyclic compounds. One of these C_3 derivatives is malonamide semialdimine or its isomer β-aminoacrylamide which by condensation with urea could be expected to yield uracil.

Because β-aminoacrylamide was not available to us, we tested some of the C_3 compounds which are formed in the experiments with electric discharges and which are considered to be intermediates in the formation of β-alanine. These intermediates are acrylonitrile, β-aminopropionitrile, and β-aminopropionanide. When each of these compounds was allowed to react with urea in aqueous ammonia systems at 130° C., the formation of small amounts of uracil was observed in each case.[146] Uracil was characterized by paper and ion exchange column chromatography and by ultraviolet spectrophotometry. The yields obtained from β-aminopropionanide were approximately 2 and 5 times higher than those obtained from β-aminopropionitrile and acrylonitrile, respectively. This is what would be expected if acrylonitrile has to undergo first amination into β-aminopropionitrile and this, in turn, has to undergo hydrolysis into β-aminopropionanide. Because this amide is, in fact, the dihydroderivative of β-aminoacrylamide it is obvious that the mechanism of the reaction must involve a dehydrogenation step either before or after the cyclization.

The mechanism of uracil formation involving β-aminoacrylamide or its isomer, malonamide semialdimire, is in line with the well known chemical synthesis of uracil from malic acid and urea in the presence of a strong mineral acid.[147,148] A strong mineral acid transforms malic acid into malonic semialdehyde which then condenses with urea to form uracil.[147] Also, in line with the above mechanism, it is known from the work of Bredereck *et al.*,[149] that the pyrimidine ring can be formed in good yield from either aminoacrolein or malonodialdehyde. In theory the 3 pyrimidines found in nucleic acids could conceivably be formed in aqueous systems under possible primitive earth conditions by the mechanism described above. In addition to β-aminoacrylamide yielding uracil, β-aminoacrylamidine could be expected to condense with urea into cytosine, and α-methyl-β-aminoacrylamide into thymine.

A possible pathway for the conversion of the symmetrical C_3 species of comets into β-aminoacrylamide or malonamide semialdimine is through the formation of carbon suboxide (C_3O_2), which has been suggested to exist in several cosmic bodies.[150] By the addition of hydrogen and ammonia to carbon suboxide, malonamide semialdehyde or malonamide semialdimine might be obtained. In fact, malonic acid derivatives have been obtained recently in the laboratory from carbon suboxide.[151] In addition to purines and pyrimidines, preliminary data have been obtained on the synthesis of other heterocyclic compounds and fluorescent pigments.[152]

Synthesis of Polypeptides

The early literature on the direct polymerization of unsubstituted amino acids has been previously reviewed in some detail.[153-155] Current studies on the synthesis of peptides and of polymers containing amino acids, under conditions presumed to have existed on the primitive Earth were initiated by Fox and

Middlebrook,[156] and by Akabori.[157] This work has been reviewed recently[158-161] and has been extended by other workers. As a result of these investigations a number of different pathways for the formation of polypeptides in a cosmic body or on the primitive Earth seems possible.

Polymers containing many of the amino acids found in proteins can be prepared by heating a mixture of these amino acids in the presence of an excess of dicarboxylic[162,163] or diamino amino acids.[164] This synthesis requires anhydrous conditions and heating at high temperatures for relatively short periods of time.

The formation of homo- and heteropolypeptides can occur also under aqueous conditions and at moderate temperatures, as shown by other workers. Thus, unsubstituted amino acids[161,165] and their corresponding amides[165-167] and nitriles[165,168,169] have been observed to polymerize directly, or by the action of basic (ammonia) or surface (silicates) catalysts.

A pathway which seems to be particularly good for the formation of polypeptides containing hydroxy acids is that of Akabori *et al.*,[160] which is based upon the condensation of aldehydes (also olefins) with polyglycine. The natural occurrence of this process would be quite probable because, as has been shown in our laboratory, polyglycines are readily formed from glycine in aqueous ammonia systems. Furthermore, in practically all of the abiogenic synthesis of amino acids studied, glycine has been found to be the predominant amino acid formed.

Another interesting pathway has been described recently by Schramm *et al.*[170] Polyarginine (mol. wt. 4000 to 5000) was prepared from arginine with the help of polyphosphate esters. Using the same method, polyleucine, polyvaline, and polyserine were prepared in our laboratory.[171]

In addition to the above pathways of polypeptide formation other observations have been made which indicate that peptides or polymers containing amino acids can also be obtained by the action of ultraviolet light[172] and electric discharges.[173] It should be added that some of the products obtained by thermal polymerization have the ability to form microspheres with internal structure,[174] and of displaying some catalytic activity.[159]

Finally, a very significant recent development is the isolation of polymers containing several amino acids from the reaction product of mixtures of hydrogen cyanide, ammonia, and water.[112] This is the same reaction mixture that has been shown to give rise to the formation of amino acids, purines, purine intermediates, and fluorescent pigments among other compounds. Because nitriles are formed in this system it is possible that the above polymers result from nitrile condensation reactions. Hydrogen cyanide has been suggested as an amino acid condensing agent by Calvin.[175] Hydrogen cyanide and also cyanamide (formed by combination of CN and NH_2 radicals), were probably abundant in the primordial cosmic bodies of the solar system. It is quite possible that these reactants were responsible for the formation of a number of polymeric compounds including polypeptides. In fact, it is known that unsubstituted cyanamide can be used for the synthesis of peptides.[176]

Synthesis of Polynucleotides

A possible abiogenic mechanism for the formation of a high energy phosphate compound, carbamyl phosphate, was proposed some time ago.[177] Form-

iminyl phosphate, obtained by condensation of hydrogen cyanide with monohydrogen phosphate, is suggested here as another possibility of a primitive high energy phosphate compound. More recently, Schramm *et al.*,[170] have shown that mononucleosides, mononucleotides, and polynucleotides can be synthesized at moderate temperatures, from their building monomeric blocks, with the help of polyphosphate esters. The polymers obtained seem to have the 3′,5′-phosphate diester linkages which are common to RNA and DNA. Strand complementarity, which is the principle of molecular self duplication, and autocatalytic activity, have also been observed in the above polynucleotides. The role that nucleic acids and other macromolecules may have played in directing prebiochemical evolution has been discussed in some detail by several authors.[175,178-180]

Conclusion

There is no doubt that carbon compounds exist widely distributed in the universe. Whether the more complex biochemical compounds described in this paper are present in cosmic bodies other than the earth will only be answered with certainty by space probes. Probes to the moon, Mars, and Venus are feasible and should provide valuable information about the organic and inorganic chemistry in these bodies. However, more information about the chemistry prevailing during the beginning of the solar system would be obtained by sending probes to Jupiter and to comets passing sufficiently close to the earth's orbit.

From the experimental studies presented here it is reasonable to say that if the Earth protoplanet had some of the simple organic constituents of comets, a large number of biochemical compounds (including carbohydrates, amino acids, purines, pyrimidines, and polymers containing amino acids) would have been spontaneously synthesized during the development of this cosmic body.

The formation of complex biochemical compounds from simple organic molecules is not in disagreement with thermodynamic principles. In fact, these syntheses can occur because the initial precursors (nitriles, aldehydes, olefins, etc.) are compounds of high energy content which, in their tendency to acquire lower energy states and to become stabilized, react and are *ipso facto* transformed into biochemical compounds.

The possibility that organic chemical synthesis may have occurred in interstellar dust and planetesimal bodies before the Earth was formed has also been suggested by Lederberg and Cowie[181] and Fowler, Greenstein and Hoyle.[182]

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EVALUATION OF RADIATION EFFECTS IN SPACE

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In addition to such radiation effects in space as nuclear transformations, the breakage of chemical bonds and other physical phenomena, the formation of chemical compounds by radiation synthesis is of great importance.

The aspects of the synthesis of more complicated organic compounds from simple predecessors are primarily to be discussed in this paper, because they offer clues to the evolution of organic compounds and to some degree to questions connected with studies on the origin of life.

FIGURE 1 illustrates the overlapping successive evolutions which occurred ever since the body of the Earth accreted. It is evident how the span of chemical evolution interacts with that of organic evolution, the period in which somewhere life began.

Radiation reactions of the kind discussed here are believed to have occurred on the primitive Earth. They proceeded in the past and still do on planets, their satellites, comets, meteors, and even particles of the smallness of interstellar grains. However, each type of reaction may not be applicable everywhere in space.

One of the first experiments carried out in this area of research is the discharge experiment of Miller.[1] Theories of Oparin[2] and also Urey[3] held for some time that the atmosphere of the primitive Earth was essentially composed of methane, water, ammonia, and hydrogen. When these compounds were subjected to an electrical discharge in the laboratory to simulate conditions in nature during a thunderstorm or in the proximity of corona discharges, a host of different biologically important compounds was detected in the reaction mixture. A number of the resulting compounds are listed in TABLE 1.

The most interesting species are the synthesized amino acids, which as is generally known are the building blocks of all proteins. It is significant that none of the complicated amino acids such as tryptophane or serine are produced in this way (FIGURE 2).

A number of similar confirming experiments were performed by Abelson[4] who used various mixtures of H_2, CH_4, CO_2, NH_3, N_2, O_2 and H_2O. Heyns, Walter, and Meyer[5] in addition to confirming Miller's work used also H_2S in their investigations and obtained ammonium thiocyanate, thiourea, and thioacetamide. Pavlovskaya and Passynsky[6] equally checked the discharge experiments.

Generally speaking, amino acids were obtained from reducing mixtures only containing an excess of either H_2, CH_4, CO, or NH_3. No amino acids could be obtained from an oxidative environment. The mechanism of amino acid production follows essentially the path of a Strecker synthesis. First HCN and aldehydes are obtained in the gas phase by the action of the electrical discharge, then these compounds give amino nitriles in the aqueous phase. Finally, hydrolysis leads to the amino acids.

The experiments on the reaction mechanism show that special conditions of

the electron bombardment are not necessary, which make it the more plausible that these radiation syntheses were responsible for the occurrence of amino acids in the oceans of the primitive earth.[7] It was there that they could be used to further evolution.

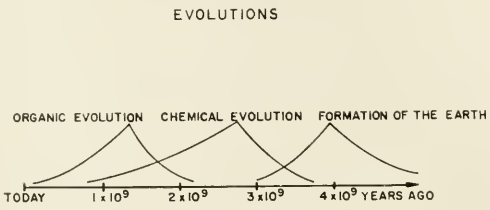

FIGURE 1.

TABLE 1

YIELDS[1] FROM SPARKING A MIXTURE OF CH_4, NH_3, H_3O, AND H_2; 710 MG. OF CARBON WAS ADDED AS CH_4

Compound	Yield (moles ($\times 10^5$))
Glycine	63
Glycolic acid	56
Sarcosine	5
Alanine	34
Lactic acid	31
N-Methylalanine	1
α-Amino-n-butyric acid	5
α-Aminoisobutyric acid	0.1
α-Hydroxybutyric acid	5
β-Alanine	15
Succinic acid	4
Aspartic acid	0.4
Glutamic acid	0.6
Iminodiacetic acid	5.5
Iminoacetic-propionic acid	1.5
Formic acid	233
Acetic acid	15
Propionic acid	13
Urea	2.0
N-Methyl urea	1.5

Because the composition of gas mixtures may be varied at will in the laboratory, conditions in the gas envelope of other planets may be approximated. Sagan and Miller[8] used hydrogen, methane and ammonia resembling the atmosphere of Jupiter. The reaction mixture contained afterward a number of different lower hydrocarbons and acetonitrile. Recent investigations on magnetic fields around Jupiter indicate that very strong ones are indeed present.[9] Therefore, Jupiter may contain currents of molten material which cause the fields. It may be deduced that the possibility of warmer zones on Jupiter has

to be reckoned with. In such zones further chemical reactions are entirely possible to yield more complicated systems.

The effects of ultraviolet light on a mixture of methane, water, and ammonia have been studied also by Miller.[10] Only a very small yield of amino acids could be obtained. Groth, and separately, Terenin, examined similar reactions.[11] Ellenbogen irradiated a reaction mixture containing FeS, NH_4Cl, H_2O, and CH_4 with ultraviolet light and observed the formation of a substance the infrared absorption spectrum of which indicated peptide bonds.[12] Apparently other similar experiments to synthesize amino acid sequences held together by peptide bonds have not yet been successful.

The effects of visible light on the formation of many different organic compounds have been examined extensively in the literature on photochemistry and need not be discussed in this paper.

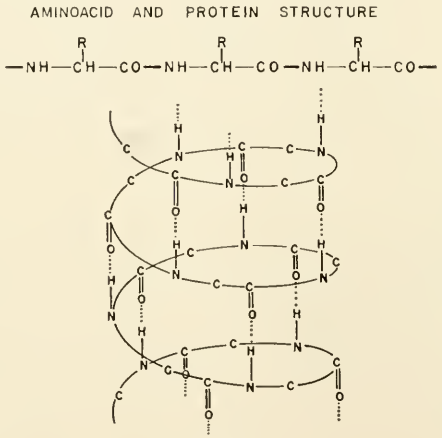

FIGURE 2.

In a number of experiments high energy particle radiation as well as x- and γ-rays were utilized. Dose and Rajewsky obtained amino acids and amines from gaseous mixtures of NH_3, N_2, H_2O, CH_4, and CO_2 with X-rays.[13] The action of 2 Mev electrons on CH_4, H_2O, and NH_3 yielded also amino acids.[14] Calvin irradiated CH_4, NH_3, H_2O, and PH_3 in the gas phase with electrons.[15] Radiochemical analysis showed the presence of small quantities of nucleic acid bases, substances which are of vast importance in genetic material (FIGURE 3).

Garrison *et al.*,[16] used 40 Mev helium ions to obtain formic acid and formaldehyde from carbon dioxide. Aqueous formic acid yielded formaldehyde and oxalic acid.[16] Hasselstrom and Henry also obtained oxalic acid from $Ca(HCO_3)_2$ and NH_4HCO_3.[17] Succinic, tricarbolic, malic, citric, and malonic acid were isolated from the reaction of aqueous acetic acid with helium ions.[18] Also, glycine and aspartic acid were the products in the 2 Mev electron bombardment of aqueous ammonium acetate.[19]

All of the previously mentioned radiation reactions occur in gaseous or liquid systems, but even reactions in the solid state may be carried out. For example, high doses of γ-rays on solid $(NH_4)_2CO_3$ yielded formic acid and glycine.[20]

In another experiment, methane, water, and ammonia were condensed to a solid icy mixture and irradiated with 12 Mev protons.[21] These conditions approximate the environment thought to exist on comet heads. Analysis of the reaction mixture indicated the presence of urea, acetamide, and acetone. The mechanism of this reaction proceeds presumably through a free radical stage. Either the radicals react with each other in the cold when radical concentrations become too high, or reaction takes place when the reaction site warms up to a higher temperature. Similar reactions may not only occur on comets and icy meteors, but also on the colder outer planets of the solar system and their satellites.

Based upon astronomical, chemical, and physical observations, it is clear that not all reactions apply to the same body in space; rather certain reactions will not occur in some instances but play a major role in others. Therefore, it is essential to consider carefully the environment of the object in

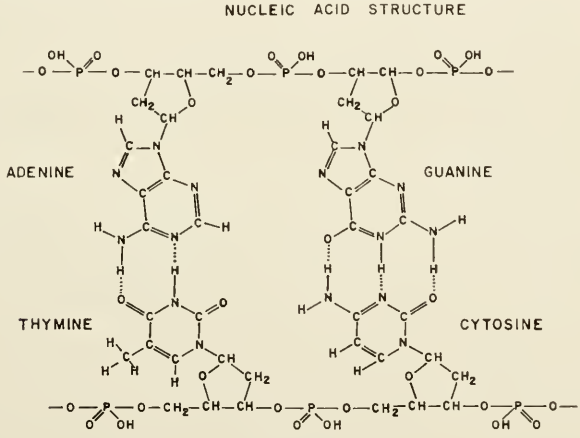

FIGURE 3.

space before assigning which reactions may predominate. Glasel bombarded solid D_2O with electrons and observed the liberation of considerable amounts of D_2.[22] Because the bond energy of the D—O bond is higher than that of the C—H bond in organic compounds, it is to be expected that over periods of time unshielded organic compounds will be destroyed in space. Therefore, organic material initially produced on cosmic grains will not remain intact. Similar destructive radiation effects will occur elsewhere; it is only there, where shielding from damaging radiation comes into play, that organic compounds will be available for further reactions.

If I may speculate a little, it may very well be that initially radiation may have been the agent which at least in part built up molecules to such systems, which finally were able to handle in a controlled manner radiation or rather light quanta. The first such successful system to use radiation energy for the synthesis of organic compounds was the beginning of photosynthesis.

In conclusion, let me say that with the aid of radiation as a form of energy, one can synthesize chemical species which are the building blocks of proteins, nucleic acids and other important biological compounds.

It is reasonable to assume that vast quantities of organic material are or were formed in space from which a fraction under special circumstances was the substrate for the evolution of life. To what extent radiation was involved is hard to assess quantitatively at the moment but the experimental evidence points to a major role in the processes leading to the creation and functioning of life.

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ABIOTIC PRODUCTION OF PRIMITIVE PROTEIN AND FORMED MICROPARTICLES*

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This presentation of results with a thermal model of origins will focus particularly on some of the properties of the microparticles which emerge therefrom. The more purely chemical aspects of the model have been treated elsewhere.[1-3] Although the significance of the particles found is much different in this context than in others, salient features of the experimentally derived scheme of origins will, however, again be reviewed here.

One of the popular assumptions which had to be abandoned before the research could proceed was the widely held belief that heating amino acids above the boiling point of water will yield only dark, unworkable products. This evaluation has been a common one in the experience of many organic and biological chemists and has been documented many times.[4] If, however, one follows the suggestions from analyses of evolution at the molecular level[5] it becomes possible simultaneously to condense thermally all of the amino acids common to protein. The products contain each of these amino acids and have many of the properties of protein. The necessary conditions are the use of a sufficient proportion of aspartic acid or lysine and an initially dry state. Heating can be at 170° for 3 hours.[6,7] The product is a light amber in color when sufficient aspartic acid is used, and, like protein, it may then be further purified by dialysis and reprecipitation by salting out the polymer from aqueous solution.

A second heresy concerns the belief that heat has generally been thought to be a reliable agent for denaturation of protein. Not so generally known is the fact that this process is "extraordinarily sensitive"[8] to the amount of water present.[9] Also, enzymes are more stable when *dry*.[10] Accordingly, the production of biologically significant polymers by heating amino acids is not precluded.

After extensive study of thermal copolymerization of simple combinations of amino acids, initial evidence that these processes could be effected simultaneously was obtained by chromatography.[6] End group assay[6,7] showed that molecular weights were above that of insulin[7] (6000 for insulin, or approximately 3000 per end group). With lysine, thermal polymers of mean molecular weight over 300,000 have been demonstrated in the ultracentrifuge.[17] The two criteria of qualitative composition and molecular weight are common to the only two textbook definitions of protein that we have found.[11,12]

Of particular interest is the fact that polymerization is aided by phosphoric acid,[6,7,13] polyphosphoric acid, or ATP,[14,15] and especially, that the minimal

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temperature for polymerization is lowered by addition of polyphosphoric acid to about 70°,[1,16] as well as its contribution to the formation of uracil.[18] The recent report of Schramm,[19] in which is claimed the polymerization of nucleotides as earlier proposed in a thermal mode,[1] occurs under similar conditions. A principal difference is Schramm's use of the ethyl ester of polyphosphoric acid.

The polyamino acids obtained are referred to as proteinoids because of molecular weight and qualitative composition, but they have in addition many properties in common with protein.[7,20]

Two properties of most interest are those of catalytic activity and morphogenicity. Catalytic activity has been found and studied for the hydrolysis of *p*-nitrophenyl acetate. This is an unnatural substrate popularly used in studies by enzyme model chemists.[21] This substrate is unstable and hydrolyzes spontaneously over a large range of pH. Histidine, which has been implicated as part of the active site of many enzymes,[20] catalyzes this hydrolysis. Simple derivatives of histidine also have this effect and some which are several times as active as histidine have been reported, *e.g.*, carbobenzoxyhistidine.[22] Proteinoids have been found to be many times as active as that, and in fact 2 of them are more than 15 times as active.

Of more interest is the fact that the catalytically active proteinoids are inactivated by heat at 100° for 20 minutes in *aqueous* buffer solution at pH 6.8. This effect has been observed in numerous repetitions and the percentage of inactivation has been found to be greatest for those proteinoids possessing the highest relative activity.

In an overall view, one interesting relationship involves the fact that catalytically powerful macromolecules are formed under almost dry conditions by heating and that this activity is later lost also by heating, but the loss occurs in aqueous solution. The significance of understanding the intimate and subtle effects of water is emphasized by this relationship. Also demonstrated is the fact that very elaborate molecules, approximately as complex as protein molecules, can be produced by a process which, although mechanistically complicated, is remarkably simple in operation.

The kind of morphogenicity observed also depends upon the intrusion of water into the system, under conditions different from those for inducing loss of catalytic activity. Acid proteinoid is typically heated in boiling water or salt solution (1 part of solid to 2000 parts of aqueous phase) for 10 seconds, the hot supernatant decanted and allowed to cool. There result, for each milligram of solid, approximately 10^7 to 10^8 microspheres of the kind shown in FIGURE 1. The fact that intrusion of water is required for formation of spherules demands a relative absence of water from the system before the macromolecules are organized into supramolecular entities.

These formed units are of interest as precell models alternative to Oparin's coacervate droplets, also studied as precell models.[23] They and derivatives are of interest also for their morphological similarity to some microfossils[24] and to formed elements found in meteorites.[25] Interesting differences between microspheres and coacervate droplets are known; for example, both the microspheres and bacteria retain their integrity on centrifugation, whereas the coacervate droplets coalesce easily.[26] The microspheres also emerge from a

continuum of conditions which can explain the origins of enzymes and of metabolism, whereas the coacervates are fabricated from such materials as gelatin and gum arabic, which arose late in evolution.

The units in FIGURE 1 are slightly less than 2.0 μ in diameter. They have the size and shape of the cocci, which have been thought of as the most primitive of the bacteria.[27]

In FIGURE 2 are microspheres which have been transferred to a solution saturated with proteinoid and containing 38 per cent calcium chloride. Two boundaries can be seen. The effects are not optical, as indicated by acentricity in some of the units. It was later learned that double boundaries could be

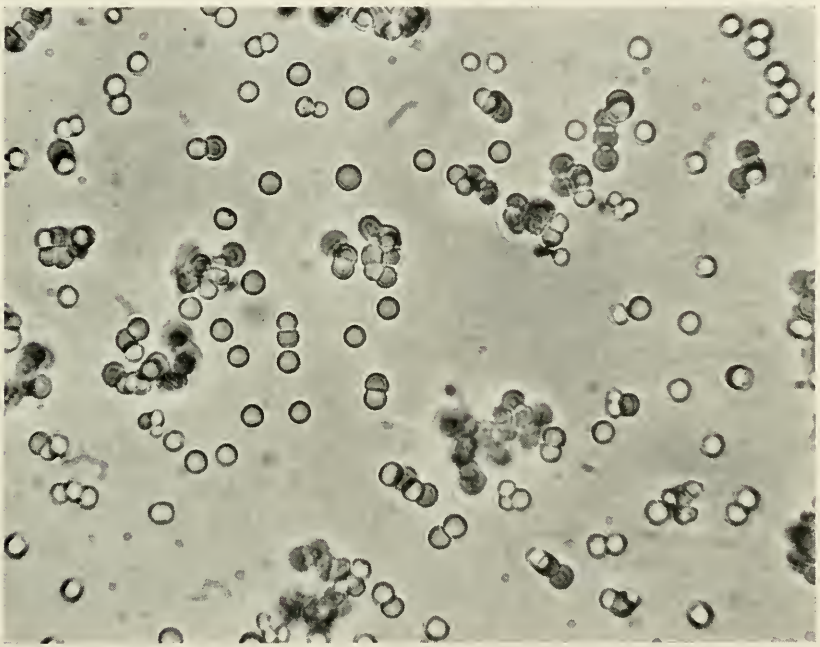

FIGURE 1. Microspheres. Photomicrograph courtesy of Dr. K. Harada. Units are approximately 2 μ in diameter.

more easily produced by raising the pH, as from 3.0 to 5.5. Time lapse photomicrographic studies demonstrate that the interior can be completely dissolved, yet the outer membrane remains. This behavior poses the provocative question of the difference between the nature of the outer membrane and the inner material.

In FIGURE 3 is seen a field in which appears a form resembling a cell in division. In fact, this one is very similar to an object carefully referred to by Claus and Nagy in figure 5 of their paper as an organized element resembling cell division. Preliminary time lapse studies suggest neither division nor fusion is occurring in the majority or all of these units. The appearance of such phenomena, however, is provocative in the sense of the properties and behavior found in the units. An additional field of twinned microspheres is

seen in FIGURE 4. This figure also shows filamentous structures which arise from proteinoid.

In FIGURE 5 are seen the effects of pressure on the microspheres. This segmentation resulted from digital pressure on the coverglass.

In FIGURE 6 are seen algal-like associations of microspheres. These were produced by making them under a coverglass on the microscope slide. The resemblance is to *Anaboena* or *Nostoc*.[28] We are indebted to Dr. Chester S. Nielsen for aid in verifying the superficial, albeit incomplete, resemblance. The resemblance of alleged fossils of this type is also imperfect.

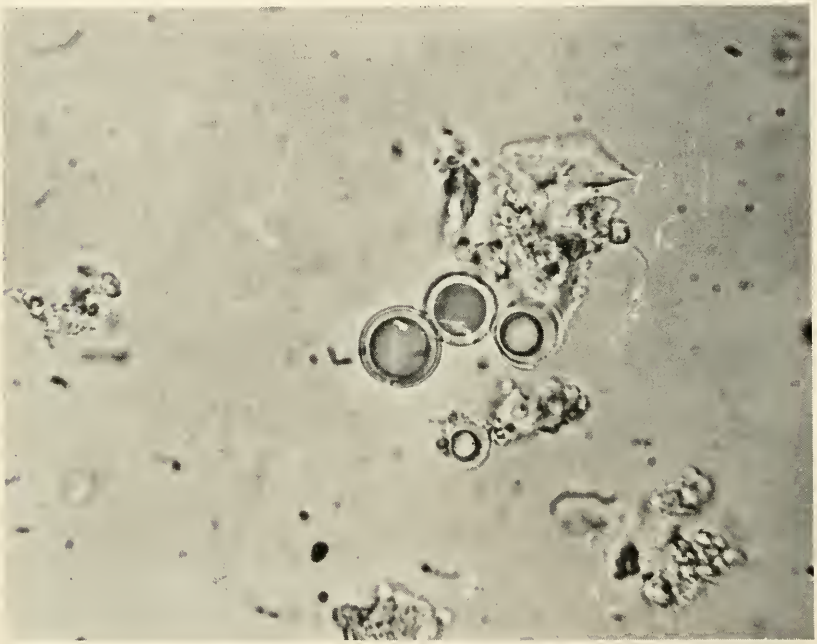

FIGURE 2. Microspheres with double boundaries following increase in pH. Larger figures are approximately 10 μ in length.

The microspheres are also found to be birefringent, indicating internal order.

When we review the results of almost a decade of experimental studies of models of biochemical origins we can perceive: (1) amino acids have been produced by many workers under many laboratory conditions and from many reactants that plausibly existed on or in the prebiological Earth; (2) in a majority of such experimental reports, the key aspartic acid appears as a product; (3) the polymerization of amino acids has now been accomplished in hundreds of variations over a range of conditions; and (4) similarly, the formation of spherular forms has been accomplished in thousands of variations in the laboratory. We now regard processes 3 and 4 as so rugged and so inexorable as to believe that they could and should have occurred on many occasions in many places in the universe. Also, the origin of the necessary amino acids seems to be inexorable, by one process or another.[29]

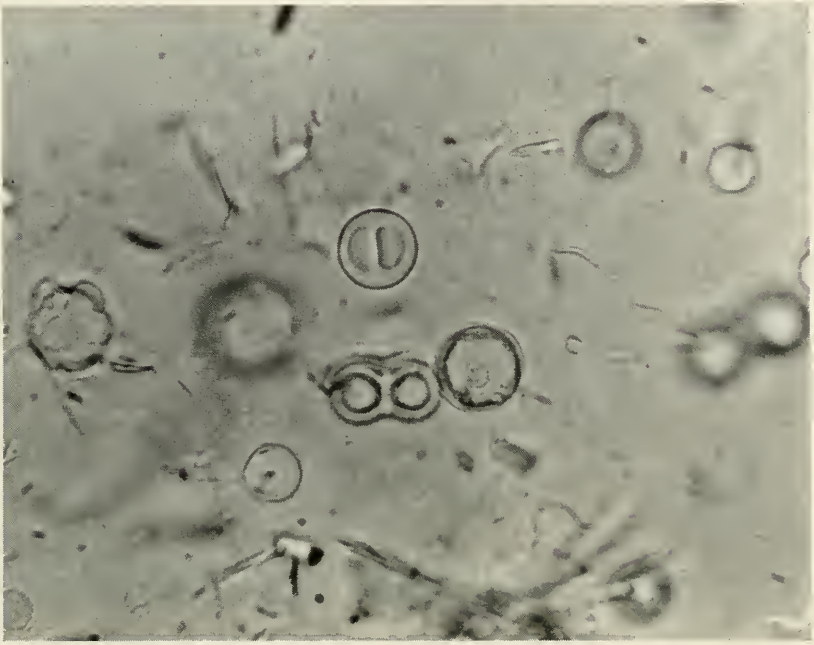

FIGURE 3. Twinned microspheres produced by rise in pH. Size as in FIGURE 2.

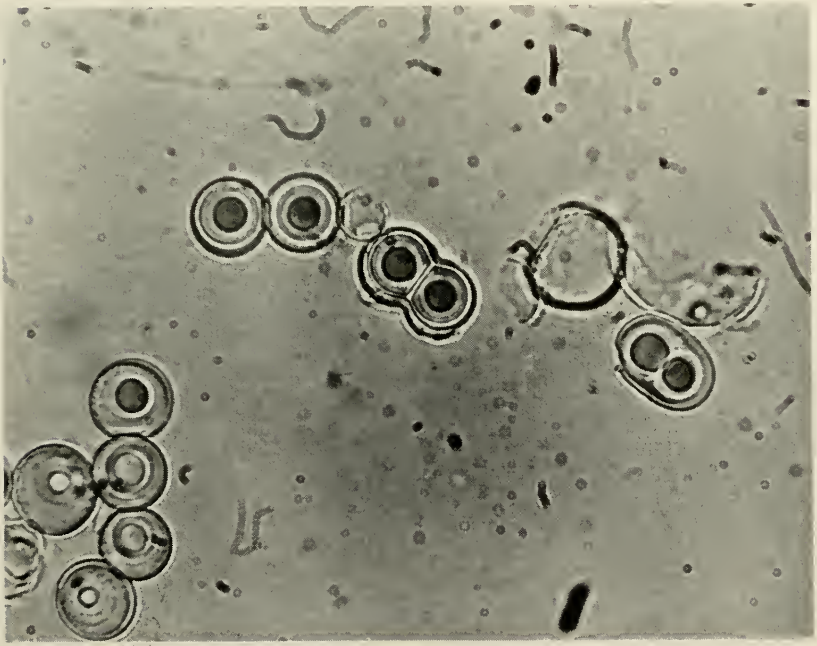

FIGURE 4. An additional field of twinned microspheres. Size as in FIGURE 2.

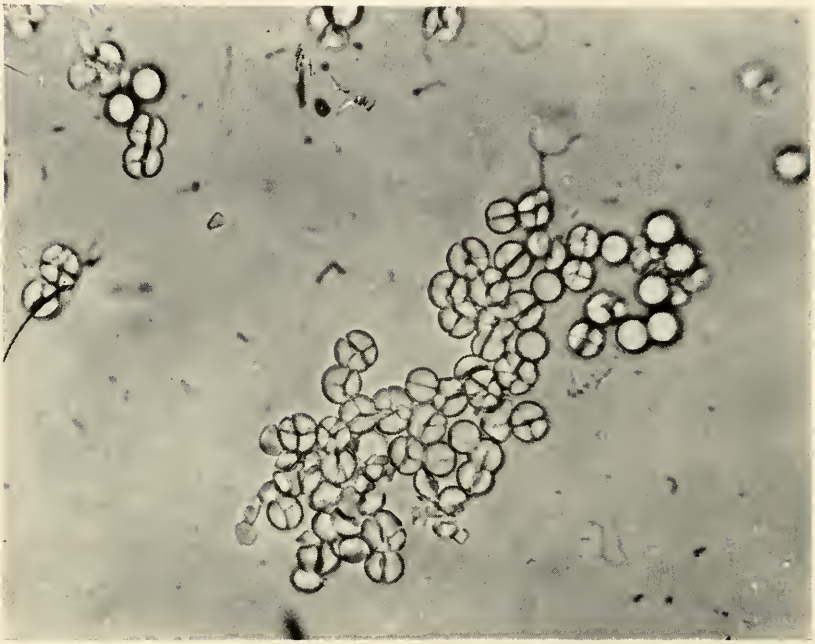

FIGURE 5. Effect of digital pressure on microspheres. Size as in FIGURE 2.

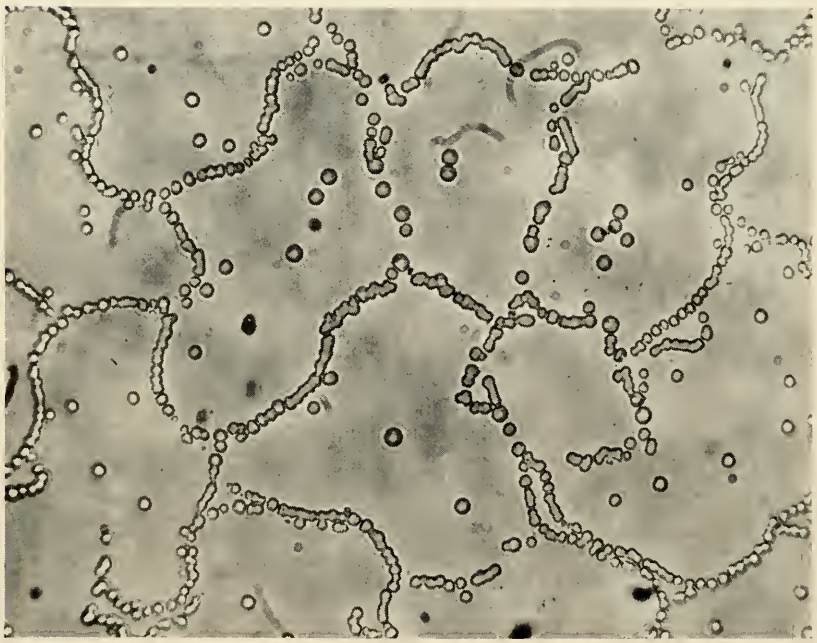

FIGURE 6. Associations of microspheres with resemblance to algae. Size as in FIGURE 2.

In the context of the origin of living units, one inference is that nature had almost endless opportunities to experiment with precellular forms until the necessary apparatus for replication was included by chance.

In the context of the present conference, the presumed protobionta observed in fossils and meteorites may actually be prebionta. If they are, they would be in one sense more significant than if they are protobionta. A third possibility is that they are meaningless artifacts, easy for nature to come by because of the simplicity of the processes leading to their formation. This point of view has a semantic flavor, because of the position that no natural experiment is truly without meaning. Also of interest is the fact that Dr. Philip Morrison independently reached the same conclusion from data presented at the Denver meeting of the American Association for the Advancement of Science.[30] In essence, however, and either with or without regard to the difficult questions of terrestrial contamination of meteorites, the conclusion at present is that there cannot yet be a conclusion on the question of whether the inclusions in meteorites are protobionta.

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OBSERVATIONS ON THE NATURE OF THE "ORGANIZED ELEMENTS" IN CARBONACEOUS CHONDRITES

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Our interest in the morphological study of carbonaceous chondrites was stimulated by reports of Claus and Nagy[1] and of Nagy *et al.*,[2] describing a variety of "organized elements" found in Class I carbonaceous chondrites. The organized elements had been classified by Claus and Nagy into 5 types on the basis, primarily, of morphology. The various properties of the organized elements are tabulated in TABLE 1. Types I and II which were circular or spherical were most numerous; the other types were much less abundant. A total of about 1700 organized elements per milligram were reported.[1,2]

In an attempt to confirm these observations and to characterize further the composition of the organized elements, we examined samples of the carbonaceous chondrites Orgueil and Ivuna. One sample of Orgueil was obtained several years ago from the Musée d'Histoire Naturelle, Paris. Another sample was obtained through the courtesy of Henderson of the U.S. National Museum, and was from the same fragment given to Nagy. A sample of Ivuna was obtained through the courtesy of Roy of the Chicago Natural History Museum. Conventional brightfield, phase contrast and fluorescence microscopy were used. Other methods included staining with biological stains, and the use of x-ray diffraction and electron microprobe analysis.

Microscopical Observations

Both samples of Orgueil and the single sample of Ivuna had crumbled apart and consisted of fragments ranging in size from a fine dust to several millimeters in diameter. Fragments were inspected visually to be certain that they were free from fusion crust, paint markings, and other visible contaminants. To minimize sampling errors, observations were made on the fine dust as well as fragments broken from larger pieces. This dust that had accumulated at the bottom of the sealed glass containers came from the surface of many individual fragments and should, therefore, be fairly representative of the meteorite as a whole. Because of the friable nature and the porosity of the carbonaceous chondrites, it is not feasible to clean the meteorite surface. For microscopy, samples of the meteorite weighing about 1 mg. were placed in a drop of glycerin on a microscope slide which had been cleaned with 95 per cent ethanol. The sample was gently crushed with a glass rod cleaned with ethanol. Samples subjected to density separation were lightly crushed in an alcohol-cleaned agate mortar.

Initially, particles were sought which had the general morphological characteristics of the organized elements. Because Types I and II elements were circular or spherical, particles with this morphology were sought. As reported

in an earlier paper,[3] the most conspicuous particles with this shape and occurring in the abundance of several thousand per milligram were opaque and highly magnetic. They could be concentrated by density separation in the fraction with a density greater than 3.33. Although opaque, many had transparent, yellow-brown mineral fragments attached to the surface. When viewed with phase-contrast microscopy, the diffraction pattern around the particles frequently gave a false impression of a double outer wall, especially when the particles were slightly out of focus. X-ray diffraction and electron microprobe studies of isolated particles of this type indicated that they were composed of troilite or magnetite.[3] Although possessing several characteristics of the organized elements, these troilite and magnetite particles were opaque. Subse-

TABLE 1
REPORTED PROPERTIES OF ORGANIZED ELEMENTS*

Organized element	Shape	Surface	Color	Size	Abundance
				μ	
I	Circular	Double wall, thickening and sculpturing	Yellow-green	4–10	Abundant
II	Circular	Spines, appendages, furrows		8–30	Abundant
III	Shield-shaped	Thickening and sculpturing		15	Less common
IV	Cylindrical	Thick wall, sculpturing		10–12 × 20	Less common
V	Hexagonal	Appendages		20	Rare

Other reported general properties:
Fluorescence in ultraviolet light
Staining with biological stains
Appearance suggesting cell division occasionally
Resistance to HF treatment

* From Claus and Nagy[1] and Nagy *et al.*[2]

quently, Nagy *et al.*,[2] emphasized several differences between these particles and the "organized elements."

Other spherical particles were found in some samples of the meteorite which had been subjected to a density separation with organic liquids. These ranged in size from about 1 to 20 μ and were transparent and colorless or yellow. Some appeared to have a double wall. These had a bluish fluorescence of the outer portion when viewed with ultraviolet light. The smaller particles had uniform bluish fluorescence. A number of tests indicated that these were hydrocarbon droplets and droplets of supercooled liquid sulfur coated with hydrocarbon.[3] They could be removed by repeated washing of the sample with chloroform or acetone and therefore did not seem to be organized elements.

A variety of hexagonal particles varying in size from about 2 to 20 μ were also found. Some hexagonal particles were transparent and yellow-brown with an opaque, irregular central area; these particles were highly magnetic. They may be goethite pseudomorphs after troilite, probably formed by preterrestrial oxidation of troilite. Other hexagonal particles were quite

small, colorless, and transparent. These were probably silicate or carbonate minerals. Other hexagonal particles were opaque and nonmagnetic. These were probably one form of troilite which is non-magnetic. None of these hexagonal particles had appendages quite like those found in the type V hexagonal particle illustrated by Claus and Nagy.[1] It should be noted, however, that the type V organized element is quite rare; only two and a fragment of a third were found by them in Orgueil.

No other particles of distinctly spheroidal shape could be found. The bulk of the meteorite consists of a brownish-yellow hydrated silicate (Orgueil LM).[4] Most of the silicate particles had a very irregular shape, but a few were roughly spherical (FIGURE 1). However, even these ovoid to spherical fragments had at least a partially irregular surface, and none had any definite internal structure or double walls. They were not magnetic. Although some variation in color and refractility was noted, the spheroidal particles had numerous irregular counterparts which matched them in every way except shape. It seems likely that all of these particles were mineral fragments.

Although each of these types of particles had some of the characteristics of the organized elements, none seemed to possess all of the primary morphological properties. However, other properties of the organized elements have been described.[1,2] These include fluorescence in ultraviolet light, staining with biological stains, and insolubility in hydrofluoric acid. Particles with these characteristics were then sought.

Fluorescence in Ultraviolet Light

Crushed, but otherwise untreated, Orgueil from the U. S. National Museum was examined with the fluorescence microscope and all of the fluorescent particles seen were photographed in visible and ultraviolet light. Based upon the information of Claus and Nagy,[1] 39 organized elements should have been found in the area of the slide examined. Actually, 15 fluorescent particles were found, but they did not seem to resemble the published illustrations or descriptions of the organized elements (TABLE 2). They were quite irregular and when viewed with ordinary illumination were colorless or slightly yellow. Two typical particles selected from the 14 photographed are illustrated in FIGURE 2.

Biological Stains

Since the organized elements have been reported to stain with various biological staining reactions including Feulgen and PAS, these as well as other staining procedures were used on samples of the meteorite. Many of the irregular yellow-brown grains stained slightly with the PAS and Feulgen reactions. Although many particles stained slightly, none stained the brilliant magenta usually achieved in biological materials, and many of the rounded grains did not appear to stain at all (FIGURE 1). Similar results were obtained with the Feulgen reaction.

To interpret these staining results it is necessary to examine the nature of the PAS and Feulgen reactions. The color in both reactions is produced by using Schiff's reagent, prepared by decolorizing basic fuchsin with sulfurous acid.[5]

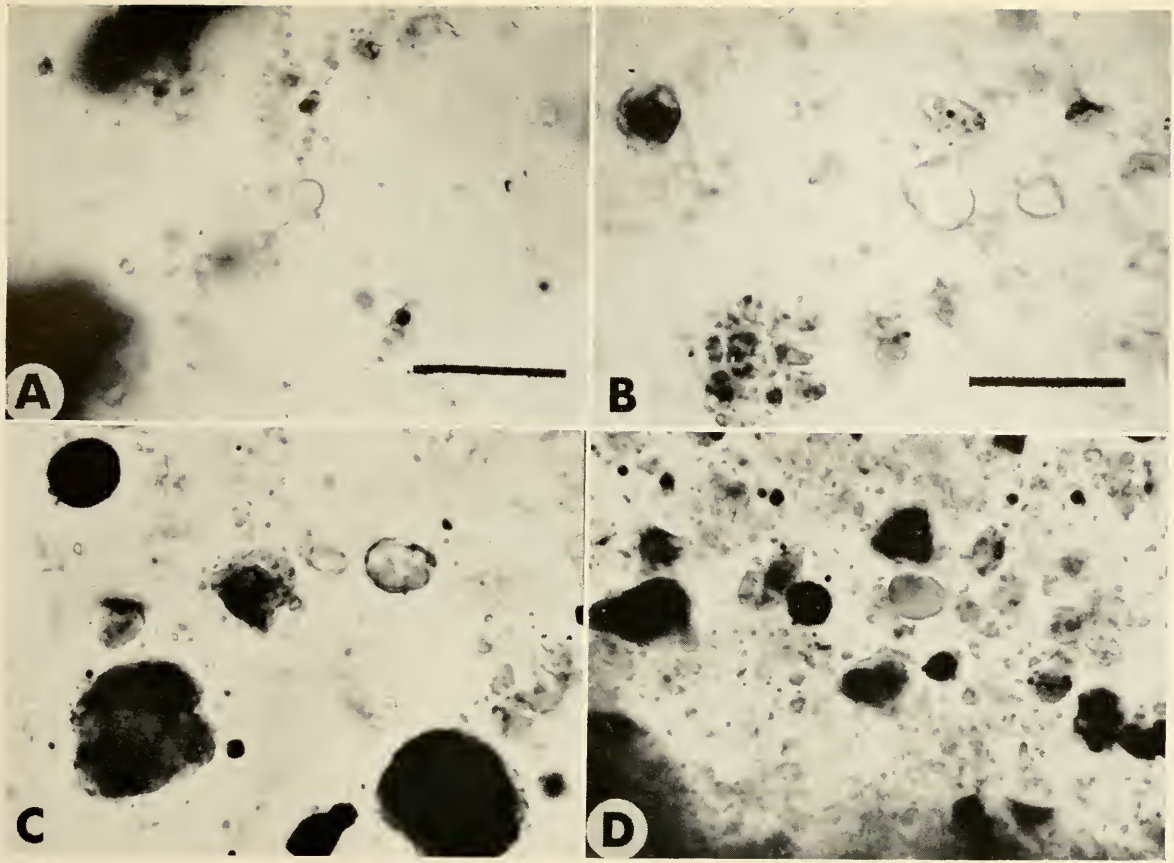

FIGURE 1. Microscopical appearance of Orgueil meteorite. Crushed sample mounted in gelatin. (*A*) Unstained preparation. (*B*, *C*, *D*) Preparation stained with PAS reaction. The opaque spherical particles which did not stain are magnetite or troilite. Some of the other irregular grains stained slightly. The spheroidal particles in the center of each field most nearly match the descriptions of organized elements but have little evident internal structure. They are relatively scarce. The *line* is 20 μ in length.

If aldehydes are reacted with Schiff's reagent, a red-violet color develops that is different from the original fuchsin. In addition to aldehydes, certain ketones, certain unsaturated compounds, and various oxidants can colorize Schiff's reagent.[5,6] The solution must be fairly freshly prepared; oxidation, aging, exposure to air, and sunlight can recolorize Schiff's reagent stored in the laboratory.[7]

In addition to any aldehyde groups present initially, Schiff's reagent will react with any artificially produced aldehyde groups. For example, periodic acid oxidizes 1,2 glycol linkages to aldehyde groups. If one of the hydroxyl groups is substituted with amino alcohol, alkylamino alcohol or carbonyl, it is also oxidized to give a positive reaction. In biological materials, the reaction is relatively specific for carbohydrates, mucoproteins and glycolipids. Unsaturated lipids which can also react are usually removed from biological samples during preparation for microscopical examination.[5,6]

Table 2
UV Fluorescence in Orgueil

Color	Size range	Number of particles	
		Regular	Irregular
	μ		
Yellow	2–10	0	5
Bluish	2–10	0	3
Bluish	10–50	0	5
Bluish	>50	0	2

Expectation value for area scanned: 39 organized elements.

In biological tissues the Feulgen reaction is usually considered to be specific for desoxyribonucleic acid (DNA).[5,6] As the first step in the procedure, DNA is partially hydrolyzed by 1 N HCl to produce the aldehyde form of desoxyribose phosphate. The aldehyde groups then react with Schiff's reagent to produce the same magenta color found in the PAS reaction. In biological samples, substances which will react directly with Schiff's reagent are usually not present. With meteorite samples it is essential to determine whether or not materials are present that will react directly with Schiff's reagent. Such substances would give a false positive Feulgen reaction and simulate the presence of DNA. To correctly interpret the results of the staining reactions on the meteorite samples, proper controls are necessary.

To control the staining reactions, sections of rat spleen tissue fixed in Carnoy's solution and embedded in paraffin, as well as samples of kimberlite and Orgueil were studied. Kimberlite, the diamond-bearing rock usually believed to have come from deep within the earth, was chosen becaues it is perhaps more similar to Orgueil in mineral composition than other terrestrial rocks. Both Orgueil and kimberlite consist primarily of serpentine-like hydrated silicates produced from olivine by alteration under aqueous, reducing conditions. For the staining reactions, samples of Orgueil and kimberlite were suspended in 6 per cent gelatin and the mixture was spread on microscope slides and allowed to dry.

FIGURE 2. Typical fluorescent particles in Orgueil meteorite. (*A*) and (*C*) show irregular particles which are fluorescent in ultraviolet light. (*B*) and (*D*) show the same particles, indicated by arrows, in visible light. These particles are 2 of 15 irregular ones found in an area where 39 organized elements should have been present. No fluorescent particles of regular outline were seen.

Gelatin was used to adhere the samples to the slides because preliminary experiments showed that there was little staining of the gelatin.

The Feulgen reaction was carried out in the routine manner on these samples with the usual hydrolysis with 1 N HCl at 60° C. for 8 minutes followed by

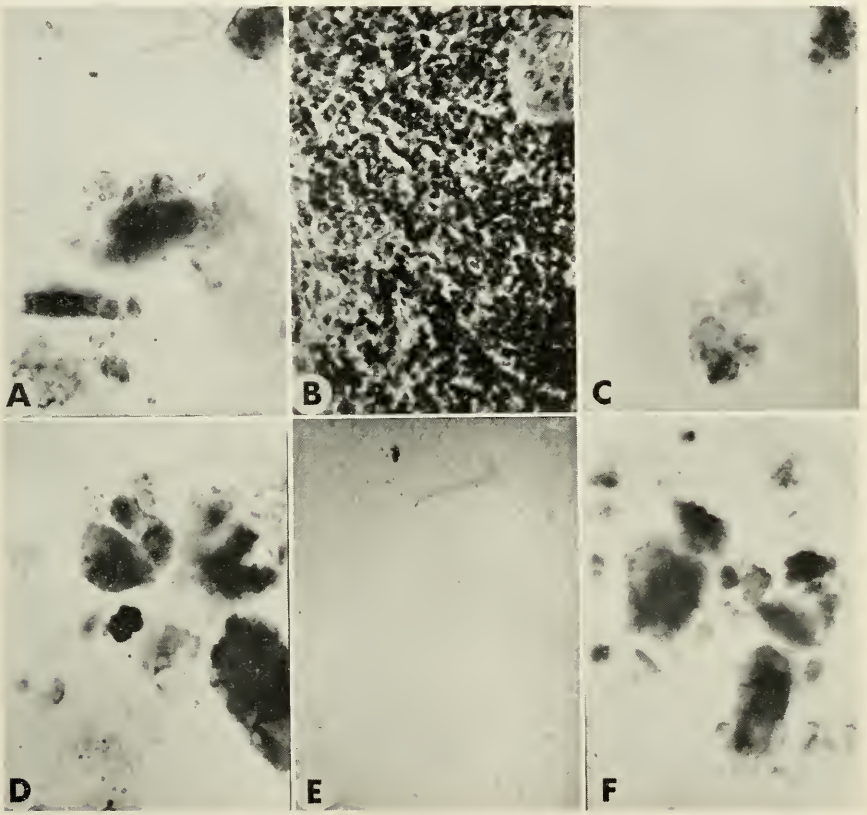

FIGURE 3. Orgueil, rat spleen, and kimberlite stained with the Feulgen reaction. (*A*) Orgueil, Feulgen reaction. Most particles stain irregularly. (*B*) Rat spleen, Feulgen reaction. Nuclei have brilliant magenta staining of DNA. Other tissue components do not stain. (*C*) Kimberlite, Feulgen reaction. Most particles stain irregularly. Some of the sample was dissolved by the HCl treatment. (*D*) Orgueil, Schiff's reagent only. Staining is as intense as with the Feulgen reaction. (*E*) Rat spleen, Schiff's reagent only. There is no staining of nuclear DNA. (*F*) Kimberlite, Schiff's reagent only. Staining is as intense as with the Feulgen reaction.

treatment with Schiff's reagent for 1 hour.[5] As a control procedure, samples were reacted with Schiff's reagent for 1 hour without previous treatment with acid. In the rat spleen sections, nuclear DNA stained brilliantly after acid hydrolysis (FIGURE 3, *B*). In sections treated with Schiff's reagent alone, no staining occurred (FIGURE 3, *E*). However, samples of Orgueil and kimberlite stained equally well whether treated with acid or not (FIGURE 3; *A*, *D*, *C*, *F*). Something is present in the meteorite and in kimberlite which reacts directly

with the Schiff's reagent. Therefore, the development of a magenta color with the Feulgen reaction is, in this instance, not specific for DNA.

Similar results were obtained with the PAS reaction. There seemed to be no additional staining produced when samples were treated with periodic acid before reaction with Schiff's reagent, as compared with reaction with Schiff's reagent alone. Attempts to inhibit the staining produced by Schiff's reagent by previous treatment of samples with aniline chloride and hydroxylamine, to block the aldehyde groups,[5] were only partly successful in the samples and in periodic acid treated starch controls. Hence, the nature of the reactive groups is not known at present.

The presence of DNA in the organized elements would be powerful evidence of their biologic origin. Because the results of the Feulgen reaction had been interpreted in published reports as indicating the presence of nucleic acid in the meteorite,[1,2,8] it was desirable to confirm this interpretation with another histochemical test for DNA. Methyl green is frequently used for this purpose.[5] The characteristic reaction of DNA with this stain is thought to be the result of binding of the dye by phosphoric acid radicals in the intact, polymerized DNA.[5] Thus, the mechanism of this reaction is altogether different from that of the Feulgen reaction.

Samples of rat spleen, Orgueil and kimberlite were stained with methyl green.[5] As a control procedure, samples were treated with 10 per cent perchloric acid for 4 hours and 30 minutes, a procedure which depolymerizes and extracts DNA from biological samples.[9] In rat spleen sections stained directly with methyl green, there was brilliant green staining of the nuclei (FIGURE 4, *B*). In spleen sections treated with perchloric acid to remove DNA before reaction with methyl green, there was no nuclear staining (FIGURE 4, *E*). However, the samples of Orgueil and kimberlite stained brilliantly with methyl green whether treated previously with perchloric acid or not (FIGURE 4; *A*, *D*, *C*, *F*).

It is evident that when biological staining reactions are applied to nonbiological materials, great care is necessary in the interpretation of results. Because of the presence of other reactive groups the usual tests for DNA are no longer specific. Positive or negative reactions of any DNA present would be masked by the intense, nonspecific staining due to other groups. Under these conditions, the staining tests cannot be regarded as evidence for the presence of DNA in the meteorite.

Treatment with Hydrofluoric Acid

The "organized elements" were reported by Nagy *et al.*, not to be seriously affected morphologically by treatment with boiling hydrofluoric acid (HF) for 15 minutes, whereas silicate minerals should be dissolved.[2] We treated a sample of Orgueil with boiling HF for 15 minutes; 49 per cent of the sample remained (TABLE 3). Because the carbon content of the meteorite is only 3.1 per cent, the bulk of this residue must have been inorganic. Consideration of the pertinent solubility products indicates that calcium, magnesium, and possibly other major constituents of the meteorite should remain as insoluble fluorides or fluosilicates. Thus, persistence after HF treatment is not a sufficient criterion for the organic nature of a particle.

To dissolve the mineral residue, the sample was first treated with HF for 17

hours at 60° C. and with 6 N HCl for 18 hours at 25° C. Treatment with HF-HCl is a standard palynological technique which leaves organic materials of biological origin, including various pollen grains, morphologically unaffected.[10] After this treatment only 3 per cent of the sample remained. X-ray

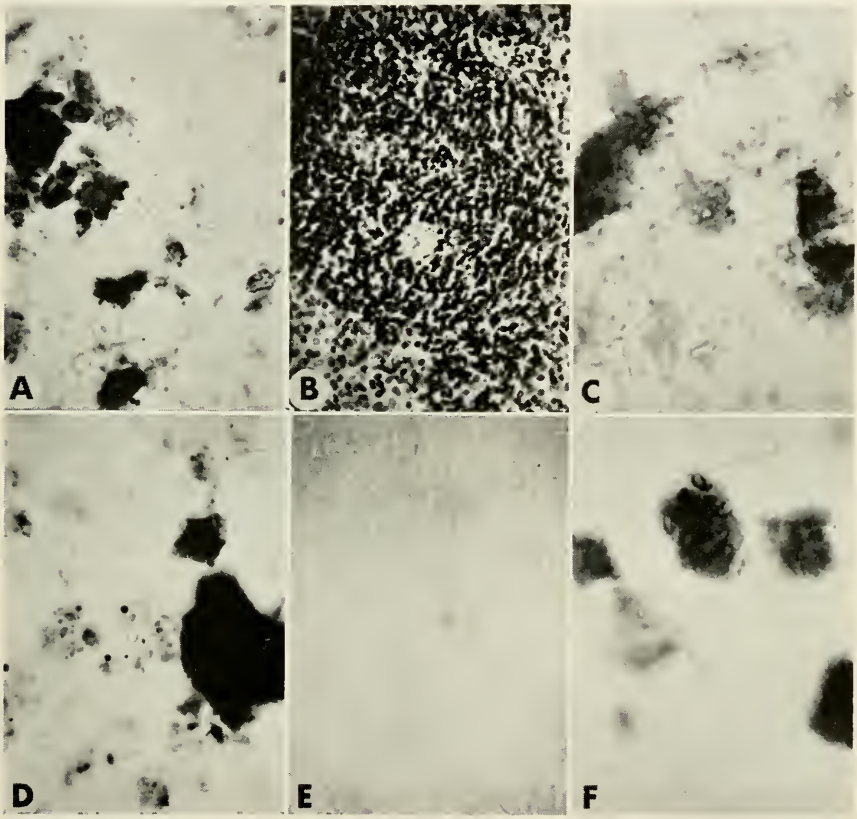

FIGURE 4. Orgueil, rat spleen, and kimberlite stained with methyl green. (*A*) Orgueil methyl green stain. Many particles stain irregularly. (*B*) Rat spleen, methyl green stain Nuclei are stained a dark green. Other tissue components do not stain. (*C*) Kimberlite methyl green stain. Many particles stain irregularly. (*D*) Orgueil, methyl green stain after $HClO_4$ treatment. Staining is as intense as before extraction. (*E*) Rat spleen, methyl green stain after $HClO_4$ treatment. There is no staining of nuclei; DNA has been depolymerized and extracted. (*F*) Kimberlite, methyl green stain after $HClO_4$ treatment. Staining is as intense as before extraction.

diffraction and infrared spectrophotometry indicate that this residue is mainly amorphous carbon with traces of MgF_2 and organic matter. Microscopical examination of the residue showed finely granular, black to brown material virtually devoid of any structure (FIGURE 5, *C*, *D*). Often, it was present in large irregular aggregates (FIGURE 5, *B*). Very rarely, spherical transparent particles were seen (FIGURE 5, *A*), but only 2 were found in an area where several thousand organized elements should have been present. Granular

material was adherent to their surface, and little structural detail could be resolved with either phase-contrast or brightfield microscopy. The possible nature of these particles will be discussed in the following section.

TABLE 3
ORGUEIL METEORITE: TREATMENT WITH HF

Reagent	Temperature	Time	Residue	Composition of residue
		hours	%	
HF 24 M	75°	24	>50	MgF_2, CaF_2, Fe_3O_4, FeS, organic matter
HF 24 M	100°	$\frac{1}{4}$	49	MgF_2, CaF_2, organic matter
HF 24 M	60°	17	3	Carbon + organic matter
HCl 6 M	25°	18		

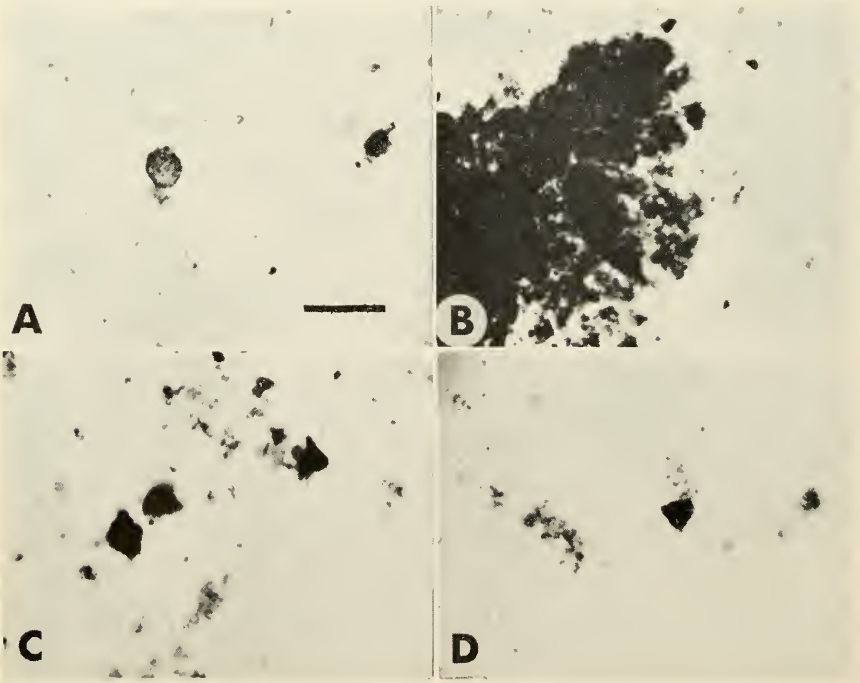

FIGURE 5. Orgueil after HF-HCl treatment. (*A*) One of 2 transparent spherical particles seen. Irregular black-brown material is adherent to the surface. (*B, C, D*) Amorphous residue remaining after HF-HCl treatment. Most of this material is amorphous carbon with traces of MgF_2 and organic matter. The *line* is 20 μ in length.

Attempts at Identification of Some Organized Elements

It is evident that there are discrepancies between our findings and those of Nagy *et al.* In an attempt to resolve these differences, we visited the laboratories of Claus and Nagy at their invitation. They examined our material and we examined their material. It became evident that there were several reasons for the differences.

First, their material contained a few particles of striking morphology which we had not found and which they did not find in our material. Examples of such particles found in their material are shown in FIGURE 6, *A* and *B*, and FIGURE 7, *A* and *B*. These were classified by Claus and Nagy as type II organized elements with double wall and spiny surface. Particles of strikingly similar morphology are illustrated in FIGURE 6, *C* and *D*, and FIGURE 7, *C* and *D*. These are common ragweed pollen grains. The particles in FIGURES 6, *A* and *B*, and 7, *A* and *B*, were suggested by them to be extraterrestrial life

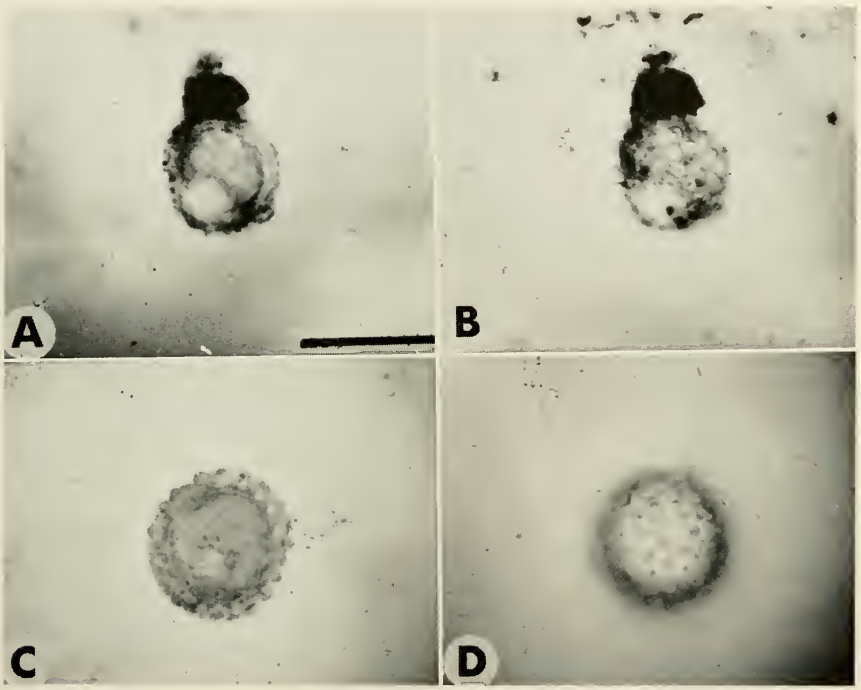

FIGURE 6. (*A* and *B*) Organized element from preparation of Claus and Nagy. The different levels of focus indicate double wall structure and spiny surface. (*C* and *D*) Ragweed pollen grain. Double wall and surface spines are shown at different levels of focus. The *line* is 20 μ in length.

forms resembling hystrichospherids, spiny fossil algae. The appearance of these algae and some pollen grains may be similar. It seems that in this instance, morphological criteria alone may not be a sufficient basis for identification.

Two other particles from their material identified by them as type II organized elements are illustrated in FIGURE 8, *A* and *B*. A third organized element of similar appearance was also seen in their material. All 3 particles were found on a slide reportedly stained with the Feulgen reaction. They show a resemblance to starch grains (FIGURE 8, *C* and *D*), stained with the PAS reaction. The difference between the Feulgen and PAS reactions may not be of significance in this instance, since we have noted that Schiff's reagent

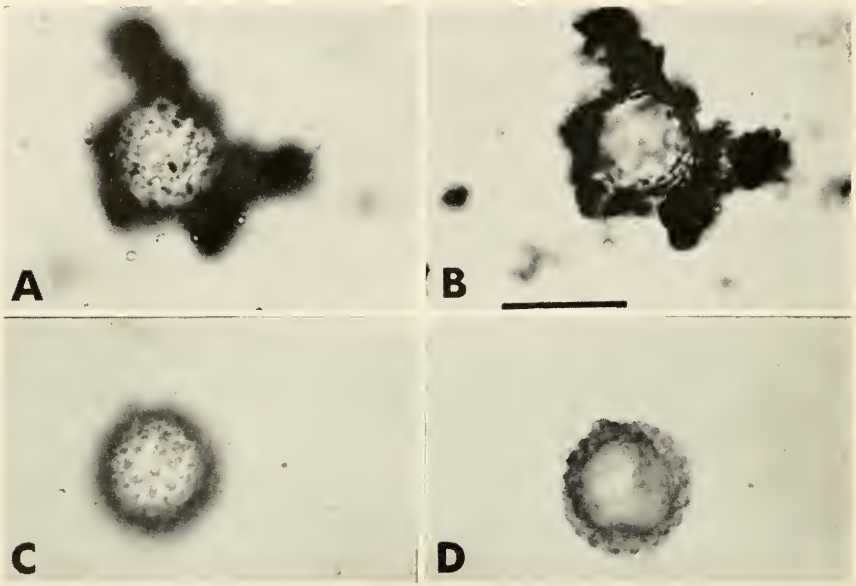

FIGURE 7. (*A* and *B*) Another organized element from preparation of Claus and Nagy (*C* and *D*) Ragweed pollen grain. The line is 20 μ in length.

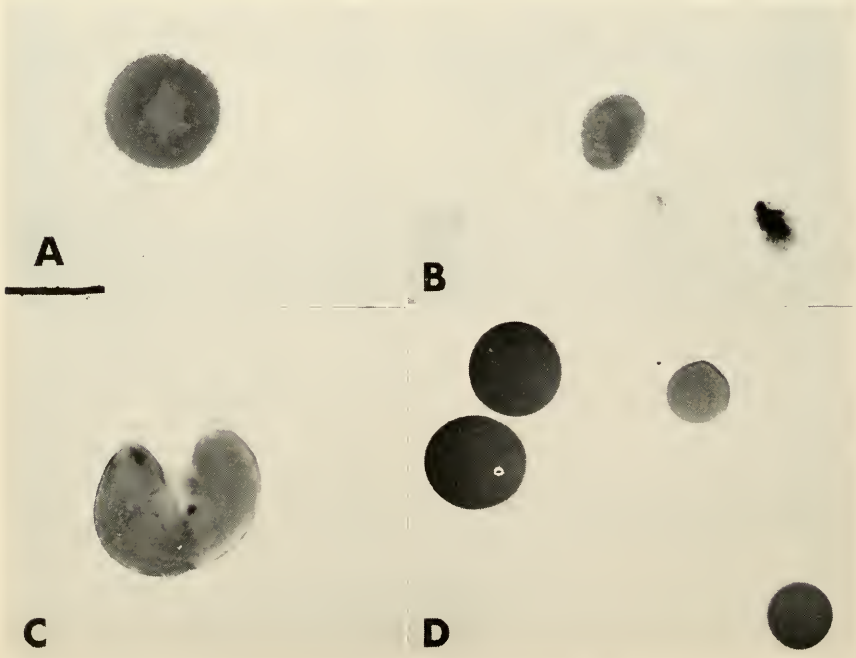

FIGURE 8. (*A* and *B*) Two different organized elements from preparation of Claus and Nagy stained with Feulgen reaction. (*C* and *D*) Starch grains stained with PAS reaction. See text for discussion of significance of staining. The line is 20 μ in length.

alone will stain some starch grains. This staining was more pronounced when an aged batch of Schiff's reagent was used, and was somewhat stronger for "Biosorb" (modified starch prepared by Ethicon Laboratories) than for potato starch. We cannot exclude the possibility that the particle in FIGURE 8, *A* is actually a juniper pollen grain. Again, morphological criteria seem to be inadequate to establish the identity of a given particle.

Another organized element, classified by Claus as a type II element resembling a *Thecamoeba*, is shown in FIGURE 9, *A*. Illustrated in FIGURE 9, *B* is an object with similar morphology found in the airborne pollen sample collected on July 20, 1961 by Siegel at the Jewish Hospital in Brooklyn, N.Y. These microscope slides, prepared for the New York City annual pollen survey, were

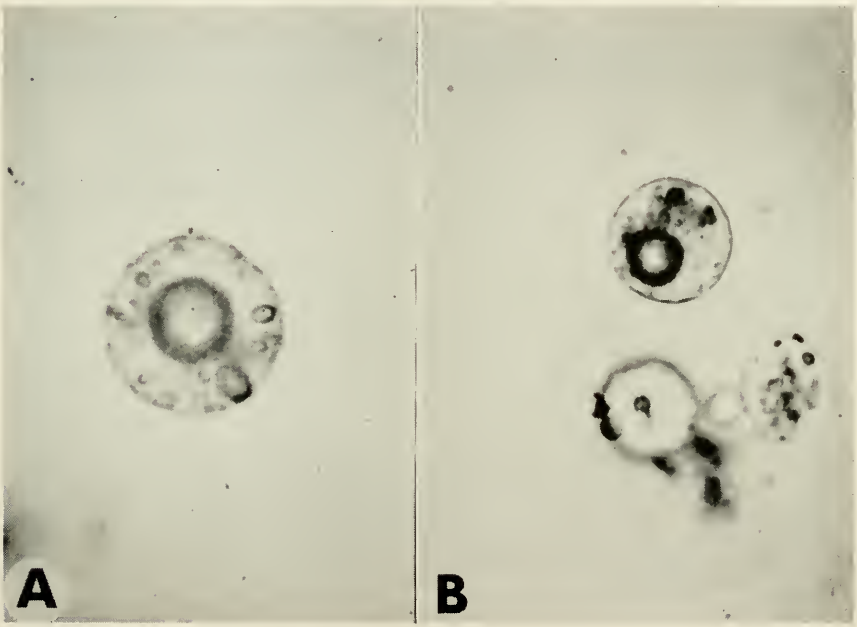

FIGURE 9. (*A*) Organized element from preparation of Claus and Nagy. (*B*) Particles with similar appearance found in pollen survey slide. See text for discussion.

kindly loaned to us by Siegel. We are not certain as to the identity of this object, but the resemblance between the organized element from the meteorite and the airborne particle is evident.* More recently, we have found several similar particles in dust samples from the American Museum of Natural History.

Pollen, mold, and fungus spores, and a variety of other objects are present in large numbers in the atmosphere at certain seasons, with daily totals of up to 100 ragweed pollen grains per cm.^{2} [11] and up to 363 mold spores per cm.^{2} [12] being

* Gregory (private communication) has suggested that these particles might be furnace ash spheres.

reported for New York City. Several of these objects are illustrated in FIGURE 10. It is extremely difficult to prevent contamination by this type of material. These types of particles are often present in great abundance in the air and are deposited as dust that later forms a secondary source for contamination.

Siegel has pointed out in personal communication that he had found it extremely difficult during the summer and fall to prepare Vaseline-coated slides free of pollen contamination, although working in a dust free, "sterile"

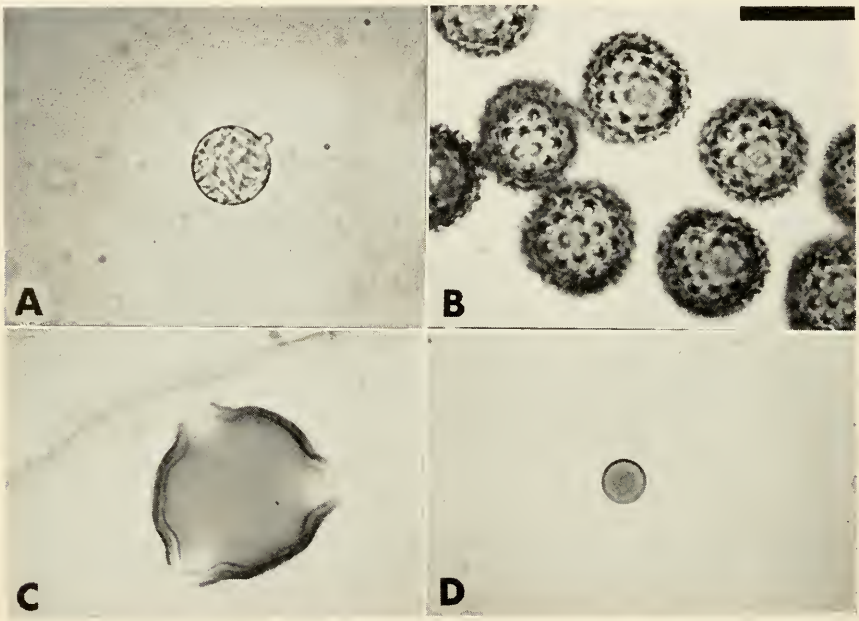

FIGURE 10. Objects found in pollen survey slides. (*A*) Unidentified object. (*B*) Ragweed pollen grains. (*C*) Oak pollen grain. (*D*) Unidentified object. The line is 20 μ in length.

room. Also, ragweed pollen grains were occasionally seen by Siegel in pollen study slides exposed long after the period of bloom, and probably represent contamination from the laboratory or other sources.

Thin Sections

Organized elements embedded in mineral veins in thin sections of the Orgueil meteorite have been described and illustrated.[2] It is extremely important to characterize these particles because they are undoubtedly indigenous to the meteorite. However, the nature of the thin sections makes adequate morphological study difficult. The sections are relatively thick, 10 to 25 μ, and although the veins are composed of transparent minerals, there are irregularities and impurities which cause optical distortion. It is difficult to be certain of fine surface detail because the practical limit of resolution for the microscope

under *ideal* conditions is only 0.2 to about 0.3 μ for the objectives that must be used with this sort of preparation. Although the organized element illustrated by Nagy *et al.*,[2] had to be viewed through a layer of optically imperfect magnesium sulfate, the presumed spines illustrated in the drawing were spaced only 0.3 μ apart.

Judging from both visual inspection and the published illustration [figure 4d in reference 2] this organized element appears to be opaque. Previously, it was emphasized that all organized elements in crushed preparations were transparent.[1,2] Also, none of the particles in the thin sections seems to have the highly structured morphology, although about 8000 organized elements should have been present in a thin section $\frac{1}{4}$ inch in diameter and 20 μ in thickness.

Some organized elements in the thin sections were described as having pink fluorescence [figure 5 in reference 2]. We encountered occasional particles in crushed preparations which appeared red against the dark background when illuminated with ultraviolet light. However, this did not prove to be true fluorescence. These particles when viewed with polarized visible light were doubly refractile. The fluorescence microscopes commonly used in biological investigations use darkfield illumination. The usual light source is a high pressure mercury arc with various filters placed in the light path to absorb the visible light. All of the 5 filters commonly used transmit ultraviolet and some blue light but they have an appreciable transmittance in the red portion of the spectrum as well.[13] Hence, doubly refractile particles should be expected to appear red when viewed with ultraviolet light in the fluorescence microscope.

Perhaps additional study of thin sections will reveal particles with a more conclusive combination of properties.* In our opinion the present evidence is inadequate to suggest a biological origin for the indigenous particles.

Discussion

Several features make it difficult to accept the highly structured particles as extraterrestrial in origin. They are absent from our preparations of Orgueil, although material from the same stone was used. They have not been observed in thin sections, and they often show a morphological resemblance to common airborne contaminants. Although a strong case can be made for the biological origin of some of these structures, the probability of a terrestrial contamination has not been ruled out in their case.

The situation is altogether different in the case of the small, brownish-yellow, somewhat irregular, roughly spherical grains which apparently make up most of the 1700 particles per milligram reported previously by Claus and Nagy[1] and Nagy *et al.*[2] Although our own experience suggests that this number represents an appreciable overestimation, there is no doubt that such particles do exist.

They are undoubtedly indigenous to the meteorite, but their morphology is so featureless that an inorganic origin cannot be ruled out. None of the other

* Additional observations on thin sections are reported in another paper (Anders and Fitch, Science, in press).

criteria for a biological origin seems to hold for these particles. They do not fluoresce and they do not take biological stains in a manner that will distinguish them from irregular silicate fragments in Orgueil and in kimberlite. Because they disappear after treatment with acids, we believe that they are most likey grains of minerals, although they are classified as organized elements by Nagy *et al.* The 2 particles remaining in our sample after HF-HCl treatment resemble terrestrial contaminants. Moreover, it must be emphasized that only 2 were seen where several thousand should have been found.

Even if organic particles should be found, a biological origin need not be inferred. Both the polypeptide particles of Fox[14] and the hydrocarbon polymer particles of Wilson[15] have an appearance at least as organized as the less structured organized elements. These materials are produced *in vitro*, by dry polymerization of amino acids,[14] and the Miller-Urey type synthesis,[16,17] respectively. In FIGURE 11 is illustrated a preparation obtained through the courtesy of Wilson in which most of the polymer occurred in the form of sheets containing thickened, round spots about 10 μ in diameter. Much of the material was fluorescent, but some of the larger spots were not.

It may well be that life did exist in meteorites, but we feel that the present evidence is not adequate to suggest an extraterrestrial biological origin for the particles found in the carbonaceous chondrites.

Criteria for Identification of Life Forms

If the present data are inadequate, what kind of information is needed to decide whether or not a particle is, in fact, a life form? This requires an initial definition of life. Life has three essential qualities. Life requires reproduction of itself with the possibility of mutations developing along the way. Regulated and integrated anabolical and catabolical chemical processes are a second feature of life. Structural organization at the molecular and supramolecular levels is a third feature. Probably for simple, small organisms, it is necessary to demonstrate all of these features—reproduction, metabolism, and organization—to establish the presence of life.

What is needed to establish that life had been present at some time in the past? Ideally, remnants of all these features should be found. In reproduction of all terrestrial forms, nucleic acids carry information from one generation to the next. Nucleic acids or breakdown products from them may remain after life has ceased. Evidence of metabolic processes frequently remains. Many carbohydrates and lipids are rather resistant and persist for long periods.

Persistence of the organization of any organism forms the basis for terrestrial paleontology. This morphology may be the result of partial or complete replacement of biological materials with nonbiogenic compounds. If replacement has been complete, probably one can never be entirely certain that a given structure was originally of biological origin. In terrestrial materials, this is occasionally an important question but it is never a critical one. For nonterrestrial materials it is a critical question.

If "fossilization" or replacement has been incomplete, then metabolical products of various sorts will remain. In pre-Cambrian rocks containing apparent fossil forms, there are, in fact, substances that resist the acid treatments used to remove the mineral materials.[10] With cytochemical as well as other

microscopical techniques, it should be possible to gain considerable information about the composition of these substances. Once characterized at the microscopical level, the substances can be isolated in larger quantities and other parameters including optical activity and isotopic composition can be measured.

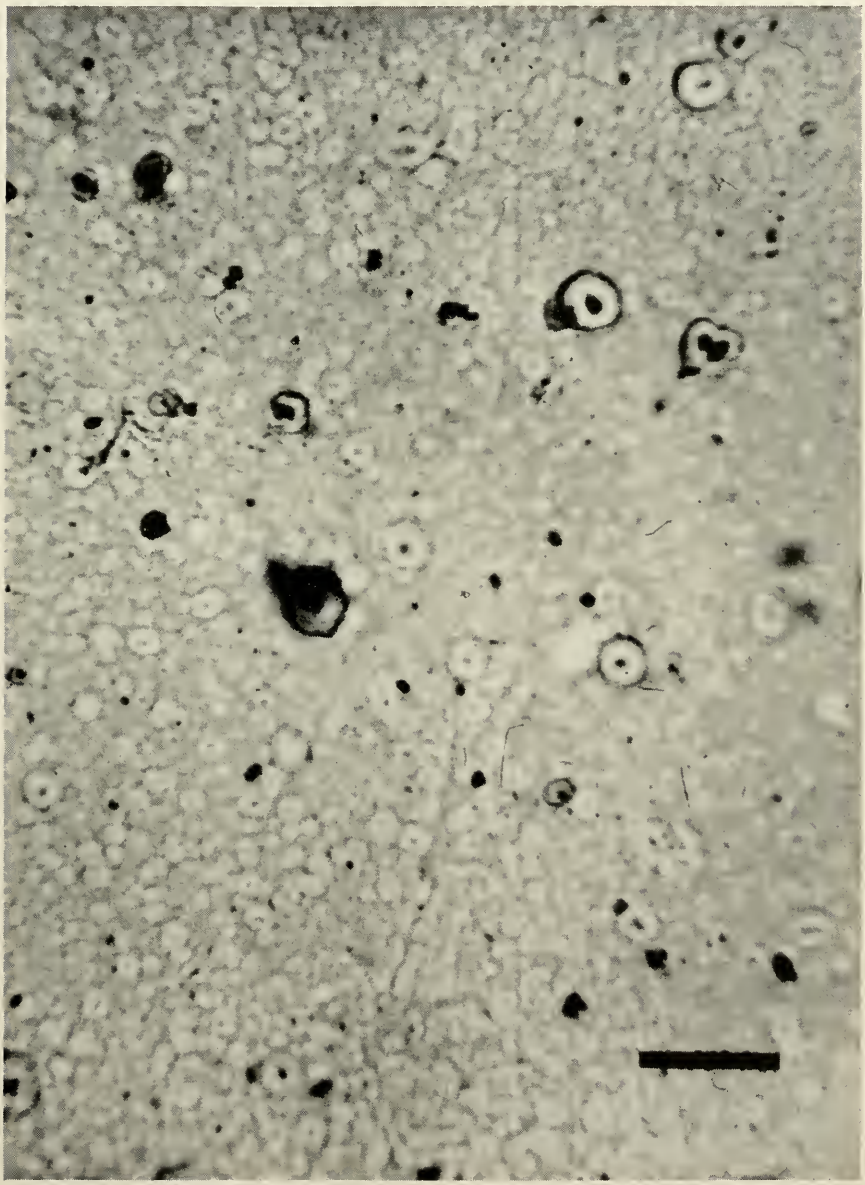

FIGURE 11. Hydrocarbon polymer prepared by Wilson (1960). Thickened spots are present in the sheet. Viewed in ultraviolet light, the spots and the sheet had a yellowish fluorescence. The line is 20 μ in length.

The observed properties of the resistant material can be compared with properties of biological compounds as well as with those of various synthetic materials including polypeptide particles prepared by Fox[14] and hydrocarbon polymer particles prepared by Wilson.[15] It is evident from their work as well as that of Miller,[16,17] Palm and Calvin,[18,19] Oró,[20] Berger[21] and others that complex organic materials can be prepared through nonbiological processes.

This approach assumes to some extent at least that extraterrestrial life resembles terrestrial life chemically. This may be a provincial idea, but comparison of unknown materials with terrestrial forms would seem to be a good starting place. It may be that even after this information is gathered and analyzed, no definite conclusions can be drawn. However, this information should provide a broader basis for critical evaluation than morphology alone.

Summary

"Organized elements" described by Claus and Nagy[1] and by Nagy *et al.*[2] are a heterogeneous group of particles which, in our opinion, are best classified into two types: those that have a highly structured morphology and those that have a much simpler appearance. The particles with highly structured morphology are less numerous than the simpler type. They have not been seen in thin sections and many appear to have a strong resemblance to common terrestrial contaminants. The particles of simpler morphology which do not fluoresce, which either do not stain or stain atypically with biological stains, and which are soluble in acids seem to be of an inorganic composition and origin. It is possible that life did exist in meteorites, but we think that the present evidence is not adequate to suggest an extraterrestrial biological origin for the particles found in the carbonaceous chondrites.

Acknowledgments

The authors express their gratitude to the Argonne Cancer Research Hospital for allowing the use of its facilities for some of the experiments, and to the staff of the Allergy Laboratory of the Jewish Hospital of Brooklyn for the loan of pollen slides. We are also indebted to Dr. George Claus and Prof. Bartholomew Nagy for permission to study and photograph their samples, in exchange for our preparations which they described in reference 2.

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ON THE ORIGIN OF CARBONACEOUS CHONDRITES*

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Carbonaceous chondrites are related to other classes of meteorites in many ways, and much of what has been said about the origin of meteorites, in general, applies to carbonaceous chondrites as well. Like all other meteorites, they are fragments of larger bodies. To reconstruct their history, we must try to learn more about the nature of these bodies, that is, their size, number, and location, and the chemical and physical processes that produced the detailed structural and compositional features of the meteorites.

Some of the principal hypotheses on the origin of meteorites are outlined in TABLE 1. (A more complete review of the subject has been given by Anders and Goles, 1961.) Each of these hypotheses can account for some 90 to 95 per cent of the properties of the meteorites, and it is only the last 5 to 10 per cent that causes difficulties. There is just as much disagreement on the origin of the carbonaceous chondrites (TABLE 2). Mason (1960, 1961) and Ringwood (1961) assume that they represent some of the primitive material from which the solar system formed; Urey (1961) believes that they are alteration products of the high iron group chondrites, which are themselves several steps removed from primitive material. Finally, Wood (1958, 1962) and others believe that they are alteration products of a hypothetical, primitive chondrite, similar to Renazzo or Ornans (Fish *et al.*, 1960; DuFresne and Anders, 1962*a*).

Clues to the Origin of Carbonaceous Chondrites

Mineralogy. Some clues to the origin of the carbonaceous chondrites can be obtained from a study of their mineralogy. Results for 9 of these meteorites are shown in TABLE 3 (DuFresne and Anders, 1962*a*). The estimated relative abundances are expressed as negative logarithms of 2; the entry 3, for example, stands for 2^{-3} or 1/8. The minerals found can be divided into three classes: conventional, "high-temperature" minerals; "characteristic" minerals peculiar to this class of meteorites; and trace minerals. In addition, these meteorites also contain appreciable amounts of sulfur, hydrated $MgSO_4$,† elemental carbon, and organic compounds. On the basis of their mineral composition, the carbonaceous chondrites can be divided into 5 subclasses. These show a fair degree of correspondence with Wiik's (1956) three classes, established on the basis of chemical composition only.

One can prove rather convincingly that the characteristic minerals are alteration products of the high-temperature minerals, rather than *vice versa*.

* This work was supported in part by the U.S. Atomic Energy Commission.

† The state of hydration varies with the temperature and the relative humidity at the time of measurement. Very probably, the $MgSO_4$ was present as the anhydrous salt or as the monohydrate at the time of fall, and became hydrated after exposure to atmospheric moisture. Boato's (1954) measurements show that the water released below 180° C. has a normal D/H ratio, and is probably of terrestrial origin.

X-ray diffraction and optical studies of composite grains of olivine and Murray F (a hydrated silicate), show that the olivine sometimes occurs in thin parallel plates of the same crystallographic orientation, although the individual plates are separated by a thin layer of exceedingly finely grained, randomly oriented, Murray F mineral. The common orientation of the olivine plates can be understood only if single crystal olivine served as the starting material (DuFresne and Anders, 1962*a*). Still, one cannot exclude the possibility that some fraction of the characteristic minerals is primordial, rather than being derived from the olivine.

Many of the other characteristic minerals, too, seem to be hydrated silicates. This fact, and particularly the occurrence of $MgSO_4$ in distinct veins (FIGURE 1)

TABLE 1
PROPERTIES OF METEORITE PARENT BODIES

	Lovering (1957)	Urey (1959)	Fish *et al.* (1960); Wood (1958, 1962)	Ringwood (1961)
Size	Planetary	Lunar	Asteroidal	Lunar
Location	2–5 a.u.	1 a.u.	2–5 a.u.	2–5 a.u.
Number	One	One	Several	Several
Heat source	Long-lived radioactivity	Chemical reactions; adiabatic compression of gases	Extinct radioactivity	Radioactivity

TABLE 2
ORIGIN OF CARBONACEOUS CHONDRITES

1. High-iron group chondrites altered by infiltration of water, carbonaceous matter, and hydrogen sulfide from some other source (Urey, 1961).
2. Primitive material accreted at low temperatures from solar nebula (Mason, 1960, 1961; Ringwood, 1961). Other chondrites were derived from this material by heating and reduction.
3. Primitive material expelled from the sun at high temperatures (Wood, 1958), accreted at low temperatures into asteroidal-sized bodies (Wood, 1958, 1962; Fish *et al.*, 1960), altered by liquid water and sulfur compounds (DuFresne & Anders, 1962*a*).

suggests that liquid water must once have acted on these meteorites. This raises three interesting questions. First, what were the chemical and physical conditions (pH, reduction potential, and temperature) during this aqueous stage, and how long did it last? Second, what was the source material of the carbonaceous chondrites, *i.e.*, where did the high temperature minerals come from? And third, in what setting did this aqueous stage occur?

Former environment. To answer the first question, one can turn to the stability diagrams of Garrels (1960), which give the stability regions for various minerals and ions as a function of pH and reduction potential (Eh). In FIGURE 2 is shown a composite diagram based upon Garrels' data. Looking up the stability regions of the principal constituents of carbonaceous chondrites on this diagram, one finds that nearly all of them [Fe_3O_4, $(Mg,Fe)CO_3$, $MgSO_4$, S, organic matter] can coexist under equilibrium conditions at pH 8 to 10 and

Eh ≥ -0.2 V. This conclusion was reached independently by Nagy *et al.* (1962*b*). The only exception is FeS, in place of which one would expect FeS_2. It is not too difficult to find an *ad hoc* assumption that accounts for this discrepancy. For example, one can argue that the FeS was first made under conditions in which it was stable, possibly even at high temperatures, and that it was then brought in contact with solid sulfur at such low temperatures that the rate of reaction was very slow.

It is quite remarkable that the carbonaceous chondrites are so close to chemical equilibrium, because intuitively one would think of an assemblage of highly

TABLE 3
MINERALOGY OF CARBONACEOUS CHONDRITES*†

	Orgueil	Ivuna	Haripura	Cold Bok.	Mighei	Murray	Ornans	Lancé	Mokoia
Wiik's class	I	I	II	II	II	II	III	III	III
Subclass	A	A	B	C	C	C	D	D	E
High Temperature Minerals									
Clinopyroxene						3			
Olivine		?	3	3	1	1	0–1	0–1	1
α-Iron			9		10	10	7	5	
γ-Iron								10	
Magnetic troilite	5	5					5	5	
"Characteristic" Minerals									
Orgueil LM	1	1							
Magnetite	1	1	3	3	11‡		?	?	2
Murray F				1	1	1			
Haripura M			1						
Mokoia HT and SW									5
Epsomite	3	3	*6*	*6*	*6*	*6*	*>16*	*10*	*6*
Sulfur	*6*	*6*	*6*	*9*	*9*	*9*	*>20*	*13*	*6*
Trace Minerals									
Dolomite	9	8							
Breunnerite	10								
Pentlandite					11				

* After DuFresne and Anders (1962*a*).

† Estimated abundances are given as negative logarithms of 2. Thus Mighei is about 50 per cent olivine and 50 per cent "Murray F" mineral, with mere traces of iron, pentlandite, magnetite, epsomite, and sulfur. Italicized values are of lower accuracy.

‡ Trace associated with metallic iron.

oxidized ($SO_4^=$, Fe_3O_4, $CO_3^=$) and reduced (S, FeS, C, organic matter) species as being far from chemical equilibrium. The source for the basic pH might be ammonia, and for the negative Eh, hydrogen ($\leq 10^{-7}$ atmos.). Both would conveniently disappear as the water evaporated.

The temperature at which the aqueous stage occurred is a little harder to determine. A lower limit near 0° C. is implied by the condition that the water was liquid; an upper limit of 200° to 400° C. is provided by various other observations, *e.g.*, the strained glass found in the Mighei carbonaceous chondrite (DuFresne and Anders, 1961). As shown in FIGURE 3, the strain disappears after annealing for 48 hours at 206° C., so that after the incorporation of this

glass into the meteorite the temperature of Mighei could never have exceeded 206° C. for as long as 48 hours. Other time-temperature combinations can be read off the graph, although it is doubtful whether any extrapolation beyond the measured points is valid. One can infer that temperatures were much lower from the fact that the characteristic minerals are quite finely grained, judging from the diffuseness of their x-ray diffraction patterns. It seems likely that the aqueous stage occurred at approximately room temperature. There is hope of obtaining a more accurate value by measuring the O^{18}/O^{16} fractionation between carbonate and magnetite (Clayton, 1962). Presumably the

FIGURE 1. A fragment of Orgueil, showing white vein of magnesium sulfate running horizontally across specimen. This vein must have deposited from water solution, thus offering evidence of the one-time presence of liquid water in the meteorite parent body. (Reproduced from DuFresne and Anders, 1962*a*, with permission of the editor.)

carbonate was made during the aqueous stage, by the action of CO_2 on basic oxides. The CO_2 was, in turn, probably evolved from the interior of the body during reduction of iron oxides to metallic iron. If the carbonate and magnetite reached isotopic equilibrium during the aqueous stage, the temperature of this stage may be determined by means of Urey's paleotemperature method.

A clue to the duration of the aqueous stage is given by the relatively high degree of ordering of the Ca^{++} and Mg^{++} ions in the dolomite from Orgueil and Ivuna. From a comparison with terrestrial dolomites, Goldsmith has estimated a formation time of $>10^3$ years.

Ancestral material of carbonaceous chondrites. It is a little harder to get an answer to the second question, concerning the origin of the high temperature minerals. Edwards and Urey (1955) and Urey (1961) have pointed out that

the carbonaceous chondrites have a variable, and frequently lower, content of Na and K than the ordinary chondrites. In the most extreme case, Nogoya, this depletion amounts to a factor of ~4. Urey, therefore, suggested that the carbonaceous chondrites were derived from the ordinary chondrites [specif-

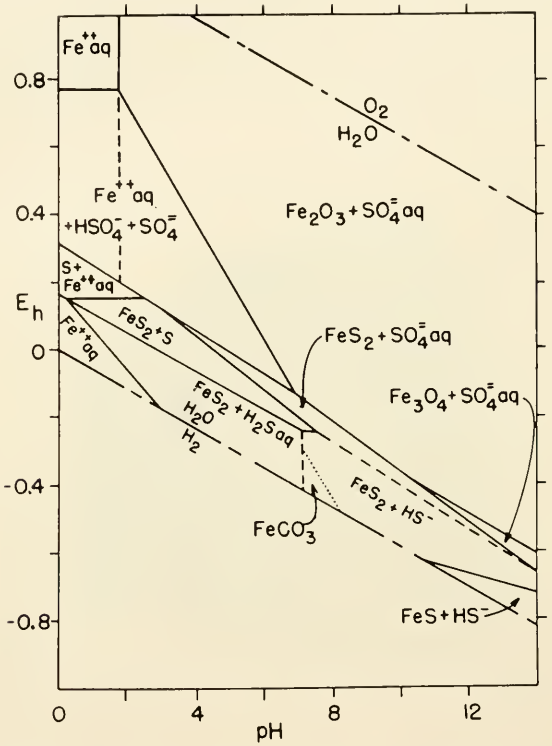

FIGURE 2. Stability relations among some of the important constituents of carbonaceous chondrites, as a function of reduction potential and hydrogen ion concentration. *Solid lines* show boundaries between solids and aqueous species at an activity of the latter of 10^{-6} M; *dashed boundaries*, those between aqueous species at 1:1 ratios. Temperature = 298° K.; total pressure = 1 atmos. Total activity of dissolved sulfur species = 0.1; of carbonate species, 0.01. Most of the constituents of carbonaceous chondrites could coexist under equilibrium conditions at Eh ~ −0.2 and pH 6 to 10. The exceptions are FeS (in place of which FeS_2 would be expected) and $(Mg,Fe)CO_3$. The absence of FeS_2 was discussed in the text. The presence of $(Mg,Fe)CO_3$ is not surprising: although pure $FeCO_3$ is unstable under the particular conditions indicated, magnesium-rich breunnerite is likely to be stable. Also, an increase in the total carbonate, and a decrease in the total sulfur activity will make $FeCO_3$ stable in the triangular field bounded by the *dotted line*. This figure has been adapted from Garrels (1960), figures 6.11, 6.18, 6.19, 6.20, and 6.21. (Reproduced from DuFresne and Anders, 1962*a*, with permission of the editor.)

ically, the high iron group, Fe/Si ≈ 0.85, Urey and Craig (1953)], by an alteration process that depleted the alkalis while introducing S, C, and a few other elements in free or combined form.

This picture has become less satisfactory now that the abundances of various trace elements in meteorites have been determined. Most elements occur in meteorites in approximately their "cosmic" abundances, as given by the semi-

empirical abundance curves of Suess and Urey (1956) and Cameron (1959). Other trace elements, including most chalcophile ones, do not conform to this pattern. They occur in approximately their predicted abundances in carbonaceous chondrites, but are depleted by factors of up to 1000 in ordinary chondrites (FIGURE 4). If the carbonaceous chondrites were derived from ordinary chondrites, as suggested by Urey, one would have to assume that the depleted elements were somehow added to the carbonaceous chondrites during the alteration process. In that case, it would be a remarkable coincidence if 6 of the 7 elements happened to be restored to just their cosmic abundances. (The seventh, mercury, may be exceptional because of its high

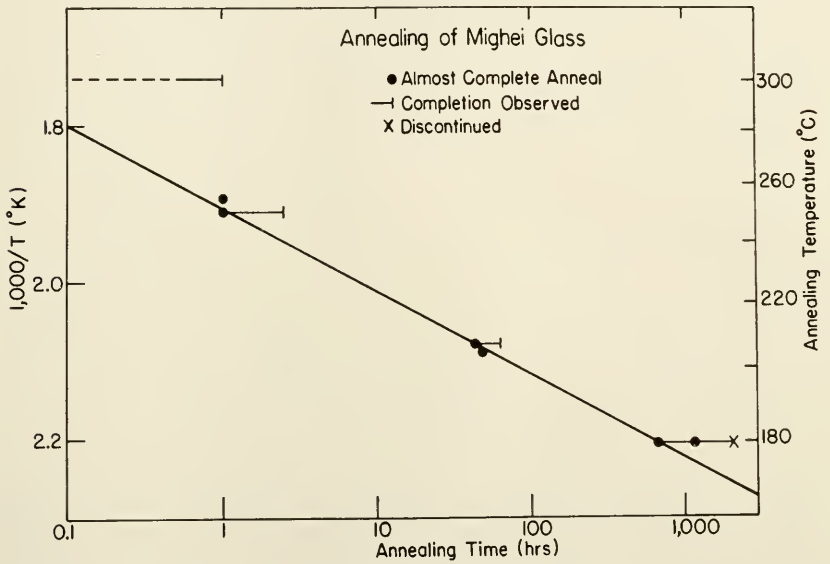

FIGURE 3. Annealing of strained glass from Mighei carbonaceous chondrite. After the incorporation of the glass, the meteorite cannot have been heated to temperatures as high as 206° for as long as 48 hours, or the strain would have disappeared. (After DuFresne and Anders, 1961.)

volatility, but it should be noted that the point in FIGURE 4 is based upon a single measurement.)

The olivine in carbonaceous chondrites has a highly variable iron content (Ringwood, 1961), whereas it is of nearly constant composition in ordinary chondrites (Mason, 1962). This factor, too, makes it difficult to derive carbonaceous chondrites from ordinary chondrites by any simple process.

Another clue comes from the primordial noble gases which seem to be present in all carbonaceous chondrites (FIGURE 5). All meteorites contain noble gases produced by cosmic rays or the decay of long lived radioactivities, but the carbonaceous chondrites also contain primordial noble gases that can be distinguished from cosmogenic or radiogenic noble gases by their isotopic and elemental composition (Stauffer, 1961; Anders, 1962*b*). With the exception of He^4 and Ar^{40}, most of which is radiogenic, the noble gases in an ordinary chondrite

are produced chiefly by the action of cosmic rays on iron, silicon, and other stable elements in the meteorite. For example, the 3 neon isotopes are made in nearly equal amounts in this process (Eberhardt and Eberhardt, 1961) whereas in primordial neon (represented by neon in the earth's atmosphere) the ratio $Ne^{20}/Ne^{21}/Ne^{22}$ is 90.8/0.26/8.9. The elemental ratios differ too, as can be seen in FIGURE 5. The bulk of the primordial noble gases once associated with the matter of the terrestrial planets and the asteroids seems to have been lost at a very early stage in the history of the solar system. It is not very plausible to assume that these gases were first lost from the ordinary chon-

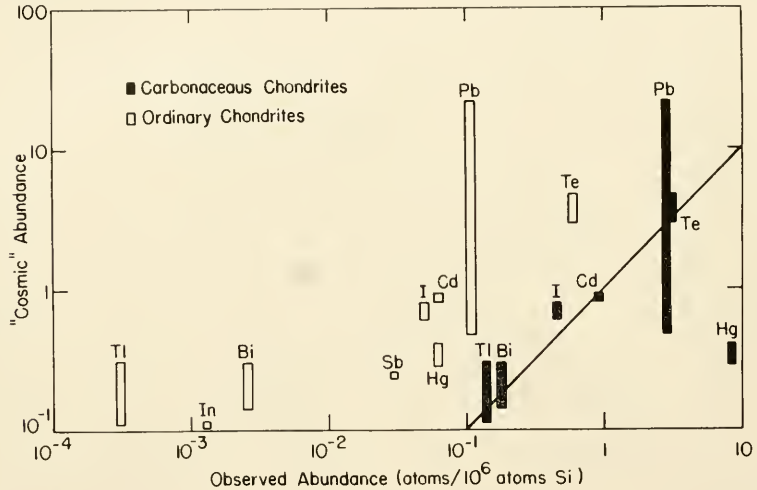

FIGURE 4. Trace element abundances in carbonaceous chondrites and ordinary chondrites. Although strongly depleted in ordinary chondrites, most of these trace elements occur in carbonaceous chondrites in nearly their "cosmic" abundances. This suggests that carbonaceous chondrites are more closely related to primordial matter than the ordinary chondrites. [Data were taken from the following sources: Bi, Hg, Pb, and Tl, Reed *et al.* (1960), and Ehmann and Huizenga (1959); Cd, Schmitt (1961); I and Te, Goles and Anders (1962); In, Schindewolf and Wahlgren (1960); Sb, Anders (1960).]

drites, then stored somewhere, and finally incorporated somehow in the carbonaceous chondrites.

The spheroidal troilite and magnetite particles found in Orgueil also suggest a high-temperature stage (Fitch *et al.*, 1962). Their chemical identification was confirmed by electron microprobe analysis (Smith, 1962). Spheroidal particles might be expected from the condensation of vapors in the liquid field, but in the presence of cosmic proportions of hydrogen, metallic iron rather than FeS or Fe_3O_4 would result (Urey, 1952). Such "primary" metal spherules might be transformed to FeS or Fe_3O_4 by the action of H_2S or H_2O at lower temperatures. It is interesting that Sztrókay *et al.* (1961) have observed spherical, opaque particles in olivine chondrules from the Kaba carbonaceous chondrite. Similar particles are found in chondrules of many ordinary chon-

drites as well (Fredriksson, 1962). Alteration of the olivine by water would release these spherules, possibly in altered form, from their chondrule matrix. But it is also possible that the spherules formed at a later stage. The particles in Orgueil are quite similar to the troilite globules in meteorite veins (Anders and Goles, 1961) and may well be of similar origin. The association of many

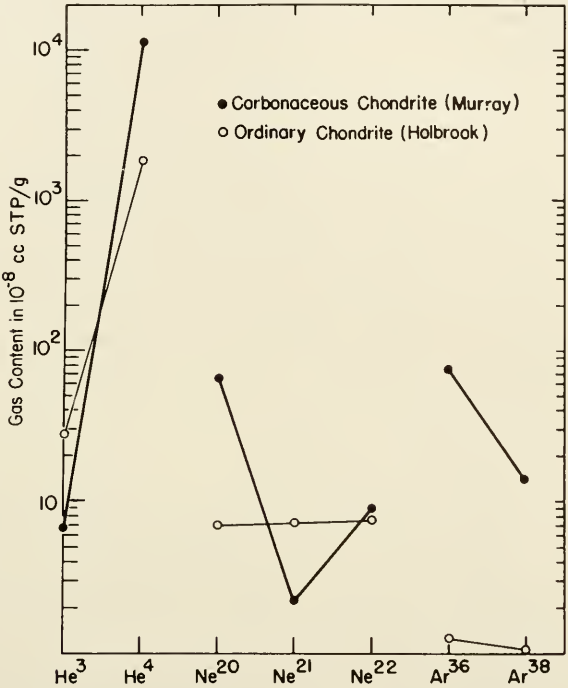

FIGURE 5. Noble gases in a carbonaceous and an ordinary chondrite. In Holbrook, these gases (except for radiogenic He^4) are produced by cosmic-ray induced spallation reactions on iron and other stable nuclides. The 3 neon isotopes are made in nearly equal abundance. In Murray, the isotopic abundances resemble those in Earth's atmosphere, suggesting that these gases, too, are of primordial origin. A small amount of cosmogenic gas is present in Murray as indicated by the increased abundances of He^3 and Ne^{21} relative to their atmospheric abundances.

of the Orgueil spherules with firmly attached silicate fragments is consistent with either hypothesis.

The trace element abundances, the variations in the olivine composition, and the primordial gas content are most easily explained by assuming that both the carbonaceous chondrites and the ordinary chondrites were derived from still more primitive ancestral matter. Perhaps the most embarrassing requirement for this material is that some of it at least must have passed through an earlier, high-temperature stage without losing its primordial gases completely.

It is possible to accomplish this in the meteorite parent body, but some special assumptions are required (DuFresne and Anders, 1962*b*). A more

attractive possibility is offered by Wood's (1958, 1962) hypothesis, according to which planetary matter, expelled from the sun at high initial temperatures, cooled by adiabatic expansion, so that progressive expansion could take place. The least volatile constituents would condense to high-temperature minerals (olivine, pyroxene, nickel-iron, and later, magnetite), which would trap some of the surrounding primordial gas. Other substances, *e.g.*, H_2O, NH_3, and carbon compounds, would condense on temperature drop. The further accretion of the (now cold) dust into solid bodies, and the separation of the solids from the noncondensable gas would proceed along the path outlined by Urey (1952, 1954, 1956, 1957, 1958) or Fish *et al.* (1960). Incidentally, if such a high-temperature stage ever took place, then cometary matter, too, must have passed through it. This raises some new possibilities in regard to the mineral composition of comets. In particular, the presence in comet tails of metal (or magnetite?) spherules, inferred from scattered light and polarization measurements (Liller, 1960), is somewhat easier to understand if part of the cometary material had a high temperature history, even though its final accretion occurred at low temperatures. This view gains further support from the discovery in cosmic dust of metal flakes with amorphous organic attachments. The fall dates of these particles seem to be correlated with several meteor showers of cometary origin (Parkin, Hunter, and Brownlow, 1962). Perhaps Herbig's (1961) suggestion that the carbonaceous chondrites were derived from comets should be re-examined in the light of this possibility.

Aqueous stage and the prerequisites for life. What about the third question, the setting in which the aqueous stage took place? This is one point in which the large planet hypothesis has an advantage over all others. A planet of terrestrial size can hold water vapor gravitationally, and can maintain bodies of liquid water, from ponds to oceans. Surely, the surface temperature must be high enough to allow liquid water to exist, but the temperature is controlled not only by the distance from the sun, but also by the composition of the atmosphere. If Venus, with its CO_2-rich atmosphere, were located in the asteroidal belt, it would have a comfortable surface temperature near 300° K., instead of the 600° K. prevailing at its present location. If it were not for the fact that the planetary hypothesis runs into so many other difficulties (Anders and Goles, 1961), one could stop here.

Of all the parent bodies discussed, the asteroids are least likely to retain liquid water *at their surfaces*, owing to their small size and consequent low escape velocities. But there is a way in which they could retain liquid water in their interiors. If the asteroids were ever heated by an internal heat source (*e.g.*, extinct radioactivity), some temperature distribution resembling the curves in FIGURE 6 would result. The surface temperature of the body would be controlled by the amount of solar radiation reaching it, and might be around 100 to 200° K. Farther inward, the temperature would rise until the melting point of ice was reached. Liquid water could exist in this zone, down to a depth at which the boiling point at the prevailing pressure was reached. In FIGURE 7 is shown the location of this zone of liquid water for a body with a central temperature of 1900° K. In this case, some 5 per cent of the volume of the body will contain liquid water.

The water will not last forever, of course. Above the zone of liquid water,

there will be a permafrost zone,* and the ice from this zone will evaporate at a rate determined by its vapor pressure (Watson *et al.*, 1961). The vapor pressure depends upon the temperature, which in turn depends on the distance from the sun. For a body with 100-km. radius, with an initial water content of 10%, these times are indicated in TABLE 4.

Unfortunately, this water zone is located in a dark, underground region, where photosynthetic organisms could not grow or reproduce. To support

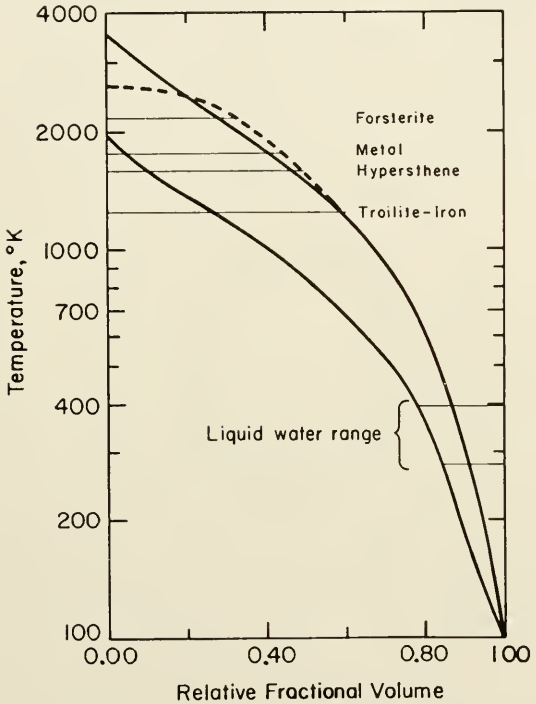

FIGURE 6. Temperature distribution of asteroids heated by radioactivity or some other uniformly distributed internal heat source. The 2 *solid curves* are calculated for different heating rates, assuming heat transport by conduction only; the *dashed curve* includes an allowance for convective heat transport as well. In all 3 cases, some 5 per cent of the body will find itself in the temperature range 273° to ~400° K., in which liquid water can exist. Melting points of important meteorite minerals are indicated by horizontal lines. (Reproduced from Fish *et al.*, 1960, with permission of the editor. Copyright, 1960 by the University of Chicago.)

life, some source of free energy must be available. Sunlight could provide this free energy indirectly, if some mechanism existed for bringing photosynthetic products from the surface to the interior. It is hard to see how this might be accomplished without a liquid vehicle. Hence, the principal remaining possibility is to derive the free energy from a local source, as first suggested by Sagan (1961). A nonequilibrium assemblage of minerals might provide such a source.

* This permafrost zone can serve to retain an "internal atmosphere" within the meteorite parent body, and may have played a role in the retention of noble gases (DuFresne and Anders, 1962*a*,*b*).

The free energy change in the conversion of high-temperature minerals to characteristic minerals cannot be calculated with any accuracy, because no thermodynamic data exist for the latter or their terrestrial counterparts, the serpentine and chlorite minerals. As a crude approximation, the following reaction may be considered:

$$Mg_2SiO_4 + H_2O\ (l) \rightarrow MgSiO_3 + Mg(OH)_2$$

for which ΔF°_{298} is -20 kcal. per mole. This corresponds to about 0.1 kcal. per gram of olivine, and because the products in this reaction are capable of

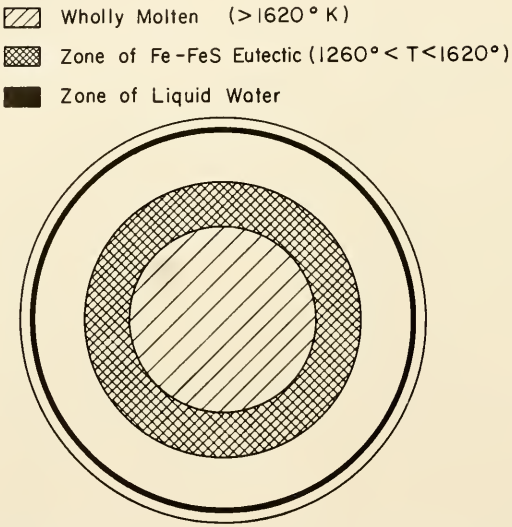

FIGURE 7. Temperature distribution in an internally heated asteroid, for a central temperature of 1900° K. The location of the zone of liquid water is indicated.

TABLE 4
TIMES FOR WATER LOSS FROM ASTEROIDS

Distance	Temperature	Evaporation, rate	Time
a.u.	°K.	*g cm⁻² yr⁻¹*	*years*
2.17	159	0.16	2.1×10^{6}
2.77	141	1.6×10^{-3}	2.1×10^{8}
4.26	113	3.1×10^{-8}	1.1×10^{13}

reacting further to give hydrated silicates, this value is probably conservative. A chondrite of the type suggested as a possible precursor of the carbonaceous chondrites, *e.g.*, Ornans or Warrenton, contains more than 75 per cent olivine on a normative basis. Thus, although possible contributions by the other minerals are neglected, the average amount of free energy released in the formation of the characteristic minerals is likely to be close to 0.1 kcal. per gram.

The extractable organic matter in Orgueil comprises about 10 per cent of the total carbon content (3.1 per cent, Wiik, 1956). Thus, approximately 3×10^{4} calories would be available for each gram of organic matter, assuming that none

of this energy is wasted by direct reactions between the minerals. At most, only a few thousand calories per gram would be required to produce biochemical compounds from simpler starting materials. If some form of life arose at this point, the remaining chemical energy could sustain it for many generations.

Any such life form would be doomed from the outset, because its energy supply, once exhausted, would no longer be replenished. But the total amount of energy available from this source is appreciable. For a liquid water zone comprising 5 per cent of the volume of a 100-km. body, as much as 8×10^{19} cal. could be stored in this manner. At a typical asteroidal distance of 2.8 a.u., this corresponds to the total solar energy received by the body in 2×10^3 years.

Of course, the futility of a doomed subterranean life form based upon a finite supply of energy makes it less appealing to the human mind than a photosynthetic form with a life expectancy approaching that of the planet or its central star. But if life arose by a spontaneous event, without guidance from above, then the probability of this event would have depended upon the chemical and physical conditions in the environment only, and not upon the perpetuity of the energy supply.

The suitability of asteroidal bodies as abodes of life would thus seem to hinge mainly on three questions. First, were the times for water retention (TABLE 4) long enough for life to arise spontaneously? All we known about this "induction period" for the origin of life is that it lasted less than 0.5 AE on Earth (Kulp, 1961). Hence the asteroids cannot be disqualified on this count alone. Second, were the necessary organic compounds present? From the work of Calvin and Vaughn (1960), and Briggs (1961), it seems that this question can be answered in the affirmative, although Degens and Bajor's (1962) observations on the bacterial production of some of these compounds may require a reevaluation of the evidence. Third, could the initial life forms learn to utilize the particular inorganic energy sources present (*e.g.*, reactions of H_2O with olivine, Fe°, etc.)? No definite answer to this question is possible, although it is perhaps relevant to point out the known, high adaptability of modern terrestrial microorganisms.*

Thus, one cannot conclude *a priori* that the asteroids were never capable of supporting life. The question of whether life ever existed in meteorites may, therefore, be examined on its own merits, because the size of the parent body does not impose any major limitations.

Isotope measurements. Further clues to the history of these meteorites come from isotope measurements, although the interpretation of the data is not always free from ambiguities. If we assume a simple, monotonic cooling history for the meteorites, the K^{40}/Ar^{40} ages in TABLE 5 give the time at which the temperature of the meteorite fell to a low enough value to permit the retention of radiogenic Ar^{40} from the decay of K^{40}. Judged from the heating experiments of Stauffer (1961), interpreted according to the model of Goles *et al.* (1960), this temperature probably lies near 200° K. Of course, short K-Ar ages would also result if the meteorite were reheated at some later stage in its

* If such subterranean life forms ever arose on the meteorite parent bodies, they are likely to have arisen on Earth and on the moon as well. This would somewhat reduce the chances of finding prebiotic organic matter on the moon (Sagan, 1961). Moreover, much of the Earth's initial endowment of organic matter would have been transformed by biological activity at a very early stage in its history.

history (*e.g.*, during close approaches to the Sun), or if its parent body happened to remain at a temperature somewhat above, say, 200° K., where slight, but continuous argon losses by diffusion would occur.

That the short exposure ages are not due to diffusion losses at perihelion has been shown conclusively at least for Cold Bokkeveld (Anders, 1962*c*). Here, the content of a nonvolatile cosmogenic nuclide, Al^{26}, is consistent with the Ne^{21}

Table 5
Ages of Carbonaceous Chondrites

Meteorite	Group	K-Ar age	Cosmic ray exposure age
		AE	*m.y.*
Cold Bokkeveld*	C	1.2	0.2
Felix†	D	4.5	56
Felix*	D	4.1	48
Ivuna†	A	1.4	1.6
Lancé†	D	<3.9	5
Mighei‡	C	4.3	
Mighei*	C	2.4	2.4
Mokoia†	E	3.4	13
Murray†	C	2.5	4
Murray*	C	1.6	4
Orgueil*	A	1.3	3

* Zähringer (1962).
† Stauffer (1961).
‡ Gerling and Rik (1955).

Table 6
Carbon Isotopic Composition in Carbonaceous Chondrites (Boato, 1954)

Meteorite	Class	C	δC^{13}
		%	‰
Ivuna	A	3.3	−6.6
Orgueil	A	2.8	−11.4
Cold Bokkeveld (London)	C	1.55	−9.4
Cold Bokkeveld (Paris)	C	1.6	−5.2
Mighei	C	2.6	−9.9
Murray	C	1.9	−3.9
Lancé	D	0.34	−15.7
Mokoia	E	0.84	−17.4
Forest City	Ordinary Ch.	0.08	−24.3
Richardton	Ordinary Ch.	0.02	−24.6

content, so that diffusion losses of the latter seem to be ruled out. The short exposure age (0.1 to 0.2 m.y.) would seem to suggest a lunar origin, as proposed by Urey (1962), but this hypothesis has its difficulties (Anders, 1962*c*).

Other isotope measurements exist that have a bearing on the origin of carbonaceous chondrites. Boato (1954) has measured the carbon isotopic composition in these meteorites (TABLE 6). The C^{12}/C^{13} ratio is variable from meteorite to meteorite, and even within the same meteorite (Cold Bokkeveld). It is known that living organisms have a preference for C^{12}, so that biogenic ma-

terials are generally depleted in C^{13} relative to the source material: atmospheric CO_2 or oceanic bicarbonate (Craig, 1953). This effect is quite pronounced if the biogenic carbon comprises only a small fraction of the total available carbon (FIGURE 8).

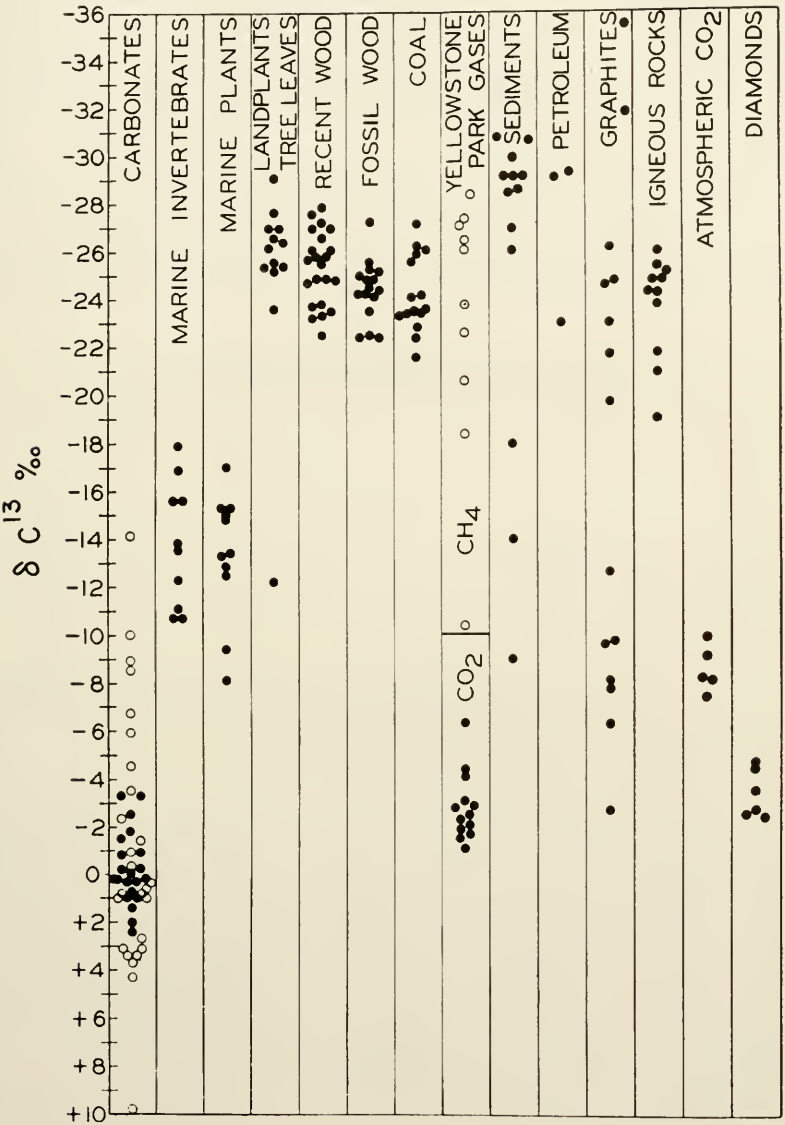

FIGURE 8. Isotopic composition of carbon from various sources. Processes involving a partial loss of carbon in the form of volatile compounds (*e.g.*, the formation of petroleum from the remains of organisms) result in the depletion of C^{13}. Such a depletion is also observed in the case of the meteorites (TABLE 6) in which the C^{13} content declines with decreasing total carbon content. [Reproduced from Craig (1953), with permission of the editor.]

Unfortunately, Boato measured only the total combustible carbon, and not the fractionation among the several forms of carbon in the meteorite. There seems to be a correlation between decreasing C^{13} content and decreasing total carbon in the meteorite. Boato suggested that this implied preferential loss of C^{13} during partial volatilization, and pointed out that a similar depletion had been observed in terrestrial processes that were accompanied by a loss of volatiles, *e.g.*, the conversion of dead organisms to petroleum. For the purpose of the present discussion, it is immaterial whether this last process is abiotic or biotic; any low-temperature process will lead to qualitatively similar fractionations.

Urey (1962) suggested that sulfur metabolizing organisms might be responsible for the oxidized sulfur compounds (S and $MgSO_4$) in the carbonaceous chondrites. However, as seen in TABLE 7, the elemental sulfur in Orgueil is enriched in S^{34} relative to the sulfate (Thode and DuFresne, 1961), whereas sulfur bacteria as well as inorganic processes occurring under equilibrium condi-

TABLE 7
SULFUR ISOTOPIC COMPOSITION IN CARBONACEOUS CHONDRITES

Object	δS^{34}(‰)	
	$SO_4^=$	S°
Orgueil*	−1.30	+3.04
Gulf Coast salt domes (11 samples)†	+41.4	+2.5
Sulfur Lake, Cyrenaica, N. Africa†	+15.8	−15.3

* DuFresne & Thode (1961).
† Thode, Wanless & Wallouch (1954).

tions tend to produce just the opposite fractionation, depleting elemental S in S^{34} (Thode *et al.*, 1954). The equilibrium constant for the reaction

$$S^{32}O_4^= + \tfrac{1}{8}\, S_8^{34} \rightleftarrows S^{34}O_4^= + \tfrac{1}{8}\, S_8^{32}$$

is 1.071 at 25° C. (Tudge and Thode, 1950), so that the sulfur and sulfate in Orgueil are clearly out of equilibrium. Perhaps the origin of the higher oxidation states of sulfur will be clarified by further isotope measurements on the troilite in Orgueil (Thode and Anders, 1962).

Boato (1954) also measured the hydrogen isotopic composition of the hydrated silicates in carbonaceous chondrites. His results (TABLE 8) show that the D/H ratio in Ivuna, Orgueil, and Mokoia was considerably higher than that in terrestrial waters. This fractionation may have been caused by kinetic isotope effects during formation of the hydrated silicates (Clayton, 1961) or by extensive evaporation of the water in the meteorite parent body.

Hydrocarbons. Finally, a few words should be said about the hydrocarbons (Nagy *et al.*, 1961). This matter has been discussed in greater detail elsewhere (Meinschein, 1961; Anders, 1961, 1962*a*; Nagy *et al.*, 1962*a*; Meinschein *et al.*, 1962). For the present discussion, only three of the most salient points will be restated.

Meinschein and his associates certainly deserve great credit for determining

the mass spectrum of the hydrocarbons in the meteorite, and for drawing attention to its possible resemblance to the mass spectra of biogenic hydrocarbons. One point on which we disagree, however, is the extent of such resemblance. FIGURES 9 and 10, plotted from their data, show the worst and the best cases, respectively. If the comparison is extended to the entire mass spectrum, and to a larger variety of biogenic reference materials, certain additional resemblances, but also certain differences appear. It seems very difficult to decide, on purely objective grounds, whether these resemblances are strong enough to prove a biological origin.

There is also a question to what extent the peak height at a given mass number may be taken as a measure of the amount of parent hydrocarbon of this mass. This is a good assumption for the $[C_nH_{2n+2}]^+$ ions derived from the C_nH_{2n+2} paraffins. But as one goes to compounds progressively poorer in hydrogen, the ambiguity increases. The $[C_nH_{2n-6}]^+$ ions are derived not only from the C_nH_{2n-6} (=tetracycloalkane) series, but also from the C_nH_{2n+2},

TABLE 8

HYDROGEN ISOTOPIC COMPOSITION IN CARBONACEOUS CHONDRITES (BOATO, 1954)

Meteorite	Class	H_2O	δD
		%	%
Ivuna	A	7.0	+35.8
Orgueil	A	7.3	+29.0
Cold Bokkeveld (London)	C	7.8	−13.0
Cold Bokkeveld (Paris)	C	8.0	−5.8
Mighei	C	8.6	−6.4
Murray	C	6.8	+9.6
Lancé	D	0.9	−7.7
Mokoia	E	0.8	+25.9
Terrestrial waters			−15 to +5

C_nH_{2n}, C_nH_{2n-2}, and C_nH_{2n-4} families, with possible additional contributions from nitrogen and oxygen compounds. Thus, it seems fair to attribute most ob the observed peak height in the C_nH_{2n+2} series to paraffins. However, just in the case of this series, the resemblance is rather poor (FIGURE 9). and the great difference between the spectra of the original Orgueil distillate (Nagy *et al.*, 1961) and the chromatographically separated hydrocarbon fraction (Meinschein, 1961) shows that even in this favorable case, some 70 to 90 per cent of the originally observed peak height came from compounds other than saturated hydrocarbons.* In FIGURE 10, the resemblance is very good, and the changes have been moderate, but as pointed out, the peaks in this series contain substantial contributions from so many different sources, that it seems unsafe to infer a similarity in parent hydrocarbon distribution from a similarity in peak heights.

Finally, one must not overlook the possibility that the observed hydrocarbon

* This sample is not strictly comparable to the original distillate, collected in the range 250° to 400° C., because it also contains the 400° to 500° C. fraction. But differences of the same order are found between the original distillate and a chromatographically separated hydrocarbon fraction of a solvent extract of the whole meteorite (Meinschein *et al.*, 1962).

distribution was made abiotically by Miller-Urey type reactions in the solar nebula. Such reactions are known to produce carbon chains of varying length, presumably by free radical reactions. The hydrocarbons in comets and the organic material in cosmic dust (Parkin *et al.*, 1962) may have been produced in this way. Meinschein (1961) has argued that such reactions would be highly nonselective, showing little preference among the billions of possible

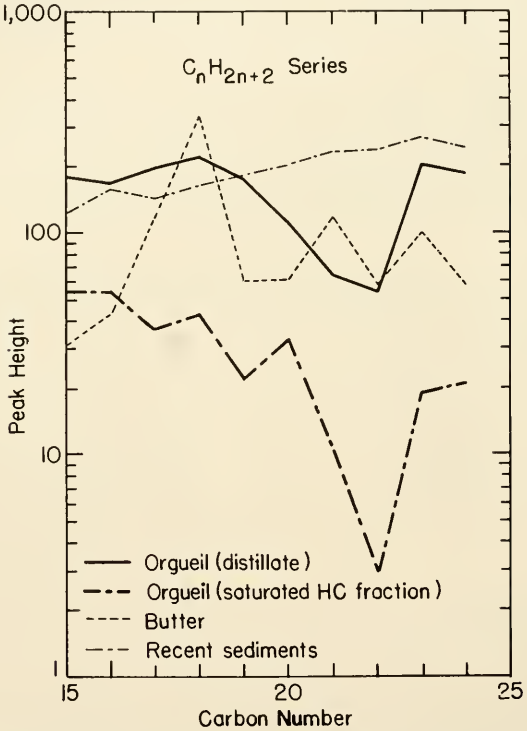

FIGURE 9. Mass spectrum of meteoritic hydrocarbons and 2 biogenic reference materials (Nagy *et al.*, 1961). The observed peak heights in the C_nH_{2n+2} series are probably due, mainly, to parent ions of saturated hydrocarbons, although fragment ions of other substances also contribute. The difference between the original Orgueil distillate and the chemically separated, saturated hydrocarbon fraction indicates that large amounts of other substances were present in the distillate.

isomers. But it is essential not to equate the concepts "abiotic" and "nonselective." Industrial chemical syntheses, from polyethylene to medicinals, are highly selective, favoring one or a few products over the multitude of others. Even Miller-Urey type reactions can be quite selective, as shown by Wilson (1960). He obtained products mainly in the mass ranges C_1 to C_5 and C_{20} and up. Although the product distribution in that particular experiment (and in the industrial Fischer-Tropsch synthesis of hydrocarbons) may not be an accurate match of the Orgueil hydrocarbon distribution, one must remember that only an infinitesimal fraction of the possible combinations of conditions

(composition, temperature, pressure, time, energy input, catalysts, availability of surfaces, etc.) has been explored.

Some chemical evidence has become available on the Orgueil hydrocarbons (Yang and Tsong, 1962). A cyclohexane extract of the meteorite shows nothing but C—H groups in its infrared spectrum, indicating that it consists mainly of hydrocarbons. The ultraviolet absorption spectrum shows a broad band near 270 mμ, but virtually no absorption above 300 mμ. Hence, aromatic ring systems larger than naphthalene or biphenyl seem to be ruled out. Presumably, 1- and 2-ring aromatic systems with aliphatic side chains are present. Chromatographic separation on silica gel resolved in the material into 5 spots, 2 of which fluoresced weakly under ultraviolet light. The material possessed a strong, terpene-like odor. More complex materials, including polynuclear

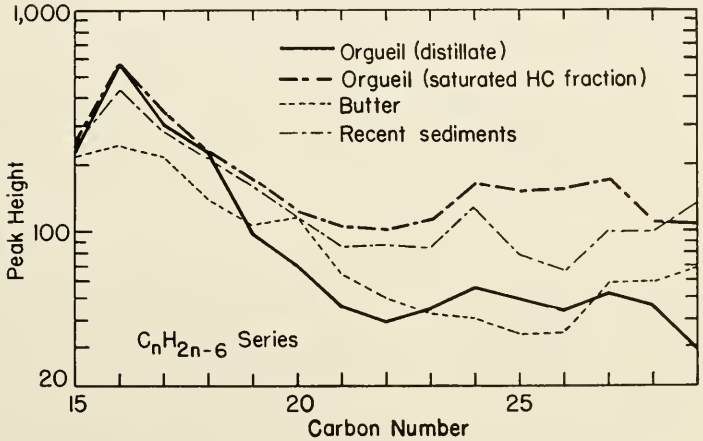

FIGURE 10. Mass spectrum of meteoritic hydrocarbons and 2 biogenic reference materials (Nagy *et al.*, 1961). In the C_nH_{2n-6} series, the meteoritic and terrestrial mass spectra show a strong resemblance to each other, but because the contribution of fragment ions to the peaks is quite large in this series, the similarity in peak heights does not necessarily imply a similarity in hydrocarbon distribution.

hydrocarbons of higher molecular weight, bearing polar substituents, were extracted from the meteorite with more polar solvents, but it seems that none of these higher polynuclear hydrocarbons were present in the free state. This relative simplicity of the aromatic hydrocarbon fraction was already noted by Meinschein *et al.* (1962), on the basis of mass spectrometric analysis.

Perhaps the hydrocarbons in Orgueil are of biogenic origin. But in our opinion, the present evidence is not sufficient to justify this conclusion.

Summary

The carbonaceous chondrites seem to have been produced by the action of liquid water on a more primitive source material. Their mineralogy implies that this exposure to water occurred at temperatures near 300° K., a pH of 6–10, a reduction potential of ≤ -0.2 volts, and that it lasted for at least 10^3 years (DuFresne and Anders, 1962). Their high content of chalcophile trace ele-

ments and primordial noble gases suggests a source material more primitive than ordinary chondrites; yet the presence of high-temperature minerals implies that this source material passed through at least one high-temperature stage. These conditions would be satisfied by material expelled from the sun in a gaseous state (Wood, 1958), and accreted to solid bodies after condensation and cooling (Urey, 1952).

The exposure to liquid water could have occurred in subsurface regions of an asteroid heated by extinct radioactivity or another internal energy source. Sunlight for photosynthesis would not reach these regions, but an appreciable amount of free energy would be available from the conversion of olivine to hydrated silicates. Although this source of energy is finite, it may have served as the basis for the evolution of a nonphotosynthetic life form. None of the isotopic data suggest the presence of life, however. The fractionation between sulfur and sulfate in Orgueil is in the opposite direction from that observed for terrestrial sulfur bacteria. The carbon data are inconclusive, having been determined on the total combustible carbon only, rather than on individual compounds or fractions. The hydrocarbon data are also not conclusive, since the degree of resemblance to biogenic hydrocarbons and the ability of nature to produce such a hydrocarbon distribution by purely abiotic (Miller-Urey) reactions are still open to dispute.

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AQUEOUS, LOW TEMPERATURE ENVIRONMENT OF THE ORGUEIL METEORITE PARENT BODY

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Sources of knowledge of the composition of the universe have been limited to (1) information which can be deduced from radiated energy and nuclear particles, and to (2) the results of the studies of meteorites. The presence of hydrous silicates, iron oxide, water soluble salts, and organic matter in the carbonaceous chondrites makes the study of this rare group of meteorites especially intriguing.

There are 19 known carbonaceous chondrites (TABLE 1). All 19 meteorites were observed to fall. They all show a few millimeters thick fusion crust. Various investigators have found, however, that below the crust the stones are unaltered. Carbonaceous chondrites usually have loose textures and many of them have a mineralogical composition indicating that they were never subjected to temperatures higher than 300 to 400° C.

The Orgueil meteorite, the principal object of the present investigation, fell at 8 P.M. on May 14, 1864. Fragments were collected in and about the villages of Orgueil, Nohic, and Campas in southern France. The appearance of the luminous meteor, the subsequent detonations and the fall were observed by the local residents. It is reported that the sound of the detonations was heard within an area of approximately 75-miles radius. The combined weight of the fragments which are now in various museum collections is approximately 11.5 kg. Based upon present knowledge of the attrition a stony meteorite undergoes when it enters the atmosphere and the loss of fragments scattered by the explosions that accompany most of these falls, it is probably safe to assume that the Orgueil stone weighed several tons before it fell to earth.

Carbonaceous chondrites are characterized by the few per cent of carbonaceous matter that they contain, by their water content, and as Urey and Craig[1] pointed out, by the highly oxidized state of their iron content. Mere traces of carbon and water, however, have been found in a large number of stony and in a few metallic meteorites, all of which had high temperature histories. Only 4 of the 19 carbonaceous chondrites have been subjected to organic analysis. As recently as 1956, Wiik[2] observed that"... the organic compounds are the least well known substances in the carbonaceous chondrites." This lack of information is probably caused by the fact that only small quantities of organic matter can be extracted from these chondrites and that this organic substance is difficult to analyze. There are a number of early investigations of varying reliability described in the literature; most of these vaguely refer to "bituminous" substances, specifying odor, color, etc.

Organic Analyses

Meteorite organic analyses may be divided into two types: (1) the classical type analyses (which may involve the combustion of the organic matter and the subsequent gravimetric determination of CO_2, the reacting of the extracts with acids or alkalies); and (2) the analyses which were based upon spectroscopical (infrared, ultraviolet, mass spectrometry) and chromatographic techniques. Many of the former type analyses are either incompletely recorded or seem to be unreliable for other reasons. Consequently, only 4 of these analyses will be discussed briefly. These are: Berzelius' analysis[3] of Alais in 1834; Wöhler's analysis[4] of Kaba, in 1858; Berthelot's analysis[5,6] of Orgueil, in 1868; and Mueller's analysis[7] of Cold Bokkeveld, in 1953.

Berzelius was the first to ascertain the presence of organic matter in a stony meteorite.* He suggested that the Alais organic matter resembled humic acids

Table 1
List of Known Carbonaceous Chondrites

Locality of fall	Date of fall	Locality of fall	Date of fall
Alais, France	1806	Mighei, U.S.S.R.	1889
Cold Bokkeveld, South Africa	1838	Mokoia, New Zealand	1908
Crescent, United States	1936	Murray, United States	1950
Felix, United States	1900	Nawapali, India	1890
Haripura, India	1921	Nogoya, Argentina	1879
Indarch, U.S.S.R.	1891	Orgueil, France	1864
Ivuna, Tanganyika	1938	Santa Cruz, Mexico	1939
Kaba, Hungary	1857	Simonod, France	1835
Lancé, France	1872	Staroye Boriskino, U.S.S.R.	1930
		Tonk, India	1911

or similar organic materials and observed that the meteorite disintegrated in water.

Within approximately 1 year after its fall, Wöhler obtained what was apparently an uncontaminated sample of the Kaba meteorite. He suggested that the meteorite may contain remnants of humic matter. One year later, in a shorter note,[8] Wöhler reported that he had identified bituminous material resembling ozocerite in Kaba, and stated that this matter "has undoubtedly organic origin." This rather important statement came 31 years after this same investigator had first discovered that a biochemical (urea) could be synthesized from inorganic matter. One must keep in mind, of course, that facilities for a comprehensive evaluation of organic compositions were somewhat limited in 1858.

Berthelot was the first investigator who obtained hydrocarbons from Orgueil.

* Berzelius' comments are of interest: ... "Es leidet folglich keinen Zweifel, dass der untersuchte Stein, ungeachtet aller seiner Verschiedenheiten im Aeussern, ein Meteorstein ist, welcher, aller Wahrscheinlichkeit nach, aus der gewöhnlichen Heimath der Meteorsteine herstammt." and "Giebt diess möglicherweise einen Wink über die Gegenwart organischer Gebilde auf anderen Weltkörpern?"

Gaseous, liquid, and solid hydrocarbons were found to be present after treatment with hydriodic acid.*

In 1953, Mueller reanalyzed the Cold Bokkeveld stone. (This meteorite had already been studied by Wöhler.) Mueller extracted a soft resinous substance. Reactions of the organic matter with alkalies suggested that the extract consisted basically of complex organic acids, containing some nitrogen, sulfur, and halogen. It must be pointed out, however, that the high organic halogen content has not yet been confirmed by other investigators. Mueller searched for but could not detect graphite in the organic substance; on the other hand, he observed 10 to 12 per cent crystalline sulfur. The Cold Bokkeveld meteorite was found to contain water; rehydration experiments demonstrated that this water was not a terrestrial contamination. This author also suggested that the extract resembled humic acid. The author was able to reject the carbide theory of hydrocarbon synthesis in meteorites on various experimental grounds. An alternate theory was proposed by Mueller, namely, that carbonaceous chondrites are fragmental aggregates, and that the organic matter is the result of low temperature condensation from the atmosphere of the meteorite parent body. He concluded that the temperature of the meteorite never exceeded 200 to 350° C.

There are few analyses of the second type. In 1959, Sisler[9] ran infrared spectra on an extract of the Murray meteorite and recorded carbon-hydrogen and the carbonyl absorptions. Calvin[10] obtained water extracts from Murray and Orgueil. The extracts probably contained some hydrocarbons and heterocyclic bases. It was reported that the ultraviolet absorption curves of the extracts, taken at different pH values, showed that there was a pH sensitive absorption at the wave length corresponding to the cytosine absorption. Amino acid analysis led to negative results; on the other hand, mass spectroscopical data showed what may have been hydrocarbons containing up to 12 carbon atoms.

Boato's study[11] of the distribution of the hydrogen and carbon isotopes in carbonaceous chondrites is of considerable interest. This author found that water, which was distilled from Orgueil, Murray, Ivuna, and Mokoia *in vacuo* and above 180° C. temperature, showed hydrogen isotope ratios that were definitely outside the terrestrial range. On the other hand the water which was distilled below 180° C. seemed to be a terrestrial contamination. Carbon isotope ratios were found to be similar to those on earth. Although Boato thought that the carbon compounds could not be derived from living things, the C^{13} depletion in the Orgueil meteorite which he observed is typical of the depletions found in some marine organisms. The author pointed out that carbonaceous chondrites are heterogeneous bodies, and suggested that the meteorite organic matter is indigenous.

In a recent publication[12] the results of an analysis of organic matter in the Orgueil meteorite were reported. Saturated hydrocarbon groups were identified, some of which contained up to 29 carbon atoms per molecule. The

* Berthelot's original statement reads: J'ai appliqué la même méthode à la matière charbonneuse de la météorite d'Orgueil. J'ai reproduit, en effet, quoique plus péniblement qu'avec la houille, une proportion notable de carbures forméniques, $C^{2n}H^{2n+2}$, comparables aux huiles de pétrole."

preliminary observation was made that the type of molecular species present in the meteorite hydrocarbon mixture and the molecular weight range of the mixture resembled in many important aspects the hydrocarbons in the products of organisms and in sediments on earth. Studies in progress are to extend the preliminary investigation.[12] The purpose of the present study is to determine whether the physical-chemical conditions on the meteorite parent body may have been suitable to sustain a form of life.

Inorganic Analyses

Carbonaceous chondrites contain only a small percentage of organic matter, the remainder consists of inorganic minerals. Their history can be determined most clearly only if one has a satisfactory understanding of both their organic and inorganic composition. Most stony meteorites, have been subjected to inorganic analyses. As early as 1878 Nordenskiöld[13] noted the pronounced uniformity in the chemical compositions of chondrites. In 1953, Urey and Craig[7] reviewed some 350 chemical analyses, selected the reliable ones, and came to the conclusion that chondrites fell into two distinct groups, a high and a low group as far as their total iron content and the oxidation state of their iron was concerned. They suggested that the cause of this phenomenon was related to the genesis of meteorites. The parent asteroids went through a low temperature accumulation process, a high temperature melting and evaporation process, a stage of collision with smaller objects and finally a collision of 2 asteroidal sized bodies. These authors, and later Wiik,[2] observed that the carbonaceous chondrites belonged to the high iron group. Urey and Craig suggested that the material forming the carbonaceous chondrites had been infiltrated on the parent body by water, carbon compounds, and hydrogen sulfide. It is generally agreed that more information is a necessary prerequisite to a satisfactory understanding of the genesis of these meteorites.

Wiik[2] has shown that there are three types of carbonaceous chondrites. The first type (Orgueil, Ivuna, Tonk) contains approximately 20 per cent water, approximately 22 per cent SiO_2 and 15 to 18 per cent "FeS." All forms of sulfur, including elementary sulfur, were hypothetically combined with the iron in the "FeS" reported, but x-ray diffraction data on Orgueil does not show any FeS. The second type (Cold Bokkeveld, Murray, Mighei, Staroye Boriskino) contains approximately 13 per cent water, 27.5 per cent SiO_2, and 9 per cent "FeS." Neither the first nor the second group contains any metallic iron, nickel, or cobalt. The third type (Lancé, Mokoia) contains approximately 33 to 34 per cent SiO_2, less than 1 per cent water and between 5 and 6 per cent "FeS." Metallic nickel and iron are present in the third group. Edwards,[14] using an analytical method developed by Edwards and Urey,[15] found that the sodium and potassium distributions in carbonaceous chondrites agreed with Wiik's classifications. They noted that there was one exception, the Murray meteorite, which gave abnormally low alkali metal values.

Inorganic analyses of meteorites point out certain important relationships, which can serve to supplement mineralogical data. Structural and synthetic mineralogy, an active field of study during the preceding 15 years, has been repeatedly applied with success to investigations concerned with determining the physical-chemical environment during rock and mineral genesis. The

identification of the mineral content of noncarbonaceous chondrites is usually a rather straightforward process. Mineral analysis in carbonaceous chondrites is more complicated.

In 1864, Pisani,[16] who was one of the first analysts of Orgueil, noted the presence of magnetite and a "serpentine-like" mineral. More recently, Kvasha[17] reported finding chlorites in Staroye Boriskino. Stulov[18] concluded that Orgueil, Cold Bokkeveld, and Staroye Boriskino contained chlorite-serpentine type minerals. Mason[19] suggested that all carbonaceous chondrites may contain chlorites. Calvin[10] found that the water soluble salts in Orgueil and Murray were magnesium sulfate and calcium sulfate, respectively. Sztrókay, Tolnay and Földváry-Vogl[20] performed ore microscopical studies and chemical analyses on the Kaba meteorite. They suggested that carbonaceous meteorites may represent an arrested phase of meteorite development.

Layer lattice silicates (such as chlorite and serpentine) lose structural water at elevated temperatures; Mueller[7] and Boato[11] came to the conclusion that water lost at high temperature was not a terrestrial contamination. Consequently, it is probably safe to conclude that the layer lattice silicates are products of the meteorite parent body.

Experimental Studies

The experiments were designed to examine the mineral composition and, through this, the parent environment. Six stony meteorites were studied: Orgueil, Murray, Ivuna, Holbrook, St. Marks, and Bruderheim. The last 3 are not carbonaceous chondrites; they were used as controls. There were 3 different samples of the Orgueil meteorite. One sample (A) was obtained from the collection of The American Museum of Natural History, New York. Sample (A) has only recently been acquired by this museum; previously it formed part of an academic collection in the United States. The hydrocarbon analysis reported in the preliminary publication[12] was performed on sample (A). The second sample (B) was broken off from meteorite specimen No. 519 of The American Museum of Natural History. Sample (B) has been in the museum collection for several years. The third sample (C) was obtained from the U.S. National Museum, Washington, D.C. It was listed as part of meteorite specimen No. 234 and it was noted that the museum originally obtained it from S. Meunier. The samples of the Ivuna, Holbrook, and St. Marks meteorites were obtained from The American Museum of Natural History. The Murray sample was received from the Institute of Meteoritics, The University of New Mexico, Albuquerque, New Mexico, where it had been labeled as I. O. M. No. CR_1-102. The Bruderheim meteorite was obtained from the Department of Geology, University of Alberta, Edmonton, Alberta, Canada; it had been part of specimen B-79. The chemical analyses of the 3 carbonaceous chondrites are listed in TABLE 2. The analysis of 1 of the noncarbonaceous chondrites (Holbrook) is included in the table for comparison. The samples were examined for visible impurities with a microscope or by visual examination, or both.

Trace Element Analysis

The origin of carbonaceous chondrites has been discussed repeatedly since Berzelius' research in 1834. Recently, Bernal[22] proposed that the Orgueil

meteorite may be part of the primitive earth "shot off some hundreds of millions of years ago and again united to its parent body." It was, therefore, deemed necessary to determine whether Orgueil is really a meteorite of extraterrestrial origin.

Chondrites, as well as sedimentary and igneous rocks on earth, have characteristic trace element distribution patterns. Fifteen trace elements were

TABLE 2
CHEMICAL ANALYSES OF 4 METEORITES

	Carbonaceous chondrites			Noncarbonaceous chondrite
	Orgueil*	Ivuna*	Murray*	Holbrook†
Fe	—	—	—	7.18
Ni	—	—	—	1.09
Co	—	—	—	0.052
FeS‡	15.07	18.38	7.67	7.94
SiO_2	22.56	22.71	28.69	40.11
TiO_2	0.07	0.07	0.09	0.14
Al_2O_3	1.65	1.62	2.19	1.90
MnO	0.19	0.23	0.21	0.37
FeO	11.39	9.45	21.08	12.01
MgO	15.81	16.10	19.77	25.18
CaO	1.22	1.89	1.92	1.74
Na_2O	0.74	0.75	0.22	0.93
K_2O	0.07	0.07	0.04	0.10
P_2O_5	0.28	0.41	0.32	0.40
H_2O^+	19.89	18.68	9.98	0.27
H_2O^-			2.44	—
Cr_2O_3	0.36	0.33	0.44	0.45
NiO	1.23	1.34	1.50	—
CoO	0.06	0.06	0.08	—
C	3.10	4.83	2.78	—
Loss on ignition (organic matter)	6.96	4.10	0.62	—
Sum	100.65	101.02	100.64	99.98

* After Wiik.[6]

† After Mason and Wiik.[21]

‡ Includes all forms of sulfur, including elementary sulfur. There is no X-ray diffraction evidence that FeS, as such, occurs in Orgueil.

Note.

H_2O, C, and S have been reduced from the value of the loss on ignition. The oxidation of FeO, Fe, Ni, and Co have been taken into consideration. The ignition loss as given, is an approximate estimate of the amount of organic matter.

H_2O^- refers to water removed below 110° C. temperature, H_2O^+ to water obtained above that temperature.

determined in Orgueil sample (A) by emission spectroscopy. Another element, phosphorus, was determined spectrophotometrically by the molybdenum blue method. A Jarrell-Ash, 3.4 m. spectrograph (15,000 lines per inch grating) was used for the trace element analysis. All determinations were made in duplicate. Germanium was used as internal standard for cobalt, chromium, copper, manganese, nickel, and vanadium. No internal standard was used for barium, gallium, lithium, strontium, zirconium, scandium, cesium, and rubidium because these elements were below the limits of detection. No internal

standard was used for titanium. Kodak SA No. 1 (2200-4650Å) and Kodak I-N (6700–9500Å) plates were used to record the spectrum. The following wave lengths ranges were covered (1) 2200–3500Å (for Co, Cr, Cu, Ga, Mn, Ni, Ti, V, Zr, and Sc); (2) 3500–4650Å (for Ba and Sr); (3) 6700–9500Å (for Li, Cs, and Rb).

The trace element data in Orgueil are consistent with the average abundances of trace elements in chondrites. Note particularly the Ni, Cr, Co, Ti, Ba, Sr, and Rb values. Table 3 also shows that the Orgueil analysis does not agree with the average abundances of trace elements in shales and in igneous rocks (the latter is commonly referred to as the crustal abundance).* The trace

Table 3
Trace Element Abundances in the Orgueil Meteorite, in Chondrites, and in Igneous Rocks and Shales

Element	Meteorites		Terrestrial rocks	
	Orgueil carbonaceous chondrite	Chondrites*	Shales†	Igneous rocks‡
Ba	<10	8	570	1,000
Co	400	800	18	20
Cr	2,600	2,200	110	100
Cs	10	0.13	5	5
Cu	200	90	18	55
Ga	<10	5.3	13	19
Li	<3	2.7	55	32
Mn	1,900	1,900	620	1,000
Ni	11,000	13,400	64	35
Rb	<10	3.7	140	115
Sc	<10	9.4	14	20
Sr	<10	10	300	450
Ti	200	790	4,920	4,400
V	30	39	120	110
Zr	<20	33	160	156
P	790			

Concentrations in parts per million.
* After Goldschmidt.[23]
† After Shaw;[24] supplemented by Taylor and Sachs[25] with recent data from the literature.
‡ After Ahrens and Taylor.[26]

element abundances support the view that the Orgueil sample had an extra-terrestrial or precrustal origin.

Electron Microscopy

Most of the mineral particles in Orgueil were too small to be visible under the polarizing microscope. Therefore, 2 of the samples, (A) and (B), were examined with North American Phillips EM-100B electron microscopes. Detailed measurements were made on sample (A) after a survey has shown that both (A) and (B) contained particles which had identical crystal habits. Specimens were prepared by dusting with a Q-tip on Formvar film. Specimens were given

* It should be noted, that it is difficult to establish average values for trace elements in shales; this was pointed out by Shaw.[24]

a light coating of carbon evaporated under vacuum for stabilization and for improved heat conductance under the electron beam. Some specimens were shadowed with platinum.

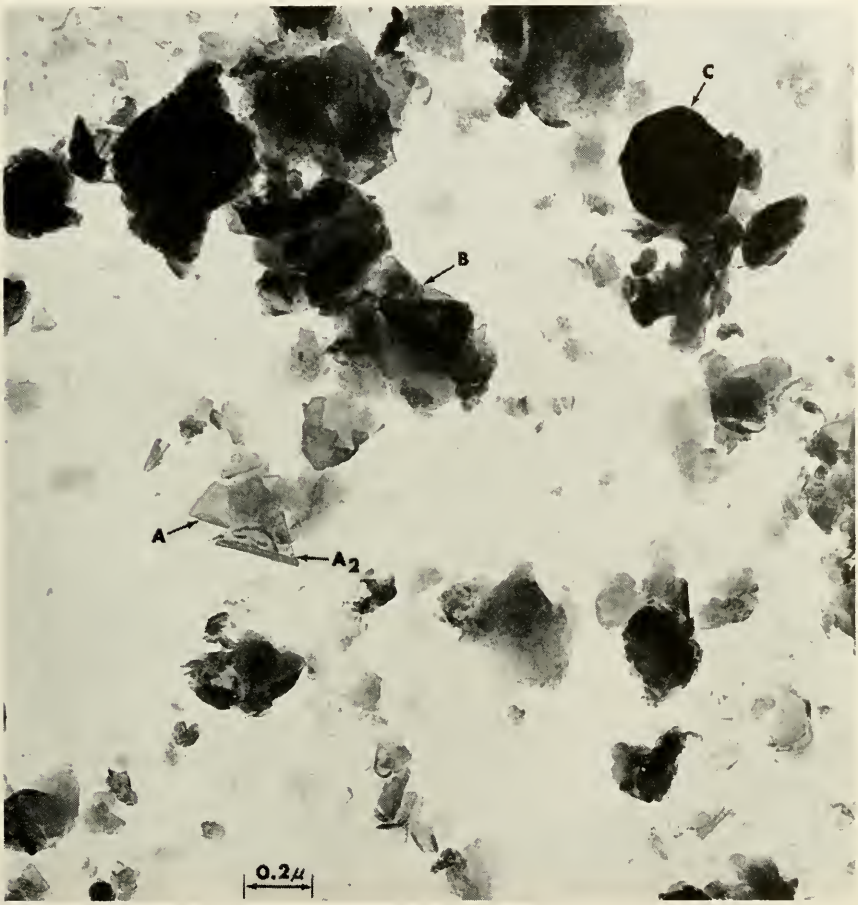

FIGURE 1. Electron micrograph of the Orgueil meteorite. *A*, micaceous particle. A_2, micaceous particle with one edge rolled up; *B*, aggregate of micaceous particles; *C*, opaque, equidimensional particle (probably magnetite).

In FIGURE 1 is shown 1 of the electron micrographs. It was found that the Orgueil meteorite consisted mainly of thin, sheetlike particles, and of the aggregates of such particles. The flaky crystals had irregular shapes. Their average particle size was approximately 0.1 to 0.2 μ. The thickness of the flakes was not estimated; it appeared that they were quite thin. Some of the flakes showed a tendency of rolling up along one or more edges under the electron beam. The flaky particles resembled layer lattice silicates, and particularly the thin, irregular, and fluffy flakes of montmorillonite clay. In addition

to the flaky mineral, a few opaque and equidimensional particles were also visible. These were probably octahedral or dodecahedral crystals of magnetite. Their average diameters were 0.2 to 0.5 μ.

X-ray and Electron Diffraction Studies

X-ray and electron diffraction techniques were used to identify the mineral matrix of the Orgueil meteorite. The x-ray data were obtained from diffractometer patterns, from manual, step-scanning counts, as well as from flat film and Debye-Scherrer photographs.

The 6 meteorites, (including 2 Orgueil samples, A and C), were x-rayed. In addition, x-ray patterns were obtained from a sample of salt, extracted with water from Orgueil, from 5 samples of Orgueil heated with water in sealed glass tubes for a period of several days at 105°, 240°, 350°, and 400° C., respectively, and, from samples of Orgueil, Murray, and Holbrook, after being subjected to rapid heating in air to 980° C. temperature. The results were compared with published data and with the diffraction patterns of the following standards: chlorite (clinochlore) from Brinton Quarry, West Chester, Pa.; magnetite from Mineville, Adirondack Mts., N.Y. (both were obtained from the Mineral Collection of the Department of Geology, Columbia University); serpentine (mainly antigorite) from Havana, Cuba (from the Genth Collection, The Pennsylvania State University), and iron (metal) powder, C.P. grade.

The carbonaceous chondrites gave poor diffraction patterns. Apparently, this was caused by small particle size and by a strong fluorescence of the sample, when subjected to CuKα radiation. Magnetite lines appeared on all Orgueil patterns; many of the silicate lines were made visible on photographic film by reducing the exposure of the diffuse background with another strip film, put in front of the one that was to be used for the diffraction record. Manual, step-scanning in the low angle region established a diffuse band related to the characteristic basal reflections of layer lattice silicates. The counting pattern, however, did not show the same resolution as the photographs, where at least one of the 00l reflections stood out as a very weak but as a still slightly noticeable line. The hydrothermal treatment of Orgueil failed to improve the quality of the diffraction effects.

Diffraction data from the Orgueil silicates are shown in TABLE 4, with some layer lattice silicates containing magnesium. Low angle counts obtained from oriented slides are shown in FIGURE 2. The oriented samples were prepared by subjecting the powdered meteorite to shearing stress with a pestle on abraded glass slides. This produced a thin, glossy film in which the mineral flakes appear to have been aligned parallel to the glass, as prescribed by earlier experiments and theory.[27] Each 0.2 degree 2θ increment was counted in the 2.0°–16.0° 2θ range for a period of 134 seconds, with CuKα radiation and a scale factor of 256 on a Norelco X-ray diffractometer unit. The statistical probable error in the counts, under such experimental conditions, is 0.4 per cent.

Because of the uncertainty in the position of the 00l reflections positive identification was not possible. Chlorite and/or montmorillonite may be present; the former is a likelier constituent, considering that chlorites rich in iron give weak 1st and 3rd order basal reflections. Reflections extending above 7 Å preclude serpentine.

TABLE 4

DIFFRACTION DATA FROM MICACEOUS ORGUEIL MINERAL AND FROM SOME LAYER LATTICE SILICATES CONTAINING MAGNESIUM

Montmorillonite		Talc		Serpentine				Chlorite				Orgueil Meteorite‡	
After MacEwan[28]		After Gruner[29]		Antigorite, after Selfridge[30]		This study		Sheridanite, after Brindley and Robinson[31]		Clinochlore, this study			
d*	I†	d	I	d	I	d	I	d	I	d	I	d	I
001 spacings variable		8.94						13.6	m	14.5	m	diffuse band	
				7.36	ss	7.32	ss	7.01	ms	7.2	ss	7.6(?)	vw
				4.67	m			4.69	ss	4.77	ss		
4.49	ms	4.57	w			4.58	w			4.63	w	4.63	w
		3.82	vw	3.64	ss	3.66	ss						
								3.52	ss	3.58	ss		
		3.06	ss					2.82	m	2.86	m	2.89	vw
2.64	mw									2.60	w	2.62	w
		2.56	vw	2.56	ss			2.57	vw	2.56	m		
		2.50	m			2.51	m	2.52	w				
						2.44	w	2.42	w	2.45	w		
2.29	vw	2.31	vw					2.35	w			2.32	vw
								2.24	w	2.27	vw		
		2.19	w	2.19	s								
		2.08	vw					2.01	mw	2.04	w	2.10	diffuse, w
								2.00	mw				
		1.92	vw					1.87	w	1.88	vw		
		1.85	w	1.85	w			1.81	w				
1.74	vw			1.79	m			1.71	vw			1.74	vw
		1.66	w					1.65	vw				
1.53	m	1.52	m	1.58	s			1.56	m	1.55	vw	1.55	m
				1.55	s								
								1.41	mw				
1.32	vw	1.38	m					1.39	m			1.34	w

* Because of the expanding montmorillonite lattice only hk reflections are tabulated. The sample was a trioctahedral soil montmorillonite.
† Intensity symbols: ss = very strong, ms = medium strong, m = medium, mw = medium weak, w = weak, vw = very weak.
‡ Electron and x-ray diffraction data; magnetite lines are excluded.

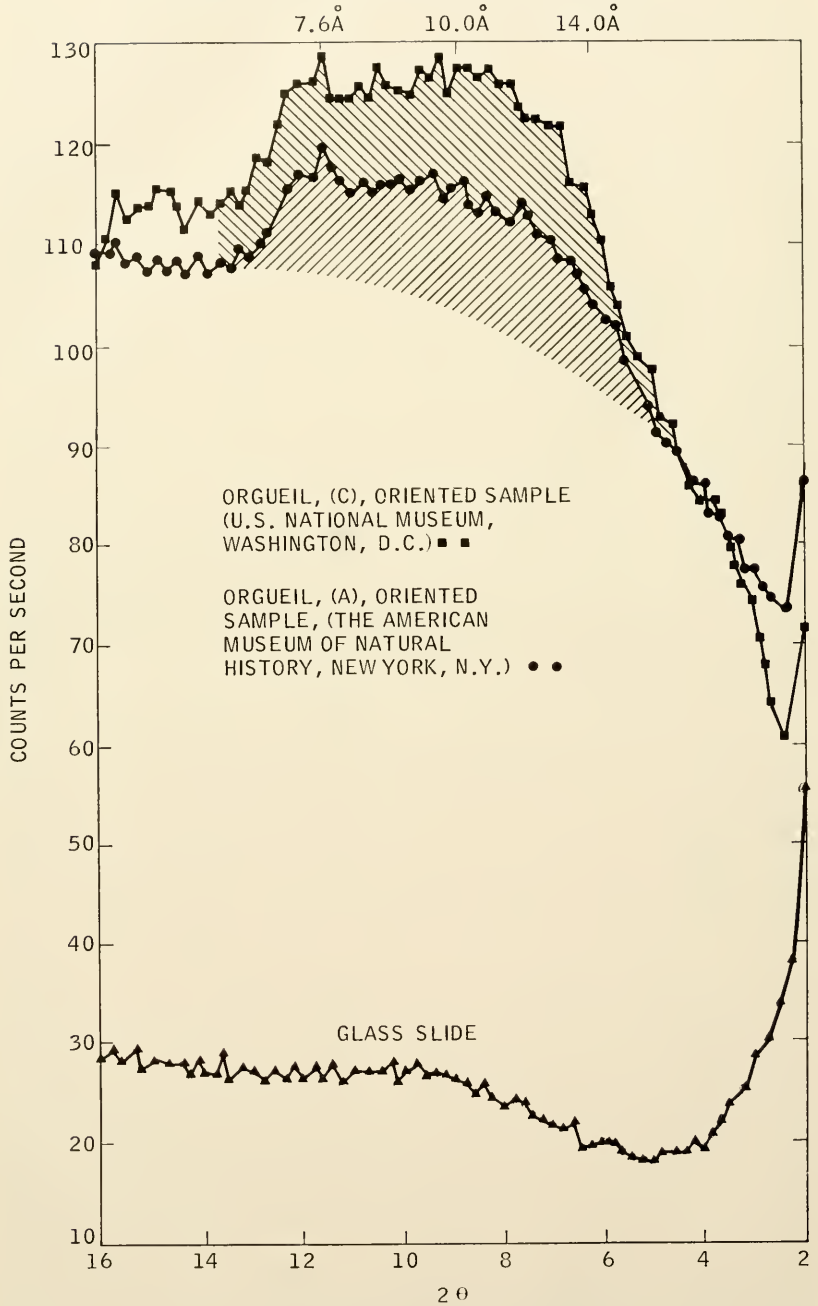

FIGURE 2. Record of manual, step-scanning counts in the low angle region. Norelco X-ray diffractometer, CuKα radiation, fixed time basis. Each point on the graph was counted for 134 seconds, and the scale factor was 256.

Magnetite (Fe_3O_4) is a component in Orgueil;* the magnetite lines were sharp enough to ensure that they were not caused by chromite ($FeCr_2O_4$), which gives a similar pattern. The 2.97 Å, 2.53 Å, 1.71 Å, and 1.62 Å magnetite lines were recorded. Chromite peaks at 4.83 Å and 1.91 Å were not observed; the strongest diffraction effect was sharp and always appeared at 2.53 Å. The x-ray diffraction patterns showed no evidence for hematite (α-Fe_2O_3), pyrite (FeS_2), troilite (FeS), pyrrhotite (Fe_7S_8), metallic iron and nickel, fayalite (Fe_2SiO_4), forsterite (Mg_2SiO_4), enstatite ($MgSiO_3$), or gibbsite ($Al_2O_3 \cdot 3H_2O$) in Orgueil. The X-ray data indicated that the mineral composition of the sample was heterogeneous to some extent.

The water soluble salt was obtained by heating the sample in water in sealed glass tubes at 104° C. for a period of 2 days, after which the supernatant liquid was poured off, filtered, and evaporated. The crystalline product was $MgSO_4 \cdot 6H_2O$; there were a few minor peaks which have not been identified. The diffraction patterns showed that subjecting the Orgueil sample to rapid heating (6.5° C. per minute) to 980° C. temperature in air, led to the formation of a limited quantity of olivine (forsterite) and hematite. When the chlorite and the serpentine standards were subjected to the identical heat treatment they seem to have fully recrystallized into the high temperature minerals.

The diffraction pattern of Ivuna was almost identical to Orgueil, but Murray showed signs of containing olivine. The diffraction patterns of the noncarbonaceous chondrites were sharp and distinct; the results were in agreement with published data.

Electron diffraction studies were conducted in an attempt to confirm the x-ray data. Specimens were prepared by dusting with a Q-tip because it was thought that this method would lead to a random orientation of the flakes. Patterns were taken in selected areas and in manipulator positions. A "beam-stop" was used for some patterns; the centers of the patterns were reduced with Farmer's reducer. Measurements were made both on plates and on enlargements.

The electron diffraction diagrams showed a series of concentric rings, with a hexagonal (or pseudohexagonal) array of spots overimposed on most rings. There were also 2 diffuse bands present. 001 reflections were not recorded.

The electron and X-ray diffraction data were in good agreement (d-values in TABLE 4 are based on both). There were only two differences. Electron diffraction diagrams did not show magnetite lines (probably because of the scarcity of magnetite in the fields that were examined). Furthermore, electron diffraction diagrams were always sharp and distinct. The hexagonal pattern of spots was related apparently to diffractions from the basic hexagonal building units of layer silicate structures.

Thermogravimetric Analysis

In addition to the X-ray and electron diffraction methods, there are 2 thermal methods of layer lattice silicate analysis: differential thermal analysis and thermogravimetric analysis. Faust[32] obtained differential thermal curves on Orgueil and Mighei but was unable to interpret the data because of the inter-

* Part of the magnetite may contain Ni, as $NiFe_2O_4$.

ference of a wide range of exothermic effects (caused probably by the combustion of organic matter). Thermogravimetric analysis was selected because it was thought that the temperature-weight curves of untreated meteorite samples could be meaningfully interpreted even if there was organic matter present. Experiments were performed with the 6 meteorites and with mineral standards, as well as with mineral-organic mixtures. A "Stanton Thermo-recording" instrument was used; the samples were heated in platinum crucibles. The instrument was calibrated for both temperature and weight effects. In addi-

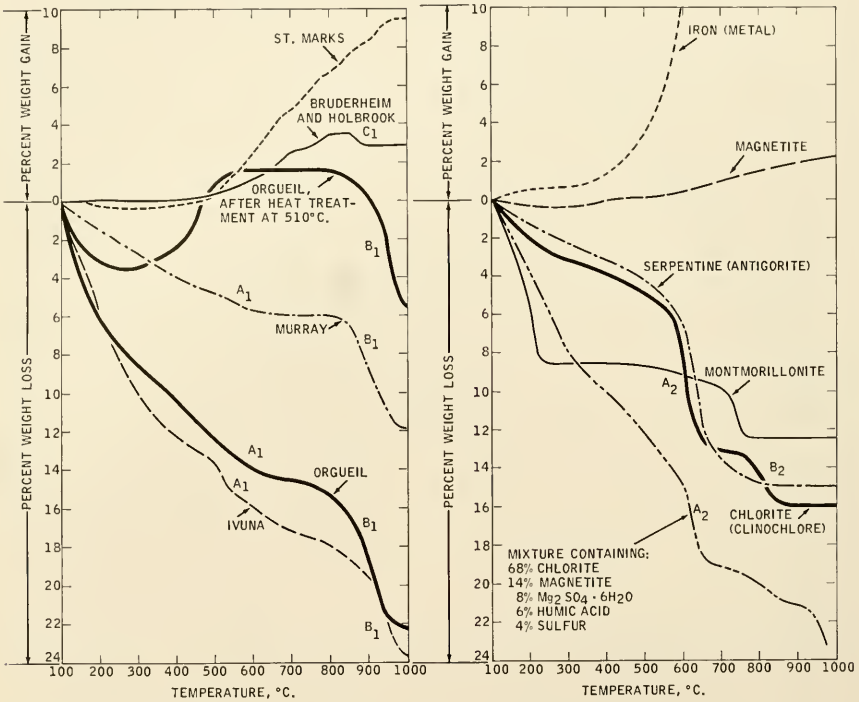

FIGURE 3. Thermalbalance curves of meteorites (*left*) and of mineral standards, and of a mineral-organic mixture (*right*). Heating rate 6.5° C. per minute; each sample weighed 0.302 gm.

tion, standard kaolinite (API-No. 17) and montmorillonite (API-No. 25) samples were run for the purpose of calibration. Each sample was run at a 6.5° C. per minute heating rate; sample weights were held identical: 0.302 g. The thermobalance curves are shown in FIGURE 3 (*left* and *right*).

The Orgueil curve shows a gradual decrease in weight to the inflection point (A_1) at approximately 600° C. temperature. At approximately 900° C. there is a second inflection point (B_1). The distillation residue of Orgueil sample (A), heated in an initial vacuum of 10^{-3} mm. Hg at 510° C. for a period of 2 hours, showed only the effect at B_1. The weight gain in the 400 to 500° C. temperature range may have been caused by the oxidation of magnetite made apparent by the removal of volatile organic matter and part of the water.

The initial weight loss of the distillation residue may have been caused by the loss of rehydrated water. The Ivuna thermobalance curve was very similar to that of Orgueil, although the position of point A_1 was less well defined. Murray lost less weight than either Orgueil or Ivuna. The 3 noncarbonaceous chondrites gained weight during heating, caused probably by the oxidation of metal. Note that the Holbrook and Bruderheim curves were actually identical. The cause of the small inflection at approximately 870° C. (C_1) is not known.

One may compare the meteorite curves with published data and with those in FIGURE 3 (*right*) in an attempt to evaluate the meteorite compositions. The thermogravimetric patterns and the differential thermal curves of chlorites are characterized by two high temperature dehydration effects; see Mielenz, Schieltz and King,[33] and Nutting.[34] Serpentine (antigorite), seems to show only one principal, dehydration reaction at high temperatures.[33] Certain chlorites, containing 2 polymorphic (14Å and 7Å) units, had been observed to exhibit 3 high temperature, dehydration effects.[35] Montmorillonite may give complicated patterns; in most montmorillonites, however, the first dehydration reaction occurs at temperatures lower than A_1. Some montmorillonite-organic complexes show endothermic reactions in the 850 to 950° C. temperature range.[36] Talc[33] seems to have only 1 principal, dehydration effect, which occurs close to 1000° C. Gibbsites seem to lose the majority of their water below 425° C. temperature.[33]

The Orgueil curve (untreated) has 2 inflection points, A_1 and B_1, similar to chlorite, A_2 and B_2. The thermobalance curve of a synthetic mixture consisting of 68 weight per cent chlorite, 14 per cent magnetite, 8 per cent $Mg_2SO_4 \cdot 6H_2O$, 4 per cent elementary sulfur, and 6 per cent humic acid,* was in part similar to the Orgueil curve. Other mixtures, containing either bituminous petroleum, asphaltene, graphite, and/or serpentine, were less similar. The thermogravimetric pattern of a chlorite sample, ground in and saturated with piperidine, has shown that point B_1 shifted to higher temperatures. Thermogravimetric analysis of mineral-organic mixtures suggests that the gradual decrease in weight below 600° C. temperature is caused by the volatilization of complex organic matter.

The following samples yielded curves which were dissimilar to Orgueil: Recent marine sediment (from the Eastern Atlantic Ocean, 35°57′N, 07°30′W, from a depth of 1350 feet, and 575 cm. below the sea bottom); top soil (from an oak forest in Hartsdale, N.Y.); and a low temperature silicate reaction product. The latter sample was prepared by mixing, in stoichiometric proportions, sodium silicate and magnesium chloride solutions and allowing them to stand for several days at room temperature. It has been claimed in the literature[37,38] that such a low temperature process might yield a product resembling serpentine. X-ray diffraction patterns of the product did not show serpentine lines and most of the weight loss occurred below 300° C. temperature on the thermobalance curve. The soil sample also lost most of its weight at low temperatures. The Recent sediment was indicative of clay minerals other than chlorites, and also probably other than montmorillonite.

* The humic acid was prepared by I. A. Breger of the U.S. Geological Survey from Minnesota peat by low temperature alkali extraction, followed by acid precipitation and dialysis.

A Discussion of Conditions on the Parent Body

Experimental data establish that the Orgueil meteorite consists chiefly of the following substances (listed in an approximate order of decreasing abundance). (1) Hydrous layer lattice silicate mineral(s), (probably chlorite or, less likely, montmorillonite); (2) magnetite; (3) magnesium sulfate; (4) organic matter; and (5) elementary sulfur.

The terrestrial occurrence of the minerals (1, 2, 3, 5) must be briefly considered before one attempts to evaluate the environment of the Orgueil parent body. As an initial consideration, one may note that hydrous, layer lattice silicate minerals can form only in the presence of water (liquid or vapor). Clearly, the parent body must have contained water.

The chlorite minerals occur in crystalline, metamorphic schists (which had high temperature histories), in altered, basic igneous rocks as well as in soils and sediments (low temperature history). Serpentine and talc have either hydrothermal origins or they are alteration products of igneous rocks. The montmorillonite minerals are known to occur both in soils and sediments and in rocks altered by hot hydrothermal solutions. Layer silicate minerals occur under a rather wide range of temperatures.

Magnetite is present in many igneous rocks (which crystallized from molten silicates), and in sediments. Epsomite is known to crystallize from (low temperature) mineral water; it is often found in limestone caves. Sulphur may be the result of either volcanic activity, of the decomposition of H_2S in thermal springs or of bacterial action in rocks and Recent sediments.

On the other hand, phase equilibria studies,[39-41] have demonstrated that certain, characteristic high temperature minerals, absent in Orgueil, beg'n to form above 450 to 500° C. temperature. This then may be safely assigned as the upper limit of the Orgueil temperature history. As to the lower limit of the parent body, one must resort to speculation. It is difficult to visualize how a great mass of crystalline silicates could have formed through solid state reactions, at temperatures below the freezing point of water.

Other considerations may narrow down the temperature range. The fact that some sulfur and hydrocarbons can be liberated from the stone at temperatures as low as 150 to 200° C., at slightly reduced pressure, suggests that the upper limit of the temperature range could not have been much higher than 200° C.* Furthermore, the composition of the organic matter seems to have been altered when the meteorite was heated with water in sealed glass tubes at temperatures substantially higher than 200° C. DuFresne and Anders[42] noted recently that some strained glass fragments found in the Mighei carbonaceous chondrite indicated that the meteorite could not have been subjected to a temperature of 180° C. for a period longer than a few weeks. The authors claimed that Mighei temperatures could not have exceeded 300° C. It was also suggested that the magnesium sulfate veins in Orgueil were produced by liquid water.

There are 2 other useful indications of environment: the oxidation-reduction potential (*E*h) and the pH. It is known from Pourbaix's[43] fundamental work

* Gas chromatographic and mass spectrometric analyses indicate that hydrocarbons as small as C_9 are present in Orgueil. The boiling point of *n*-nonane is 150° C. at atmospheric pressure.

on the thermodynamics of dilute aqueous solutions that *E*h-pH relationships may be used to define mineral stability. *E*h-pH diagrams had been used to deduce environmental conditions from low temperature mineral paragenesis and from sedimentation data; Garrels[44] presented a comprehensive treatise on the geological aspects of *E*h-pH relationships.

Because of a lack of information, an evaluation of the parent environment in

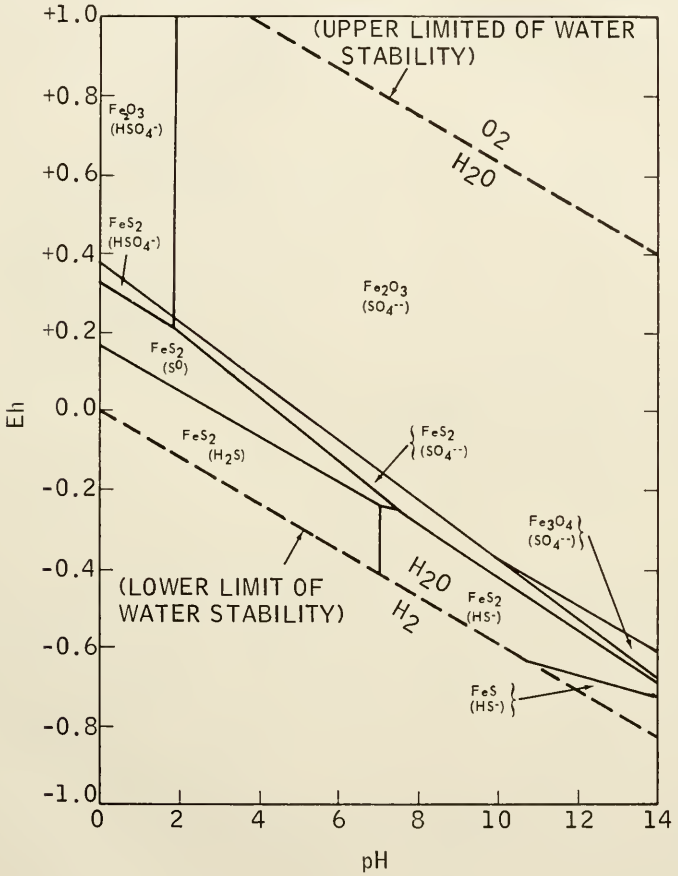

FIGURE 4. Stability relationships of iron sulfides and oxides in water at 25° C. and 1 atmos total pressure and total dissolved sulfur activity of 10^{-1}. After Garrels.[44]

terms of *E*h-pH relations must be basically speculative in nature. Speculation is possible, however, if it can be assumed, that: (1) the temperature range was approximately 0° to 200° C.; (2) organic matter and other minor components do not substantially affect known *E*h-pH relationships; and (3) minerals of the Orgueil suite are gentically related. It is known[44] from the stability of iron oxides in water that pressure has only a limited affect on *E*h-pH relationships. A change of the temperature from 25° to 100° C. causes a shift in the stability fields of solids relative to the *E*h-pH axes but it does not affect the shape and size of the fields.

In FIGURE 4 is illustrated a common geological phenomenon, *i.e.*, the interrelations between iron oxides and iron sulfides in water at different *Eh* and pH values. The stability relationships on the diagram were calculated by Garrels[44] for 25° C. temperature and for 1 atmos. pressure at a total dissolved sulfur activity of 10^{-1}. Under such circumstances, magnetite is stable under mildly reducing conditions and at a pH higher than 7. The $SO_4^{=}$ ion is stable

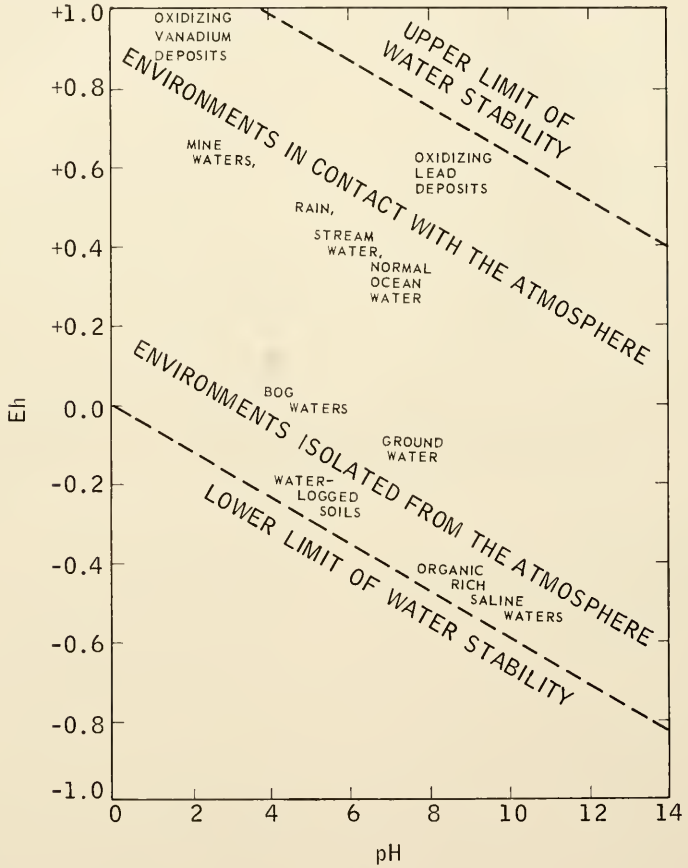

FIGURE 5. Approximate position of some natural environments as characterized by *Eh* and *p*H. After Garrels.[44]

in the magnetite field, but the stability field of elementary sulfur extends toward a more oxidizing environment and an acidic pH. The missing mineral phases in a given suite are also indicative of environment. Note that pyrite forms at the same pH as magnetite but under a more reducing environment than magnetite; whereas, hematite forms under a more oxidizing environment. Neither pyrite nor hematite has crystallized out in Orgueil, although their elementary components are present. Layer lattice silicates are known to occur under conditions similar to the magnetite environment in FIGURE 4.

The relationship shown in FIGURE 4 may be applicable, in general, to the Orgueil meteorite parent body. From this relationship one may then speculate that the Orgueil parent body had an aqueous, low temperature, slightly alkaline and slightly reducing environment. It seems that sulfur was formed by some unrelated process.

The approximate positions[44] of some terrestrial environments as characterized by *E*h and *p*H are shown in FIGURE 5. It is interesting that the proposed Orgueil environment resembles those terrestrial environments which are isolated from the earth's atmosphere (organic rich saline waters).*

Summary

The Orgueil meteorite has long been known to contain bound-water, organic matter, and sulfur, in addition to silicate, iron oxide, and magnesium sulfate. Trace element data in Orgueil, obtained during the present study, were found to be consistent with the average abundances of trace elements in chondritic meteorites but they appeared dissimilar to average abundances in terrestrial shales and igneous rocks. Electron microscopy showed that the meteorite consists mainly of micaceous minerals. X-ray, electron diffraction studies and thermogravimetric analysis confirmed the occurrence of hydrous layer lattice silicates and of magnetite, in addition to some magnesium sulfate. The mineral suite prescribes an aqueous environment of the parent body. The parent body temperature seems to have been low to moderate and one may speculate that the environment was slightly reducing and that the *p*H was slightly alkaline.

Acknowledgments

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* Sagan[45,46] suggested that indigenous organic matter may exist buried under the surface of the moon. He observed that "organisms shielded from solar illumination, perhaps in congealed dust matrix interstices, might survive cosmic radiation for 10^9 years or more; lunar subsurface temperatures are too low to impede survival." The Orgueil meteorite may represent the remnant of such an underground habitat, but the experimental data gathered in this study do not preclude the possibility that the parent body was of sufficient size to hold an atmosphere and thus, bodies of water.

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EVIDENCE IN METEORITES OF FORMER LIFE: THE ORGANIC COMPOUNDS IN CARBONACEOUS CHONDRITES ARE SIMILAR TO THOSE FOUND IN MARINE SEDIMENTS

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Recently, the composition of the hydrocarbons in the Orgueil carbonaceous chondrite has been proposed as evidence for biological activity in the parent body.[1] This apparently novel use of hydrocarbons has created great interest and is the subject of appreciable controversy.

The proposal that certain meteorites were once a part of an extraterrestrial biosphere is not new. Analyses of carbonaceous substances in chondrites were published first more than 120 years ago. Berzelius,[2] in 1834, speculated about and decided against the possibility that the humic acid type substances in the Alais meteorite were biological products. Wöhler,[3,4] however, thought that the ozocerite type constituents in the Kaba chondrite, which he investigated, in 1858, were "undoubtedly of organic origin;" but Berthelot did not share Wöhler's belief that a resemblance to terrestrial organic matter was proof of a biological origin. Berthelot[5,6] hypothesized a reaction of metallic carbides and water to explain the presence of "petroleum-like" hydrocarbons in the Orgueil stone. Although Mueller[7] noted experimental evidence against the carbide theory of Berthelot, the "chlorobitumens" which were reportedly isolated from the Cold Bokkevelt by Mueller[7] were not suggested as organic products. All of these observational and elemental analyses were far less definitive than the analyses that have been made possible by modern techniques and instruments and recent acquisition of paleobiological reference data. Investigations of terrestrial biotic matter and modern analytical methods, now, provide a basis for speculations about extraterrestrial life.

Spectrometric, chromatographic, and new microscopic methods were not utilized in the study of the carbonaceous substances in meteorites before 1954. Boato[8] measured the abundances of stable carbon and hydrogen isotopes in 14 meteorites. He noted that "the carbonaceous material is, of course, not derived from living sources, but it is noteworthy that the range of variation in C^{13} in the meteorites is of the order of the depletion observed in a terrestrial process involving loss of volatile compounds."[9] Recent investigations, however, do not support the view that a loss of volatile compounds leads to a depletion of C^{13} in sedimental organic matter. Silverman and Epstein[10] and Park and Epstein[11] have found that ecology exerts the principal control on terrestrial, organic C^{13} contents. Lipids, which are the major volatile constituents of plants and animals, have lower C^{13} contents than other organic compounds.[11]

Boato's deuterium determinations provided evidence of the extraterrestrial origin of meteorites. He found much greater concentrations of deuterium in the combined water which were removed from the Orgueil stone at temperatures

exceeding 180° C. than have been found in natural waters on earth. Neither deuterium[8] nor trace metal[12] abundances in the Orgueil meteorite support Bernal's[13] contention that this carbonaceous chondrite "may be a part of the primitive earth shot off hundreds of millions of years ago and again united to its parent body."

Calvin[14] obtained the ultraviolet absorption spectra of water extracts of the Murray and Orgueil meteorites in acidic and basic solutions, and he concluded that the absorption wavelengths and the variations in these lengths induced by the addition of acids were suggestive of cytosine, a building block of nucleic acids which are essential components of all living cells. Briggs[15] reported evidence of purines and imidazoles in the Orgueil, Murray, and Mokoia meteorites.

Detailed analyses of the amino acids, amino sugars, and sugars in the Bruderheim chondrite and the Murray carbonaceous chondrite have been run by Degens and Bajor.[16] They determined the quantities of 20 amino acids, 3 sugars, and 2 amino sugars as well as the presence of cytosine, uracil, and/or hypoxanthine in the 2 samples.[16] "After complete extraction of all hydrolyzable matter in the meteorite," the investigators[16] found that "amino acids and sugars could be generated within 3 weeks' exposure to open air in quantities about 20 per cent of the original values." Because of the regeneration, composition, and stabilities of the amino, sugar, and nucleic acid constituents in the Bruderheim and Murray, Degens and Bajor[16] proposed that the "organic constituents analyzed are with great probability terrestrial in origin rather than fossil remnants of extraterrestrial life."

Claus and Nagy have reported "organized elements" in the Orgueil and Ivuna[17] and have observed similar elements in the Alais and Tonk carbonaceous chondrites.[18] These "organized elements" are dissimilar to any known mineral forms but resemble, yet are not identical to certain species of algae."[17] Recognizable, well-preserved terrestrial type organisms, also, were seen in the 4 carbonaceous chondrites, but the latter species were present in much smaller numbers than the microfossil-like elements.[17] The terrestrial type organisms were assumed to be contaminants acquired on earth, and they represented the only specimens resembling microorganisms that were found in the Bruderheim and Holbrook chondrite.[17]

Palynological treatments have provided additional information about some of the "organized elements" in meteorites. Staplin[19] added: (1) hydrochloric acid to remove the carbonates; (2) hydrofluoric acid to remove the silicates; and (3) Schulz solution (nitric acid and potassium chlorate) to bleach the residue that he had obtained by these acid treatments of an Orgueil fragment. The residue contained "recent (organic) contaminants, a very few well-preserved Cretaceous microfossils, and relatively numerous less well-preserved microfossils of unknown age or affinities. The unidentified microfossils, mostly in the 10 to 100 μ size range, superficially resemble certain of the unicellular algae if size, texture, and the presence of an acid resistant pellicle are considered."

Fox[20] has made abiotically double walled carbonaceous particles which he believes may be formed in shapes and sizes that resemble the "organized elements" in meteorites. Fitch *et al.*,[21] noted that the "organized elements" in

carbonaceous chondrites may be minerals or sulfur droplets; whereas Briggs and Kitto[22] conclude that the "complex organic microstructures" in the Mokoia meteorite may be either of biogenic or abiogenic origin. It remains to be demonstrated, however, that these abiotic products can duplicate the fluorescence, size, and numerical distributions, structural details, biological stain acceptance, and behavior during palynological treatment which have been reported[17,19] for the microfossil-like "organized elements" in meteorites. In the opinion of Nagy *et al.*,[23] no organic particles have yet been prepared that possess all the properties of "organized elements" or cell remnants. Bernal[24] states, "the question of whether the objects admittedly composed of sulfur or mineral fragments are or are not identical with the 'organized elements' . . . clearly requires for its resolution careful comparisons by a panel of impartial experts." Urey[18] feels that "although the present evidence is not conclusive, there are good reasons for exploring possible origins of lifelike forms in the carbonaceous chondrites other than contamination after their arrival on earth."

Biological Indicators

Living things may be grossly regarded as unique assemblages of parts or molecules that possess efficient means of synthesizing, using highly select arrays of complex molecules, and of reproducing their specie. Plants can convert several per cents of the solar energy that they receive into molecular energy or food. Compounds which form a major portion of the constituent parts of organisms comprise an exceedingly small fraction of the compounds which theoretically can be made by abiotic reactions. Sagan[25] reports "the most optimistic extrapolation from existing laboratory ultraviolet experimental data" for the quantum yield of organic molecules by Miller-Urey[26] type syntheses is 1 part in 100,000 parts, and the products of these syntheses are neither solely nor entirely the compounds made by living things. Organisms are apparently in excess of a thousand times more efficient than abiotic reactions which may have occurred in a primordial environment.[26]

Although some nonbiological process under some presently undefinable conditions may duplicate the productive capacities of living cells, available data support the view that detectable concentrations of complex molecular mixtures composed of compounds resembling those in living cells are products of life.[27]

Because organisms are efficient and apparently unique producers of certain arrays of molecules, plant and animal matter has probably exerted a major control on the compositions of many carbonaceous substances in terrestrial sediments for the last 2 or more billion years.[28] Either the preserved or the altered biosynthetic products in Earth's sediments may provide a valuable, legible record of prehistorical life and its evolution. Analyses of extracts of terrestrial sediments indicate that ancient plants and animals have left evidence of their existence and that some extractable substances of natural samples may be used as biological indicators.

In this investigation, the compositions of the benzene extracts of soils and marine sediments from various regions on earth have been used as references. It is postulated that the terrestrial extracts retain evidence of biological activity, and it is assumed that similarities between terrestrial and meteoric extracts

constitute evidence that the meteorites were either contaminated while on Earth or a part of a parent body which supported life. Careful consideration will be given to the compositions of the extracts of the sediments and meteorites so as to determine, as well as these compositions permit, whether the extractable fractions of carbonaceous chondrites are indigenous or contaminants.

Experimental Procedure

Solvents and glassware. Reagent Grade solvents were used exclusively. Before use, solvents were distilled through 6 plate glass helices columns, and 100 gm. aliquots of each solvent batch were blown to constant weight in the sample recovery system. Solvents accepted for use contained less than 0.1 mg. residue per 100 gm. of solvent and these residues did not absorb detectably in either the 2 to 15 μ or 220 to 400 mμ regions. All glassware and porcelain used in preparing and analyzing meteorite samples were cleaned with acid and carefully rinsed with the accepted solvents.

Blanks. A blank, which omitted only the meteorite sample, was run on each step of sample preparation and analysis.

Extractions. Consolidated fragments of the 1-Orgueil(B) (1.7 gm.), 2-Orgueil(C) (14.5 gm.), 1-Murray (1.9 gm.), 2-Murray (10.2 gm.), and Holbrook (1.8 gm.) meteorites were placed individually on glass wool plugs in 5 glass funnels.[29] Each fragment was rinsed separately with several portions of a 1 volume methanol to 9 volumes benzene (9:1 benzene-methanol) solvent. Meteorite samples smaller than 2 gm. were rinsed with 25 ml. of solvent, and the 2-Orgueil and 2-Murray fragments were rinsed with 80 and 50 ml. of solvent respectively. The rinses from each meteorite were analyzed separately.

The rinsed fragments were crushed to 20 to 40 mesh size, placed separately on a glass wool plug above a sintered glass partition between a boiling flask and a water-cooled condenser in an all glass, single piece, Soxhlet-type extractor.

Small (<2 gm.) and large (>10 gm.) fragments were extracted by slightly different procedures. A 25-ml. aliquot of 9:1 benzene-methanol was added to each of the extractors containing the crushed small fragments. After 6 hours at reflux, the extracts were withdrawn and a second 25 ml. of the solvent was added to each unit. The extractions were continued an additional 14 hours. In this manner, a rinse, a 6-hour extract, and a 6- to 20-hour extract of each small fragment was obtained. The large 2-Murray and 2-Orgueil fragments were extracted for 20 hours with 50 ml. of solvent, so that only a rinse and a 20-hour extract of each of these samples was recovered.

Sample recovery. Solvents were evaporated from rinses, extracts, and from the eluates of colloidal copper and silica gel columns. Sample bottles containing organic solutions of meteorite rinses, extracts, or eluates were placed in receptacles or aluminum cups in a constant temperature bath maintained at $40 \pm 1^\circ$ C. Nitrogen filtered through silica gel was blown over the organic solutions for 4 to 6 hours. This recovery procedure removes the solvents and most organic compounds from the meteorites that have vapor pressures greater than C_{13} *n*-paraffins. Thus, the hydrocarbons recovered in the meteorite samples consisted primarily of C_{14} and larger molecules.

In TABLE 1 are presented the weights of the extracts recovered from the sulfur removal step.

Although a semimicro balance was used in all weighings, 1 or more removals of solvent and 2 or more weighings were required to obtain a residue weight. Therefore, the weights listed are probably accurate to only ±0.2 mg.

Sulfur removal. Elemental sulfur was removed from all meteorite rinses and extracts by means of colloidal copper columns.[30]

Chromatographic separations. The 6-hour extracts of 1-Orgueil and 1-Murray, and 20-hour extracts of 2-Murray and 2-Orgueil fragments were fractionated on 9-gm. silica gel columns.[31] The Holbrook extract was too small to fractionate. This method of chromatographic separation on silica gel has been previously investigated. Thousands of crude oils and organic extracts of sediments and organisms have been fractionated by this chromatographic procedure. Infrared, ultraviolet, and mass spectrometric analyses, elemental analyses, and aluminum chromatographic analyses[32-34] of numerous fractions of these silica gel eluates have established: (1) the *n*-heptane eluates

TABLE 1
ORGANIC RESIDUES
(Weights in milligrams)

Sample	Rinses	6-Hour extracts	6 to 20-Hour extracts
1-Orgueil (1.7 gm.)*	0.1	6.0	0.6
2-Orgueil (14.5 gm.)	0.3	75.0 (20-hour)	†
1-Murray (1.9 gm.)	0.1	1.1	0.1
2-Murray (10.2 gm.)	†	6.7 (20-hour)	†
Holbrook (1.8 gm.)	0.2‡	0.4§ (0.0¶)	0.1
Blank	0.1§	0.1¶	0.0

* A portion of sample lost when solvent "bumped."
† Not determined.
‡ Nonvolatile residue—inorganic salts.
§ Contained visible traces of colloidal copper from sulfur removal step.
¶ Estimated from mass spectra peak heights.

are composed primarily of saturated hydrocarbons; (2) carbon tetrachloride fractions contain saturated hydrocarbons, olefins, traces of some nonpolar organic nitrogen and sulfur compounds, and alkyl- and cycloalkylbenzenes; (3) benzene eluates contain most of the aromatic hydrocarbons, some organic esters, alcohols, and other organic nonhydrocarbons;[31] and (4) methanol eluates are composed predominately of organic nonhydrocarbons.

Weights of the chromatographic fractions of 1-Orgueil, 1-Murray, 2-Murray, and blanks, which were in most cases too small to be determined accurately, are presented in TABLE 2. The 2-Orgueil extract was of sufficient size for a triplicate analysis, and the chromatographic data on the 3 aliquots of this sample are given in TABLE 3.

Infrared spectroscopy. All blanks, rinses, extracts, and the methanol eluates of 1-Orgueil and 2-Murray were scanned in the 2 to 15 μ region. Infrared spectra were obtained of the *n*-heptane, carbon tetrachloride, benzene, and methanol fractions of 2-Orgueil. Scans were run on a Baird Associates Model 4-55 spectrometer with a sodium chloride prism. Sample and blank cells had 0.1-mm. cell lengths and were equipped with sodium chloride windows.

Blanks, rinses, Holbrook extracts, and 1-Murray 6- to 20-hour extract did not absorb significantly in the 2 to 15 μ infrared region. The infrared spectra of the total blanks, and total Holbrook, 1-Orgueil, and 2-Murray organic extracts are presented in FIGURE 1. In FIGURE 2 are shown the spectra of the individual chromatographic fractions of the 2-Orgueil extract.

Ultraviolet and visual spectroscopy. All blanks, extracts, rinses, and chromatographic fractions were scanned in 220 to 400 mμ region. Scans were run on a Cary model 14 spectrometer with matched cells of 1 cm. in length and methanol as solvent. Visual spectra were run on the benzene and methanol chromatographic fractions of the 1-Orgueil, and 1-Murray. Absorption decreased continuously from 400 to 800 mμ in all of these fractions. The

TABLE 2
SILICA GEL CHROMATOGRAPHIC FRACTIONS
(Weights in milligrams)

	n-Heptane	Carbon tetrachloride	Benzene	Methanol
1-Orgueil (6.0)	0.1 (0.3*)	0.2	0.6	4.4
1-Murray (1.1)	0.0 (0.1*)	0.1	0.0 (0.1*, †)	0.7
2-Murray (6.7)	0.4	0.1	0.4	4.3
Blank	0.1 (0.0*)	0.0	0.0	0.1 (0.0†)

* Estimated from mass spectra peak heights.
† Estimated from ultraviolet absorption.

TABLE 3
SILICA GEL CHROMATOGRAPHIC FRACTIONS OF 2-ORGUEIL
(Weights in Milligrams)

2-Orgueil aliquots	*n*-Heptane	Carbon tetrachloride	Benzene	Methanol	Left on column
A wt. 24.50	2.32 (9.5%)	1.46 (6.0%)	0.76 (3.1%)	15.00 (61.2%)	4.96 (20.2%)
B wt. 25.09	2.45 (9.8%)	1.32 (5.3%)	0.82 (3.3%)	15.92 (63.4%)	4.58 (18.3%)
C wt. 24.19	2.56 (10.6%)	1.17 (4.8%)	0.76 (3.1%)	15.62 (64.6%)	4.08 (16.9%)

Orgueil extracts absorbed the strongest in the visual range, but the visual spectra of all extracts lacked any suggestion of a specific absorption at a particular wavelength.

The blanks did not absorb and the chromatographic fractions of the Holbrook absorbed only slightly in the 220 to 400 mμ range. The ultraviolet spectra of the 1-Orgueil and 2-Murray chromatographic fractions are presented in FIGURES 3 and 4, respectively. In FIGURE 5 are given the ultraviolet spectra of the total Holbrook extract and the total procedure blank. The Murray and Orgueil extracts fluoresced in ultraviolet light.

Mass spectroscopy. Blanks and the rinse; 6-hour and 6- to 20-hour extracts of Holbrook; and carbon tetrachloride, benzene, and methanol eluates of the Orgueil distillate did not produce measurable peaks at masses greater than 150 in the mass spectrometer. Measurable mass spectra were obtained of the 4 individual chromatographic fractions of the 6-hour extract of 1-Orgueil and

of the 2-Murray extract; of the composited *n*-heptane, carbon tetrachloride, and benzene eluates and the methanol eluate of the 6-hour extract of 1-Murray; the rinses and 6- to 20-hour extracts of 1-Orgueil and 1-Murray and of the *n*-heptane eluate of the Orgueil distillate. Additional fractionations and analyses are being run on the 2-Orgueil fractions. All spectra except those of the 2-Murray eluates were obtained on a Consolidated 21-103C mass spec-

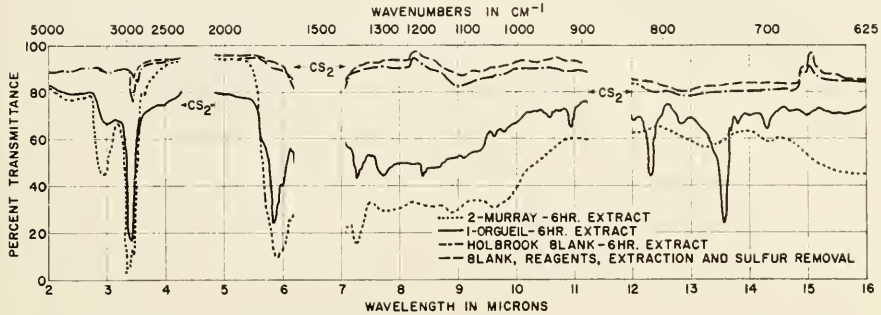

FIGURE 1. Infrared spectra of total benzene-methanol extracts (free sulfur removed) and blanks. Only minor absorptions appear in the spectra of the blank and Holbrook extract. Infrared absorption bands in the 2-Murray and 1-Orgueil extracts are similar to the bands observed in the benzene extracts of some terrestrial sediments.

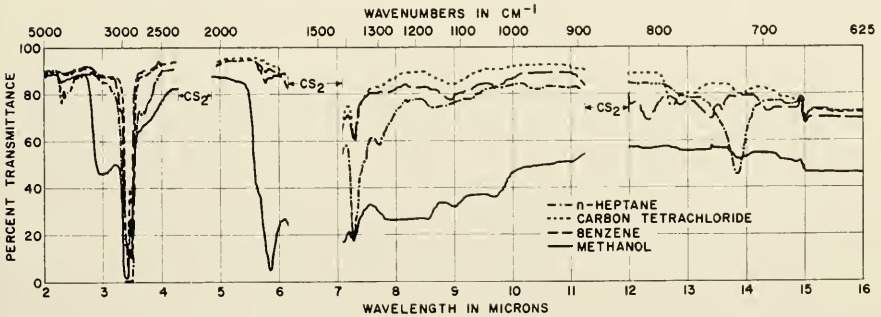

FIGURE 2. Infrared spectra of silica gel chromatographic fractions of the 2-Orgueil benzene-methanol extracts. That *n*-heptane and carbon tetrachloride eluates absorb only at wavelengths that may be attributed to C—C and C—H bonds. The benzene eluate has a small carbonyl absorption near 5.8 μ and absorption bands at 9.7, 12.3, and 13.4 μ. These latter bands appear in the infrared spectra of the benzene eluates of ancient sediment extracts, crude oils, and many recent marine sediment extracts. Absorption bands in the methanol eluate are typical of the bands found in the spectra of the methanol eluates of many terrestrial sediment extracts.

trometer which is modified for the analysis of high molecular weight organic compounds. Operating conditions for the Consolidated instrument were: ionization potential, 70 volts (benzene and methanol eluates of 1-Orgueil were run also at 12 volts); ionization current, 45 μamp.; magnet current, 1.30 amp.; scan rate, accelerating potential 3300 to 4000 volts in 20 minutes; temperature, ionization chamber, 250° C., and inlet system, 300° C.

The mass spectra of the 2-Murray eluates were obtained by the Analytical Research Division of Esso Research and Engineering Company with a General

Electric 12-in. mass spectrometer that is modified for high mass analyses. Operating conditions of the General Electric instrument were: ionization potential, 31 volts (benzene and methanol eluates of 2-Murray were run also at 12 volts); ionization current, 50 μamp.; accelerating potential, 2500 volts; scan rate, magnet current 50 to 500 mamp. in 20 minutes; temperatures, sample evaporator 255° to 262° C., volume 233° to 240° C., inlet lines 213° to 217° C., ionization chamber 174° to 177° C.

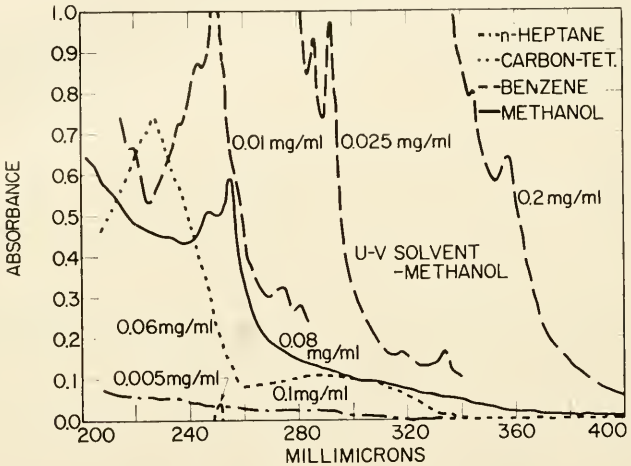

FIGURE 3. Ultraviolet spectra of the silica gel chromatographic fractions of 1-Orgueil 6-hour extract. Saturated hydrocarbons do not absorb in the ultraviolet range. Aromatic hydrocarbons do absorb. These spectra indicate that *n*-heptane eluate is composed primarily of saturated hydrocarbons and that the aromatics in the 1-Orgueil extract are concentrated in the benzene eluate.

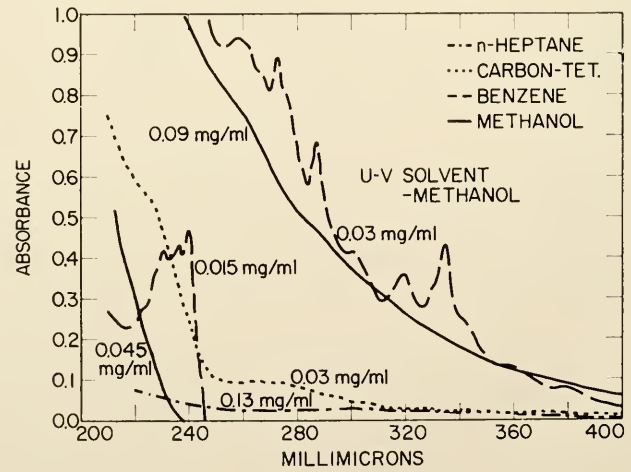

FIGURE 4. Ultraviolet spectra of the silica gel chromatographic fractions of the 2-Murray 20-hour extract. Chromatographic fractions of the 2-Murray extract are similar in composition to those of 1-Orgueil extract, but the relative concentrations of the aromatic hydrocarbons in these extracts vary in much the same manner as do the aromatic contents of different terrestrial sediments.

The Consolidated and G.E. instruments can be used for accurate analyses of mixtures of known hydrocarbons. Concentrations of individual compounds can normally be determined within ±2 per cent of the true concentrations.

Preparation of mass spectral data sheets. The mass spectra were obtained as photographic records of the galvanometer deflections produced when the ions of each particular mass are brought sequentially into focus. The deflections were measured and the heights of the individual peaks were recorded. These measured peak heights were corrected by a computer for ions containing C^{13} and H^2 (corrections made on the basis of terrestrial abundances of C^{13} and H^2). The computer totaled and normalized the corrected peak heights to a value of 300,000 and printed these heights as a 14-column array.

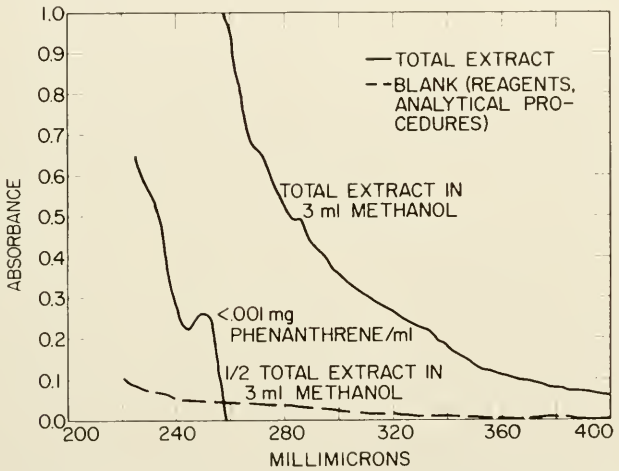

FIGURE 5. Ultraviolet spectra of total Holbrook 6-hour extract and total procedures blank. The blank does not absorb significantly in the ultraviolet. This indicates that no aromatic contaminants were added to the meteoritic extracts during their analyses. Absorption of the total Holbrook extract is very small. The amount of aromatics acquired by the Holbrook fragment in almost 50 years of storage could not have exceeded a few micrograms.

Descriptions of the 14-column array have been published.[31,35,36] The computer arbitrarily labels the 14 columns from left to right with values of x which range as integers from -11 to $+2$. Use of 14 columns results in the placement of homologous ions (*i.e.*, ions of the same structural type which differ by CH_2 or 14 mass unit groups) of a particular type in a single column. The integers heading the columns may represent x values in the general hydrocarbon formula C_nH_{2n+x}. Each horizontal row of peak heights is marked with a value of n or C# which may indicate the number of carbon atoms in the ions forming the various peaks.

The C# and x values assigned to the rows and columns of the spectra in TABLES 4, 5, and 7 are correct in most cases because saturated hydrocarbons in nature yield ions with x numbers that are predominantly in the $x = -11$ to $+2$ range. However, the x values and thus the C# shown in TABLES 6 and 8 are subject to a different interpretation. Aromatic molecules in the meteoritic extracts have x values ranging chiefly from $x = -25$ to -12, and the columns in TABLE 6 and 8 would be more accurately labeled by x values that are 14 less

and C# values that are one greater than the values shown. These apparent discrepancies in x values and C# values in the mass spectral data sheets of aromatic fractions are commonly accepted because they simplify the problem of

TABLE 4

MASS SPECTRUM OF THE *n*-HEPTANE ELUATE FROM SILICA GEL OF THE 1-ORGUEIL 6-HOUR EXTRACT. SATURATED HYDROCARBON FRACTION

Total ionization = 58022.28

Normalized isotope corrected peak heights (300,000)

	−11	−10	−9	−8	−7	−6	−5	−4	−3	−2	−1	0	+1	+2	
								0	0	−0	−0	−0	−0	−0	3
4	−0	0	0	0	−0	−0	−0	−0	−0	−0	−0	−0	−0	−0	4
5	−0	0	−0	−0	−0	−0	−0	−0	9389	3350	19430	5997	16219	74	5
6	175	265	223	221	2025	573	3593	1234	10154	4045	13601	2908	10411	67	6
7	263	86	285	229	5338	2511	3087	1175	9135	3219	11029	2118	2317	70	7
8	110	112	267	290	1885	436	2621	1208	5861	1812	6230	1651	1639	47	8
9	479	183	603	258	1418	405	1880	854	3944	1173	3360	1079	1338	376	9
10	440	175	617	245	872	361	1706	707	2465	734	1418	813	1192	176	10
11	354	161	566	171	687	312	1682	572	1768	642	921	713	985	131	11
12	270	137	433	141	581	266	1354	462	1409	479	698	650	867	114	12
13	208	101	286	142	544	280	1178	482	1112	433	555	580	751	103	13
14	234	74	263	127	585	263	1166	373	982	410	461	511	668	59	14
15	172	139	252	265	1099	335	962	252	626	324	344	422	585	29	15
16	120	64	233	116	574	293	594	296	515	284	296	385	519	44	16
17	96	62	248	105	429	215	472	220	429	254	248	350	532	10	17
18	106	59	222	87	344	176	427	178	394	215	237	318	478	67	18
19	192	49	252	88	382	158	386	156	360	208	194	319	459	44	19
20	108	60	168	73	262	114	318	152	303	196	195	265	436	39	20
21	180	53	162	72	292	83	312	124	312	185	215	294	477	60	21
22	173	41	174	63	262	103	286	121	265	203	209	292	385	200	22
23	118	44	163	79	273	133	255	176	219	262	193	312	294	274	23
24	78	56	155	84	229	218	201	221	164	287	101	260	177	189	24
25	85	81	142	126	170	210	171	211	108	265	59	214	132	129	25
26	65	82	121	140	180	188	107	192	72	171	69	152	85	96	26
27	49	150	85	167	114	261	80	169	36	114	44	88	57	59	27
28	29	69	73	106	59	129	32	80	33	77	81	67	45	42	28
29	24	66	32	98	37	105	8	57	0	73	0	477	25	157	29
30	0	31	13	38	5	0	1	0	−0	−0	−0	−0	−0	−0	30

Humble paraffin-naphthene type analyses in percentage

Paraffins	17.57
Noncondensed naphthenes	28.86
2-Ring naphthenes	17.16
3-Ring naphthenes	11.83
4-Ring naphthenes	11.25
5-Ring naphthenes	6.41
6-Ring naphthenes	6.91
Total	100.00

programming the computer. No problem other than a simple arithmetic calculation enters the interpretation of these data sheets. All peaks in TABLES 6 and 8 are recorded at their proper mass positions, and the masses and sizes of these peaks supply in conjunction with the ultraviolet spectra of the meteoritic aromatics all of the information subsequently discussed.

Mass spectral data. Mass spectra of complex saturated hydrocarbon mixtures suggest the structures and molecular weight distributions of certain of the compounds and compound types that comprise the mixtures. Spectra

TABLE 5

MASS SPECTRUM OF THE CARBON TETRACHLORIDE ELUATE FROM SILICA GEL OF THE 1-ORGUEIL 6-HOUR EXTRACT. SATURATED, OLEFINIC, PLUS MINOR CONCENTRATIONS OF AROMATIC HYDROCARBONS

Total ionization = 18452.57

Normalized isotope corrected peak heights (300,000)

	−11	−10	−9	−8	−7	−6	−5	−4	−3	−2	−1	0	+1	+2	
								0	1	−0	−0	−0	−0	−0	3
4	−0	0	0	0	−0	−0	−0	−0	−0	−0	−0	−0	−0	−0	4
5	−0	0	−0	−0	−0	−0	−0	−0	5456	2229	15614	5938	13054	169	5
6	325	395	244	253	2155	795	3026	849	7492	3040	11285	2830	9047	66	6
7	297	0	271	70	4296	981	2525	999	6527	2071	7929	2087	2119	66	7
8	226	169	466	586	3859	661	2426	899	4490	1235	4827	1999	1766	187	8
9	1434	535	1203	653	4590	626	2041	649	2911	871	2267	933	1483	1157	9
10	1366	505	1670	604	1966	538	1923	591	1836	600	1250	729	2035	754	10
11	1150	419	1658	294	1280	356	1860	350	1206	836	973	812	1708	592	11
12	917	307	1132	231	1043	309	1082	286	1361	477	862	671	1344	377	12
13	615	211	705	167	792	236	1448	628	995	315	881	578	1056	290	13
14	916	142	595	176	789	308	1156	412	892	370	706	513	1060	310	14
15	486	137	534	505	2732	380	3586	237	655	286	457	368	690	150	15
16	366	122	519	160	478	427	626	900	475	204	421	354	558	165	16
17	313	160	413	157	672	222	444	247	443	214	433	347	701	66	17
18	522	51	331	56	285	130	320	90	311	206	223	384	611	140	18
19	279	208	334	130	349	177	306	201	367	172	258	325	439	154	19
20	244	163	260	143	297	249	61	136	287	235	276	235	422	154	20
21	307	64	230	52	219	136	232	98	306	179	252	238	500	130	21
22	242	44	187	141	165	177	222	80	276	200	213	163	402	85	22
23	205	36	140	54	154	82	276	99	252	120	182	186	362	73	23
24	239	57	133	167	188	35	205	84	240	202	207	142	241	54	24
25	100	93	61	70	69	167	58	231	138	158	155	302	98	84	25
26	79	32	0	79	53	57	56	56	154	44	153	126	178	50	26
27	85	79	80	0	114	74	161	39	72	35	126	68	163	55	27
28	67	35	109	23	79	98	106	21	128	82	92	74	96	25	28
29	95	26	94	124	111	227	109	515	97	358	114	279	75	150	29
30	21	92	0	121	51	100	87	105	100	117	46	101	0	6	30

Humble paraffin-naphthene type analysis in percentage

Paraffins	16.78
Noncondensed naphthenes	22.52
2-Ring naphthenes	9.86
3-Ring naphthenes	15.87
4-Ring naphthenes	17.00
5-Ring naphthenes	7.10
6-Ring naphthenes	10.87
Total	100.00

shown in TABLES 4, 5, and 7 indicate the relative numbers of ions of various masses that were formed when gaseous, predominantly saturated hydrocarbons were bombarded by 70-volt electrons. The ions measured consisted chiefly of fragment and "parent" ions. Fragment ions are made by the rupture of carbon-to-carbon and/or carbon-to-hydrogen bonds of hydrocarbon molecules.

"Parent" ions are formed when a single electron is removed from a molecule. Only the positively charged ions with masses greater than 66 are recorded in TABLES 4 through 8.

A saturated hydrocarbon may yield a variety of ions. Any compound can form a greater number of small than of large fragments, and large molecules can produce more fragments than can small molecules. It is for these reasons that the values (peak heights) in TABLES 4, 5, and 7 generally decrease from the

TABLE 6

MASS SPECTRUM OF THE BENZENE ELUATE FROM SILICA GEL OF THE 1-ORGUEIL 6-HOUR EXTRACT. AROMATIC HYDROCARBON FRACTION

Total ionization = 3748.80 | Ionizing potential = 12 volts

Normalized isotope corrected peak heights (300,000)

	−11	−10	−9	−8	−7	−6	−5	−4	−3	−2	−1	0	+1	+2	
								0	3	−0	−0	−0	−0	−0	3
4	−0	0	0	0	−0	−0	−0	−0	−0	−0	−0	−0	−0	−0	4
5	−0	0	−0	−0	−0	−0	−0	−0	56	1493	1254	0	0	0	5
6	0	0	0	33	0	0	628	1375	1565	1150	1017	0	0	0	6
7	0	0	0	86	0	329	672	1204	2602	2412	503	0	0	0	7
8	0	0	0	0	0	11	136	364	504	2171	982	121	0	0	8
9	0	0	0	0	391	0	0	538	563	558	1598	0	452	0	9
10	0	0	0	1105	815	200	589	150	196	0	620	0	463	0	10
11	0	6	0	379	90	123	120	0	300	3141	551	447	477	315	11
12	0	0	0	137	358	8	52	0	1180	12303	890	1918	1150	526	12
13	0	215	185	0	62	0	0	38880	706	9265	379	871	83	515	13
14	0	0	0	0	0		815	2653	36	2021	461	442	221	336	14
15	0	51	134	1074	917	130	1062	438	0	295	7	211	341	0	15
16	0	0	182	428	0	308	243	969	121	0	0	0	0	0	16
17	0	1128	0	416	0	308	560	1546	0	141	271	83	0	0	17
18	0	244	0	129	0	446	614	4647	0	0	0	2460	284	593	18
19	89	506	0	970	16	673	0	494	0	0	0	599	96	664	19
20	0	256	0	378	0	0	0	1074	0	133	0	313	157	895	20
21	0	167	0	226	0	702	0	320	0	686	0	555	18	1799	21
22	0	496	0	954	0	65	0	639	953	1487	0	954	707	4257	22
23	0	585	0	403	0	1376	0	947	1498	8527	0	1308	341	5767	23
24	0	239	0	1275	0	2294	29	3413	101	14557	167	2635	187	6071	24
25	0	533	0	4600	0	2176	0	6957	44	6348	0	3832	0	5896	25
26	0	758	0	2588	0	1820	98	5077	22	4726	0	3537	0	6088	26
27	0	3057	0	1957	0	1185	0	2664	0	1676	0	3262	0	1772	27
28	0	1077	0	525	0	710	0	722	0	722	0	1628	0	1788	28
29	0	847	0	1036	0	1069	0	49	0	−0	−0	−0	−0	−0	29

Humble aromatics type analysis in percentage	
Alkyl benzenes	5.11
Naphthalenes	16.56
Acenaphthenes	17.75
Acenaphthylenes	11.10
Phenanthrenes	20.95
Diacenaphthylenes	4.01
Pyrenes	1.43
Chrysenes	0.55
Acepyrenes	1.09
Benzpyrene and other organic compounds	21.45
Total	100.00

top toward the bottom of each x column. However, there are exceptions. Certain ions have peak heights which exceed or approach in size peaks immediately above them on the data sheets. These anomalously large peaks provide structural and distributional information about some of the compounds in mixtures of saturated hydrocarbons.

TABLE 7

MASS SPECTRUM OF *n*-HEPTANE ELUATE FROM SILICA GEL OF THE 2-MURRAY 20-HOUR EXTRACT. SATURATED HYDROCARBON FRACTION

Total ionization = 127759.59

Normalized isotope corrected peak heights (300,000)

	−11	−10	−9	−8	−7	−6	−5	−4	−3	−2	−1	0	+1	+2	
								0	0	−0	−0	−0	−0	−0	3
4	−0	0	0	0	−0	−0	−0	−0	−0	−0	−0	−0	−0	−0	4
5	−0	0	−0	−0	−0	−0	−0	−0	2678	1227	8838	3342	12892	186	5
6	110	105	52	46	189	96	719	763	5669	3153	9558	2430	9621	151	6
7	101	48	38	20	475	177	1155	984	6975	3298	10467	1765	3703	87	7
8	73	38	53	70	581	233	1361	950	5738	2329	7673	1671	2742	81	8
9	98	51	164	99	624	295	1645	911	4587	1738	4935	1409	2134	85	9
10	127	58	256	108	616	326	1941	963	3490	1243	2556	1104	1702	79	10
11	139	67	296	114	595	326	2120	826	2654	939	1700	913	1385	77	11
12	147	71	281	108	615	345	1803	710	2252	773	1302	835	1175	68	12
13	131	60	274	122	670	342	1622	659	1849	664	1049	793	1108	64	13
14	135	49	282	118	761	348	1657	613	1578	629	815	697	932	48	14
15	134	66	283	159	773	325	1138	483	1135	511	633	613	793	45	15
16	146	65	329	147	845	400	990	396	899	468	523	550	707	49	16
17	142	64	324	154	698	340	845	360	787	439	432	549	706	45	17
18	155	64	346	146	624	295	765	337	731	394	367	510	661	38	18
19	179	88	360	162	651	261	725	304	646	366	343	499	648	59	19
20	187	76	347	128	565	233	611	283	582	345	363	450	600	53	20
21	189	78	360	142	589	227	563	280	511	359	318	449	581	169	21
22	196	111	345	159	543	269	522	312	455	409	285	476	518	260	22
23	207	131	335	197	486	339	478	378	414	438	250	471	444	317	23
24	192	147	311	224	425	381	426	389	332	437	209	424	365	247	24
25	183	132	275	226	364	327	358	250	288	380	164	343	294	205	25
26	156	141	260	212	346	274	306	299	222	303	137	292	230	160	26
27	147	129	236	226	290	318	243	257	174	250	109	235	177	140	27
28	135	116	204	175	250	251	191	191	132	184	86	175	138	106	28
29	110	98	176	213	181	250	156	155	105	152	57	138	112	109	29
30	90	91	122	175	137	182	114	123	76	117	43	122	83	73	30
31	74	66	95	116	88	117	81	85	52	84	24	71	60	64	31
32	54	57	77	81	79	89	62	78	34	63	17	55	44	37	32
33	47	51	56	65	54	60	37	48	22	52	19	36	32	33	33
34	26	42	36	47	36	46	24	41	13	30	11	34	17	26	34
35	17	34	21	32	21	32	16	22	7	21	0	24	4	15	35
36	8	19	17	13	17	16	9	19	0	2	0	−0	−0	−0	36

Humble paraffin-naphthene type analysis in percentage

Paraffins	16.49
Noncondensed naphthenes	17.28
2-Ring naphthenes	18.06
3-Ring naphthenes	13.87
4-Ring naphthenes	13.18
5-Ring naphthenes	10.12
6-Ring naphthenes	10.99
Total	100.00

TABLE 8

MASS SPECTRUM OF BENZENE ELUATE FROM SILICA GEL OF THE 2-MURRAY 20-HOUR EXTRACT. AROMATIC HYDROCARBON FRACTION

Total ionization = 38025.84 | Ionizing potential = 12 volts

Normalized isotope corrected peak heights (300,000)

	−11	−10	−9	−8	−7	−6	−5	−4	−3	−2	−1	0	+1	+2	
								0	0	−0	−0	−0	−0	−0	3
4	−0	0	0	0	−0	−0	−0	−0	−0	−0	−0	−0	−0	−0	4
5	−0	0	−0	−0	−0	−0	−0	−0	121	114	272	334	433	57	5
6	149	64	0	0	0	36	47	9	249	286	417	290	416	53	6
7	85	4	0	0	93	137	78	122	356	274	540	259	304	57	7
8	21	0	0	130	315	172	112	221	424	288	512	264	237	59	8
9	75	129	344	252	489	292	176	244	419	236	435	179	219	82	9
10	175	235	402	241	447	267	270	200	373	228	298	141	245	235	10
11	148	307	453	214	368	224	343	188	332	276	511	340	289	280	11
12	274	280	452	201	296	214	216	161	326	257	1180	277	350	276	12
13	246	257	389	228	289	203	239	439	363	364	814	244	314	222	13
14	306	213	384	162	312	283	444	325	428	342	749	237	286	191	14
15	308	273	379	20919	891	675	545	1124	447	1317	629	322	326	335	15
16	296	316	881	7558	966	1345	604	1996	522	637	622	426	374	2359	16
17	444	5966	1060	5834	715	1750	524	3305	561	843	612	748	410	1584	17
18	476	2587	741	4114	556	1731	518	1972	489	956	518	10436	665	2274	18
19	437	1837	552	4092	510	1718	398	1475	438	1180	484	2912	409	1534	19
20	375	1401	384	2298	375	1365	374	4454	487	1659	460	2195	337	1808	20
21	362	1319	363	1761	237	1219	270	1619	413	1449	416	1610	315	1243	21
22	284	1107	291	2101	270	1233	281	1357	360	1594	312	1434	286	1074	22
23	265	1095	211	1310	204	1013	271	1147	322	1169	267	1119	233	940	23
24	193	915	203	1004	199	830	246	941	230	934	201	894	157	734	24
25	193	791	153	877	154	708	200	780	195	794	174	752	147	635	25
26	173	638	141	724	117	649	124	663	155	695	140	627	144	258	26
27	148	838	82	588	104	497	131	509	142	544	91	487	142	419	27
28	121	522	102	577	108	422	132	477	83	443	24	407	54	375	28
29	46	379	34	406	58	365	69	379	80	343	67	357	32	344	29
30	53	322	57	329	54	329	46	284	61	290	35	251	41	219	30
31	28	241	43	226	47	217	65	243	48	224	23	178	41	183	31
32	23	220	16	196	57	143	49	165	19	167	28	148	42	152	32
33	31	155	45	157	0	159	0	121	12	147	0	106	4	97	33
34	0	134	0	123	27	110	5	95	13	85	0	99	0	102	34
35	3	104	8	110	0	108	0	90	0	102	0	27	0	89	35
36	0	92	0	94	0	94	0	56	0	45	0	36	0	31	36
37	0	69	0	54	0	37	0	39	0	71	0	61	0	44	37
38	0	3	0	−0	−0	−0	−0	−0	−0	−0	−0	−0	−0	−0	38

Humble aromatic type analysis in percentage

Alkyl benzenes	4.73
Naphthalenes	3.65
Acenaphthenes	7.72
Acenaphthylenes	2.81
Phenanthrenes	6.36
Diacenaphthylenes	4.75
Pyrenes	13.14
Chrysenes	7.57
Acepyrenes	2.93
Benzpyrenes & other organic compounds	46.34
Total	100.00

Large peaks may appear at masses equal to those of molecules, fragments containing stable ring systems, or fragments of different structure and common mass. *n*-Paraffins and polycycloalkanes form more molecular or "parent" ions than do branched chain paraffins or monocycloalkanes. Fragments containing the stable, partially hydrogenated phenanthrene ring system frequently give large peaks. Isoprenoids usually have a number of methyl branches or substituents, and several different fragments of the same mass can be formed when an isoprenoid loses a methyl group. Common mass ions of isoprenoids, also, yield large peaks in the mass spectra of naturally occurring saturated hydrocarbons.

"Parent," stable ring, or common mass ions can be identified in most cases. For convenience, the whole number atomic masses of C = 12 and H = 1 are used in calculations of ion mass numbers, and fragment ions generally have odd mass numbers. Therefore, stable ring and common mass ions are found predominantly in odd numbered x columns. All "parent" ions of hydrocarbons have even mass numbers, and these ions appear in even numbered x columns. Common mass ions normally appear a row above and one column to the left of "parent" ions; whereas most stable ring fragment ions have masses 50 or more units less than the masses of the molecular ions. Thus, stable ring ions appear chiefly in odd x columns several rows above the "parent" ion region.

Some stable ring ions, however, are formed by breaking 2 bonds. These ions have even mass numbers. An example of an even mass stable ring ion is the $x = -6$ and $C_{\#} = 16$, mass 218, ion in the cholestane[31] mass spectrum. The even mass numbers of some fragment ions lead to ambiguity in the identification of "parent" ions. This ambiguity can be removed in many cases by additional fractionations or by information gathered from the mass spectra of reference compounds. Studies of thousands of mass spectra of saturated hydrocarbon mixtures from plant and animal lipids, sedimental extracts, and crude oils have shown that certain fragment and "parent" ions commonly yield large peaks at particular masses. Some of these large peaks may be absent in the mass spectra of highly paraffinic fractions, but further separations usually yield cycloalkyl concentrates in which these large peaks appear as they do in most biological and sedimental hydrocarbons.

The peaks that are normally large in the spectra of naturally occurring saturated hydrocarbon mixtures have been mentioned in previous publications,[37-39] and many of these peaks will be indicated, again, in the subsequent interpretation of the meteoritic hydrocarbon spectra. But, it is noteworthy in the spectra of cholestane[31] that the $x = -7$, $C_{\#} = 26$ (common mass ion) and 17 (stable ring ion); $x = -6$, $C_{\#} = 27$ ("parent" ion) and 16 (stable ring ion); and $x = -5$, $C_{\#} = 11$ (stable ring ion) peaks are large. These same mass peaks have been observed to be large in either total or refined saturated hydrocarbon fractions which have been isolated from extracts of terrestrial sediments. Peaks at $x = -6$, $C_{\#} = 28$, 29, and/or 30, which may be "parent" ions of compounds structurally related to parent sterol hydrocarbons other than cholestane, are also prominent in most naturally occurring alkane fractions.

Mass spectra of the meteoritic hydrocarbons presented in TABLES 6 and 8

were obtained at an ionization potential of 12 volts. At this low potential aromatic hydrocarbons yield chiefly "parent" ions. This technique cannot be used in the analyses of saturated hydrocarbons because the energies required to break bonds and remove electrons are approximately equal in alkanes.

Nearly all of the large peaks in TABLE 6 are produced by molecular ions, but apparently the wide range of electron energies in the ion source of the General Electric instrument caused appreciable fragmentation. In TABLE 8 the data on many large fragments as well as "parent" ions are presented. Ultraviolet spectra provide a valuable assistance in the interpretation of the mass spectra of aromatic hydrocarbons. Many of the aromatic hydrocarbons in naturally occurring aromatic mixtures can be identified by the combined use of mass and ultraviolet spectroscopy.

Blanks and the Holbrook meteorite. Because limited amounts of carbonaceous chondrites are available, minimal sample sizes were used in these investigations. To ensure that laboratory contaminants did not significantly affect the results of the analyses obtained on the minimal sized samples, an elaborate system of blanks was used. In addition, a high temperature meteorite, the Holbrook, which should not have contained significant amounts of indigenous organic matter served as an indicator of the type of contaminants a meteorite might acquire during its fall to earth, contact with earth, and storage in a museum. None of the blanks contained organic matter that could be detected by infrared, ultraviolet, or mass spectrometric analyses; and the Holbrook extracts showed very small infrared and ultraviolet absorptions as shown in FIGURES 1 and 5. These controls indicate: (1) that laboratory contaminants did not measurably alter the meteorite analyses; (2) that the amount of organic matter acquired by meteorites (Holbrook fell in 1912) may be negligible.

Rinses and extracts. The process of first rinsing and of then extracting the meteorites was used to detect contaminants. It was assumed that surface and near surface contaminants could be rinsed from the surfaces of the stones. Analysis of the rinse fractions (all of which were relatively small) and the extracts did not show any marked changes in concentrations of hydrocarbons between the exterior and interior portions of the meteorites. These analyses suggest that the surfaces of the meteorites had not been contacted or contaminated by significant quantities of extractable organic matter during storage.

Authenticity of meteorite fragments. A complementary publication[12] lists the reliable sources of the fragments studied in these investigations. This complement[12] also reviews and presents data that indicate that meteorites are of extraterrestrial origin and that the samples used in this study are authentic meteorites.

Records of Life on Earth

Terrestrial organisms or their remnants represent the only established references for detection of biological materials. Variations in the appearances, behaviors, and compositions of organisms make it apparent that qualitative, rather than precise, quantitative measurements or observations provide the best means of recognizing previously unseen or unanalyzed forms or remnants of life. Presently the extensive data on the fossil remains and organic matter

in the sediments on earth are the most acceptable standards for the identification of former life in meteorites.

Numerous soils and marine sediments have been analyzed by methods analogous to those used in this investigation of the Murray and Orgueil carbonaceous chondrites. Additional analyses have been run on saturated hydrocarbons from sediments, plants, and animals. A review of these investigations of terrestrial samples will serve as a basis for evaluating the analyses of the meteoritic hydrocarbons.

Smith[40] found that geologically young hydrocarbons (C^{14} ages 9000 to 14000 years) isolated from recent sediments have optical activities, infrared spectra, elemental and type compositions, and chromatographic properties equivalent to hydrocarbons in ancient crude oils. Oakwood[41] plotted optical activities *versus* distillation temperatures for hydrocarbons from kelp (a seaweed) and crude oil, and he observed that these similar plots peaked in the same temperature region.[42] Saturated hydrocarbons in mixtures of plant and animal lipids, recent and ancient sediments, and crude oils seem to have equivalent chromatographic properties and infrared spectra, and similar mass spectrometric cracking patterns.[43] C^{14} ages[40,43] and *n*-paraffin distributions[33,34,39,43,45,46] provide the only reported means of distinguishing between the C_{14} and larger saturated hydrocarbons in recent sediments and those in crude oils.

Wax-esters, closely resembling beeswax, have been found in a variety of types of soils from arid and humid areas of tropical and temperate regions of the world.[31] Blumer[46] has identified in soils the same aromatic hydrocarbons that have been identified in marine sediments.[34] Hunt[47-49] and Brenneman[50] have reported similarities between hydrocarbons dispersed in ancient sediments and concentrated in crude oils. Bray[51] has observed that the aromatic fractions of all crude oils absorb near 12.35 and 13.45 μ in the infrared.[45] He has referred to these absorptions as "oil bands."

2,6,10,14-Tetramethylpentadecane, pristane, is a norisoprenoid hydrocarbon constituent of fish oils.[52,53] Pristane forms 0.2 and 0.5 per cent, respectively, of the two crude oils in which it was measured.[54] 2,6,10,14-Tetramethylhexadecane, phytane, is a diterpenoid or isoprenoid which is, also, a common component of fish and crude oils.[55] The concentrations of phytane in 10 Iranian oils is to be reported.[55] It is of interest that the first C_{14}, or larger, branched paraffins, pristane and phytane, isolated from crude oils are of an isoprenoid type.[34]

When the possible complexity of petroleum is considered, the relatively high concentrations of pristane and the measurable quantities of phytane in crude oils is noteworthy. There are in excess of 100,000 possible isomers of C_{19} and 366,319 possible isomers of C_{20} paraffins.[56] Because crude oils contain hydrocarbons composed of more than 50 carbon atoms, the number of paraffins, cycloalkanes, and aromatic compounds that might form petroleum is astronomically large and of the order of 10^{18} different hydrocarbons. Because of the diversity of compound types and the large range of carbon atom contents of the hydrocarbons in petroleum, the abundances of pristane and phytane in crude oils suggest a highly selective synthesis of these compounds. The common presence of the precisely structured pristane and phytane in fish and crude oils may be more than fortuitous. Possibly certain of the stable saturated

hydrocarbons from prehistoric life have been preserved for geological periods of time in nature, and these compounds may be used to define and study the existence of ancient organisms.

It has been suggested that saturated isoprenoid type hydrocarbons synthesized either by living things[42,43] or from sterol and isoprenol remnants of organisms[34,49,54,57,58] are major sources of naturally occurring alkane hydrocarbons. Disagreement on the origin of terrestrial alkanes centers about the issue of whether living things[40-43,59] or chemical reactions acting on plant and animal remains[45,54,57,58,60,61] make most of the saturated hydrocarbons in nature. Either of these sources would yield biosynthetically controlled products which could serve as reliable biological indicators.

Similarities and differences in the benzene and analogous extracts of terrestrial sediments may be generally summarized and partially explained. An average sediment contains between 20 and 80 parts per million of C_{14} and larger hydrocarbons.[31,40,45,49] These hydrocarbons usually comprise between 10 and 30 per cent of most extracts, and the hydrocarbons can be separated chromatographically from the organic oxygen, nitrogen, and sulfur containing molecules which make up 70 to 90 per cent of the extract.[31,40,45] Hydrocarbons are eluted primarily in the *n*-heptane, carbon tetrachloride, and benzene eluates; whereas the organic nonhydrocarbons appear chiefly in the methanol eluates from silica gel chromatographic columns.

The extractable organic nonhydrocarbon fractions from sediments normally show hydroxyl or amino (2.9 to 3.0 μ), carbon-hydrogen (3.3 to 3.5 μ), carbonyl (5.7 to 5.9 μ) and broad absorptions in the 7 to 15 μ regions of the infrared. Saturated and aromatic hydrocarbons from sedimental extracts show the usual carbon-hydrogen absorptions in the 3.3 to 3.5 μ and 7.2 to 7.8 μ regions. In addition, the saturated hydrocarbons in most cases absorb at the carbon chain frequency near 13.9 μ which is indicative of *n*-paraffins, and aromatics generally absorb at the "oil band" frequencies of 12.4 and 13.4 μ.

Nonlinear polyring aromatics are dominant in sedimental hydrocarbon mixtures. Phenanthrenes, chrysenes, pyrenes, and perylenes appear with and without alkyl and cycloalkyl substituents in many soils and marine sediments. Anthracenes, naphthacenes, and larger linear polyring aromatics have not been identified in extracts of soils or marine sediments. Carruthers[62] has isolated some alkylanthracenes from crude oils; but in petroleum also, phenanthrenes are much more abundant than anthracenes.[63]

Sedimental extracts can be divided into two broad classes: (1) soil or aerobic, and (2) marine sediment or anaerobic. Overlaps do exist. Anaerobes and aerobes both live in soils and marine sediments, but aerobes appear dominant in most soils. Soil extracts normally contain more wax-esters and less free sulfur and aromatic hydrocarbons than do extracts of marine sediments.[31,43] Although the same molecular structures appear to be present[34,39,46] in sedimental hydrocarbons, the ratio of saturated to aromatic hydrocarbons is usually greater in soils than in marine sediments.[31,34,40,43]

Apparently, the compositions of sedimental extracts can be grossly explained on the basis of the stabilities of plant and animal constituents in different natural environments. Chemically and/or biochemically active carbohydrates, proteins, fats, oils, and porphyrins (hemin, etc.) comprise all but a

small fraction of the substances in most living cells. Yet, only traces of these substances are found in some sediments.[16,63-68] Stable saturated hydrocarbons, extremely minor constituents of the lipid fractions of most organisms[41-43,52,53,69-74] are present in measurable concentrations in essentially all sediments. Likewise, wax-esters, which form thin protective coatings of plants and insects,[73,74] are apparently stable in aerobic environments and are found commonly in soils.[31]

Differences in the compositions of the extractable fractions of soils and marine sediments may be traceable to the anaerobic activity in sea bottoms. Organic acids and alcohols combine to form wax-esters. Anaerobes utilize these acids and alcohols and sulfate ions.[75] Conversions of olefinic steroid and isoprenoid acids and alcohols into stable aromatic molecules result in large energy releases. Anaerobes need energy. Most common foods are almost completely consumed by aerobes in surface sediments. To survive, anaerobes must frequently use unusual energy sources and reduce sulfates to hydrogen sulfide which is oxidized to free sulfur.[75] Marine sediments may contain more free sulfur and aromatic hydrocarbons and less wax-esters than soils,[43] merely, because marine environments are normally more anaerobic[75] than nonmarine environments. Thus, analyses of sedimental extracts may serve as environmental as well as biological indicators.

Meteoritic Extracts

Because minimal sample sizes were used in this investigation, most meteorite extracts were too small to provide reliable data by all of the analytical methods used. Only the 2-Orgueil extract was of sufficient size to permit accurate chromatographic analysis and to supply *n*-heptane, carbon tetrachloride, and benzene fractions which absorbed significantly in the 2 to 15 micron regions (TABLE 3, FIGURE 2). However, because the ultraviolet and mass spectrometric data (FIGURES 3 and 4 and TABLES 4, 5, 6, 7, and 8) indicate that the Murray and Orgueil extracts are related much as are terrestrial sediment extracts, analyses of the 2-Orgueil sample may be approximately representative of other extracts of carbonaceous chondrites.

The 2-Orgueil chromatographic data in TABLE 3 fall in the terrestrial sediment range.[31,40,45] Infrared spectra of the total meteorite extracts in FIGURE 1 and of the chromatographic fractions of the 2-Orgueil in FIGURE 2 indicate that all of the major absorption bands may be traceable to hydroxyl or amino (2.9 to 3.1 μ), carbon to hydrogen (3.3 to 3.6 and 7.1 to 7.9 μ), carbonyl (5.6 to 6.0 and 8.0 to 9.0 μ), aromatic or olefinic (10 to 14.5 μ), and carbon-to-carbon chain (13.8 to 13.9 μ) groups. The carbonyl absorptions in the Murray and Orgueil extracts suggest a complex mixture of carbonyl compounds and the mass spectra (TABLES 6 and 8) show that these samples do not contain appreciable concentrations of either fatty acids or beeswax-like esters (absence of large peaks at odd carbon numbers in $x = -10$ column). Ultraviolet (FIGURES 3 and 4) and mass (TABLES 6 and 8) spectra show that the Murray and Orgueil extracts contain significant quantities of aromatic hydrocarbons. The Murray extracts contained small and the Orgueil extracts contained copious amounts of free sulfur. The preceding cursory examination of chromatographic and spectrometric data indicate that the Murray and Orgueil extracts resemble

terrestrial marine sediment extracts. Like most marine sediments and unlike most soils, the Murray and Orgueil meteorites have benzene extractable fractions which contain complex mixtures of carbonyl compounds (which are not wax-esters) and significant concentrations of aromatic hydrocarbons and free sulfur.

Further consideration of the spectrometric data provides evidence of additional similarities and some dissimilarities between meteoritic and terrestrial sedimental extracts. In FIGURE 2, the 12.3 and 13.4 μ absorption bands of the benzene eluate of 2-Orgueil are similar to the "oil bands"[51] found in the infrared spectra of the aromatic fractions of all crude oils,[45,51] but the ultraviolet (FIGURE 5) and mass spectra (TABLES 6 and 8) show that the meteorite aromatic fractions are unusually simple. Aromatic mixtures in crude oils and ancient sediments[34,39,43,45] greatly exceed in complexity these meteoritic fractions. Apparently, certain recent sediments are the only terrestrial samples[34, 39,45,46] containing naturally formed aromatic mixtures which even approach in simplicity the aromatic hydrocarbons from these meteorites.

Structural types of the major aromatic species in the Orgueil and Murray fractions can be deduced from the ultraviolet (FIGURES 3 and 4) and mass (TABLES 6 and 8) data. The ultraviolet spectra indicate the possible aromatic nuclei, and the mass data permits the elimination of some possible nuclei which do not yield large parent ions in the mass spectra. Based upon the ultraviolet and mass data the principal aromatic nuclei are in order of decreasing abundance: (1) Phenanthrenes, pyrenes, and chrysenes in the Orgueil extract. Most abundant aromatic hydrocarbon is phenanthrene. See 178 mass peak in $x = -4$ column at C # = 13 in TABLE 6. (2) Pyrenes, chrysenes, benz(j)-fluoranthenes (indicated but not completely identified) and phenanthrenes in the Murray extract. Most abundant aromatic hydrocarbon is pyrene. See 202 mass peak in $x = -8$ column at C # = 15 in TABLE 8.

Large "parent" ions appear in the 22 to 29 carbon number range of the even x columns in TABLE 6. These ions are made from complex aromatic molecules many of which differ in carbon and hydrogen content from aromatics reported in the literature. It has been proposed[34,43] that naturally occurring aromatics may be products of transformations of isoprenoids and steroids, and this proposal has recently been supported by the identification of 21 aromatic compounds in petroleum. Mair and Martinez-Pico[76] note that "most of the ('21 aromatic') compounds are related to steroids. The results . . . give strong support to the theory that steroids and other natural products related to phenanthrene are petroleum precursors." Conversions of olefinic steroids and terpenes to aromatics necessitate the loss of some alkyl, usually methyl substituents, from the highly substituted isoprenoid ring systems. Consequently, aromatics formed from terpenes and steroids would contain a lower number of carbon atoms than their precursors. The high concentrations of largely unreported C_{23} through C_{28} phenanthrenes, chrysenes, and pyrenes indicated by the large peaks in TABLE 6 suggest that C_{27} through C_{30} steroids and triterpenoids may have been a source of the aromatics in the Orgueil meteorite. This suggestion is amplified by the common prevalence of the same types of nonlinear polyring aromatics in meteoritic and sedimental

hydrocarbon mixtures, as is further indicated by the "oil band" absorptions of the benzene eluate in FIGURE 2.

C_{23} through C_{28} aromatics are less abundant in the Murray (TABLE 8) than in the Orgueil. The lower abundance may indicate that the Murray was subjected to a high temperature, more severe environment than the Orgueil and the polyalkyl substituted aromatics may have been partly degraded in the Murray. Olivine, a high temperature mineral, is a constituent of the Murray meteorite. The simplicity of the aromatic fractions in these meteorites may be explained by assuming that more restricted varieties of organisms existed in the meteorites than are normally found in recent terrestrial sediments.

n-Paraffin[33,34,39,43,45] and polycycloalkane distributions have been cited as evidence of the biologic origin of some of these compounds.[29,35,36,38] Most sediments and organisms contain greater abundances of: (1) odd- than even-carbon number *n*-paraffins in the C_{21} to C_{35} range. (2) C_{24}, C_{27}, C_{28}, C_{29} and/or C_{30} than of other C_{17} and larger tetra-, penta-, and higher polycycloalkanes. Although alterations, which change some organic molecular structures and distributions, decrease the features characterizing biologically derived hydrocarbons, these features apparently persist even in the hydrocarbons from ancient sediments.[34,39,43,54]

Many of the features noted previously for terrestrial hydrocarbons appear in the mass spectra of the meteoritic hydrocarbons. In the $x = +2$ columns, the 23 and 29 carbon number peaks in TABLES 4 and 7 and the C_{25}, C_{27}, and C_{29} peaks in TABLE 5 are larger than the peaks immediately above and below them in the $x = +2$ column. Peaks in the $x = +2$ are produced by ions that have masses equal to paraffins or heptacycloalkanes. Branched paraffins do not produce "parent ions" to a measurable degree in the mass spectrometer,[35,54] and most heptacycloalkanes would contain more than 25 carbon atoms in their ring nuclei. Therefore, the above designated peaks probably contain *n*-paraffin "parent peaks," and the "peakings" at odd carbon numbers in the C_{23} to C_{29} range in these $x = +2$ columns of TABLES 4, 5, and 7 are similar to "peakings" observed in the mass spectra of saturated hydrocarbons from various ancient terrestrial sediments.[78]

Other "peakings" and "inflections" (anomalously small differences in sizes between successive peaks in an x column) in TABLES 4, 5, and 7 are noteworthy. These are: C_{20} through C_{30} peaks in the odd numbered x columns of TABLES 4, 5, and 7 are approximately as large as the even numbered x peaks in this carbon number range. These large odd x peaks and certain of the "peakings" in the odd x columns are indicative of poly alkyl-substituted or branched chain alkanes such as isoprenoids. Saturated hydrocarbons, made by hydrogenating products of the abiotic Fischer-Tropsch synthesis, do not yield as large odd x peaks as do meteoritic and terrestrial alkanes. A comparison of abiotic and meteoritic hydrocarbons will be presented in a subsequent publication.

Because of the chromatographic properties of the fractions and the odd mass numbers of the ions the large peaks at $x = -7$, $C\# = 15$ in TABLES 4 and 5 and at $x = -5$, $C\# = 13$ and 15 in TABLE 5 are produced apparently

by nonbasic cyclic nitrogen compounds. These compounds are slightly more polar than alkanes and are more concentrated in the carbon tetrachloride eluate (TABLE 5) than in the *n*-heptane eluate (TABLE 4). In TABLE 4, the peaks at $x = -6$ and -7, C# = 16 are larger than those of homologous ions at C# 13, this suggests that the nitrogen ions may have obscured the "peakings" at $x = -6$ and -7, C# = 16 which are generally observed in the mass spectra of the terrestrial alkanes. "Peakings" at $x = +1$, C# = 21 in TABLES 4 and 5 have an odd mass number and also are suggestive of nitrogen compounds. "Peakings" in the 19 to 25 carbon number ranges of the $x = 0$, -2, and -4 columns which appear in TABLES 4, 5, or 7 are uncommon in sedimental hydrocarbon spectra but are present in the mass spectrum of the saturated hydrocarbons from oysters.[79] Neither crude oil nor sedimental organic contaminants are probable sources of the mono-, di-, and tricycloalkanes which form the ions producing the latter peakings. Nevertheless, differences in the carbon numbers at which the "peakings" maxima occur in the various columns of TABLES 4, 5, and 7 as well as the alternate high or low values of odd and even C# "parent ions" in TABLE 5 may be more suggestive of a biological product than of an abiotically formed mixture that is thermodynamically at equilibrium.

Contamination

Carbonaceous chondrites are friable, seemingly porous stones. Olivine, a mineral that forms at temperatures above 400° C. is present in the Murray but apparently not in the Orgueil stone. Associated minerals in the Orgueil suggest that it may have formed in an environment resembling an organic rich saline environment on Earth,[12] and the compositions of the extractable carbonaceous fractions of the Murray and Orgueil, also, are indicative of marine type sedimentary deposits. Although the compositions and the intimate associations of the mineral and carbonaceous materials in carbonaceous chondrites are not incongruous with a marine ecology, meteorites are likely to be contaminated by terrestrial substances. It is important to consider the most probable contaminants and the effect that they may have upon the composition of the carbon constituents of meteorites.

All meteorites accepted as carbonaceous chondrites were observed during their fall to Earth. Many of the carbonaceous chondrite fragments that have been collected are partially coated with a heat altered layer. Charred crusts were apparently formed on the lead surfaces of the meteorites when these areas were heated to incandescence on entry into Earth's atmosphere.

In transit to Earth carbonaceous chondrites break-up. Boato's[8] measurements show that meteoritic waters released at temperatures above 180° C. apparently have not been exchanged with terrestrial waters. Carbonaceous chondrites give off sizeable quantities of water at temperatures in excess of 180° C.,[8] and additional evidence has been presented[12] that these meteorites did retain substances in space which normally boil below 180° C. The volatile constituents of carbonaceous chondrites suggest that they have restricted the egress of gases to the vacuum of space. Perhaps, the interiors of these fragments are less accessible than their porous structures may indicate, but regardless of the permeabilities of carbonaceous chondrites, their fall was over

quickly and, in the thin molten surface layers of the falling meteorites, temperatures far in excess of 180° C. were reached. Even from these thin melts, sizeable volumes of gases may have been expelled. During their plummet to Earth, carbonaceous chondrites probably lost more volatile carbonaceous substances then they received from the atmosphere.

When the meteorites struck Earth, their hot surfaces may have distilled or decomposed organic matter, and cool portions of the chondrites may have condensed and collected the vapors. But, the burned crusts of recovered fragments of carbonaceous chondrites cover only a fraction of the stones. These crusts are very thin, and the fragments are friable. It is doubtful that the heat or impact energies of these stones could have vaporized more than a trace of terrestrial organic matter. If carbonaceous chondrites are contaminated appreciably, they probably acquired most of the contaminants after the fragments were collected.

Analyses of the Holbrook chondrite show that this stone was not greatly contaminated either during its fall or almost 50 years of storage on Earth. Nevertheless, the carbonaceous matter in the Murray and Orgueil meteorites may have been defiled. Carbonaceous chondrites are more porous than ordinary chondrites, and the Murray and Orgueil contain higher concentrations of carbon than the Holbrook. Organic materials may be strongly adsorbed on carbonaceous substances. Thus, the Murray and Orgueil fragments were probably more susceptible to contamination than the Holbrook. Notwithstanding, the high concentrations of benzene extractable materials in the Murray and Orgueil cannot be easily explained by natural contaminants. Why in 10 years should the Murray, or in 100 years should the Orgueil stones accumulate much more extractable carbonaceous substances than an average soil collects in thousands of years? It seems likely that most of the carbon compounds in the Murray and Orgueil fragments were either indigenously formed in the parent body or carelessly added by man.

Meteorites are handled, and some are marked for identification. Oily hands, paints, wax pencils, polishes on display cases, plasticizers in plastic storage cases, microorganisms, pyrolysis products of fossil fuels in urban atmospheres, lacquer coatings, and other carbonaceous substances may have contacted and contaminated the Murray and Orgueil fragments. Contamination has been considered a major problem throughout this investigation, and appreciable attention has been paid to this problem.

Fragments of the Orgueil meteorite were obtained from two museums and the Murray fragments came from another collection. Contaminants from these various locations should have been quite different, but none of the variations in the compositions of the meteoritic extracts suggested significant organic contamination. All of the fragments were carefully inspected and no evidence of markings or coatings were observed. Microscopical examinations[17,18] show that recent terrestrial type organisms are present in the Murray[80] and Orgueil in numbers that are two or more orders of magnitude less than in the average terrestrial sediment. Microorganisms usually contain from 1 to 3 parts per thousand by weight of hydrocarbons. These concentrations are only slightly greater than those of the hydrocarbons in the Orgueil (TABLE 2). Terrestrial organisms which have existed in the meteorites, seem to have been

too small in number to have supplied more than a trace of the meteoritic hydrocarbons.

Analytical data provide additional evidence against significant contamination of the Orgueil and Murray fragments. Benzpyrenes are common pyrolysis products. The air in urban areas contains from 1.5 to 25.5 parts per trillion by weight of benzpyrenes.[81] Pyrolysis products of wood include methyl alcohol, ketones, organic acids, C_9 and smaller alkanes, as well as olefinic hydrocarbons. Colored markings usually contain pigments that absorb sharply in the visual range. Wax pencils are frequently composed of wax-esters or petroleum waxes. Drying oils in paints and lacquers are mixtures of olefinic compounds which have carbonyl functional groups. Crude oil distillates and polishing oils have limited boiling point ranges and contain chiefly C_{20} and smaller compounds. The aromatic fractions in petroleum are more complex than the aromatics in recent sediments or the meteorites.[34,39,43,45]

Analyses of the Murray and Orgueil extracts show that they contain: (1) negligible concentrations of olefins (and in the Orgueil extract of benzpyrenes); (2) no substances which absorb sharply in the visual region; (3) alkane and aromatic hydrocarbons which are distributed as they are in terrestrial marine sediments; (4) hydrocarbons and benzene extractable nonhydrocarbons in the same relative abundances that they are found in sedimental extracts. Because the Murray contains about 5 times and the Orgueil more than 50 times the amount of benzene extractable materials that is found in an average sediment, it is unlikely that these extracts could have been obtained from terrestrial sediments which are the only previously reported sources of extracts of these compositions. The low recent organism counts[17,18] make it improbable that terrestrial organisms were a source of the extracts. Qualitative and quantitative considerations support the view that the Murray and Orgueil carbonaceous extracts were predominantly indigenous.

Nonbiological Sources of Hydrocarbons

Anders[82] suggests that hydrocarbons resembling those in terrestrial organisms may have been made abiotically in the solar nebula and incorporated later in the bodies of the solar system. When life evolved on Earth, he believes that these hydrocarbons favored the evolution and survival of organisms which could utilize and synthesize hydrocarbons of the types which are now present in most solar bodies.[82] Innumerable other speculative sources of hydrocarbons may be proposed. Many of these proposals are neither clearly supported nor directly denied by experimental data.

Although the extensive literature on organic reactions define what many reactants will form under various conditions, our imaginations may specify reactants and conditions that are either untried or unobtainable on Earth. Nevertheless, organic chemistry is, in part, a record of the means that have been devised by intensive study and extensive research to synthesize biotic type products. This record clearly attests that it is extremely difficult by the use of abiotic reactions to duplicate most of the individual biological compounds that have carbon numbers in the range covered by the compounds in the benzene extracts of carbonaceous chondrites. After failing to synthesize a pristane reference, Bendoraitis *et al.*,[54] isolated this hydrocarbon, which is a

minor constituent of fish oils. To avoid the problems of total synthesis, Dean and Whitehead[55] used phytol, a biological product, and exchanged a single hydroxyl group for a hydrogen atom to make phytane. Thermodynamically, it is, also, difficult to define feasible conditions under which hydrocarbons in terrestrial sediments may have formed abiotically. Amossow and Wassojewitsch[83] have observed that the equilibrium temperatures calculated from the abundances of various hydrocarbons in crude oils range between 0° and 225° C. for a Nebit-Dagh oil, 90° and 1075° C. for a Kara-Tschuchura oil, and −70° and 225° C. for a Kostschage oil. These temperature ranges are in great disagreement with the temperatures that are believed to have existed in sedimentary basins during petroleum formation.

Research efforts carried out over a 100-year period have failed to provide any evidence that abiotic, radiological, or chemical reactions were a significant source of hydrocarbons in terrestrial sediments. A summary of the API research on the origin of hydrocarbons notes the inability of radioactive induced reactions to make products similar to those found in nature.[84]

Conclusions

Aromatic hydrocarbons have been identified as common constituents of meteoritic and terrestrial sedimental extracts. Saturated hydrocarbons isolated from the Murray and Orgueil carbonaceous chondrites have infrared spectra, molecular weight ranges, and cracking patterns in the mass spectrometer that resemble those of sedimental saturated hydrocarbons. The relative amounts of hydrocarbons and nonhydrocarbons, the infrared spectra of the nonhydrocarbons, and the free sulfur contents of the benzene extracts of the Orgueil and terrestrial marine sediments are similar. Except for the relative simplicity of the aromatic fraction from the Orgueil fragment, analyses of both the Orgueil and Murray extracts fall within the range of compositional variations observed in terrestrial sediment extracts of plant and animal hydrocarbons.

Although further research may provide an alternative explanation for the amounts and overall compositions of the benzene extracts of the Murray and Orgueil carbonaceous chondrites, many similarities of these extracts to the extracts of terrestrial marine sediments have been demonstrated. Lacking another experimentally established explanation, we propose that the amounts and compositions of the benzene extracts of the Murray and Orgueil are evidence for biological activity in the parent body of these meteorites. Because of the apparent stabilities of certain hydrocarbons in natural environments, these compounds may provide a means of tracing the evolution of life in primordial times.

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FURTHER OBSERVATIONS ON THE PROPERTIES OF THE "ORGANIZED ELEMENTS" IN CARBONACEOUS CHONDRITES

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Independent studies, conducted at various laboratories, indicate that the "organized elements" do not seem to be terrestrial contaminants. This evaluation is based mainly upon a consideration of fine morphological criteria. New experiments with biological stains revealed that the organic microstructures in carbonaceous meteorites may selectively take stains in the presence of mineral matter.

Claus and Nagy (1961, 1962) and Nagy *et al.* (1962), described organic microstructures that were found embedded in some of the indigenous minerals of certain carbonaceous meteorites. These findings have been confirmed, or partially confirmed by independent investigators. Reimer (1961), Staplin (1962), Palik (1962, 1963), Cholnoky (1962), and Skuja (1962) examined the same meteorite sample as did Claus and Nagy (1961, 1962). Briggs and Kitto (1962) described what they thought to be indigenous, stainable microstructures in the Mokoia meteorite. However, the last two authors were unable to reach a conclusion regarding the origin of these particles. Ross (1962) examined another sample of the Orgueil meteorite, from the collection of the British Museum, and found microstructures which he believed were of biological origin and which were most likely indigenous to the meteorite. Recently, Engels (1962) isolated HF-resistant pellicles from another sample of the Orgueil meteorite. Timofeev (1962) found fossilized and indigenous microflora in the Mighei carbonaceous chondrite. The microscopical preparations of Claus and Nagy have been examined by approximately 100 microbiologists including Erdtman, Bourrelly, Papp, Deflandre, Palmer, Durham, Dienes, and Gregory. Tentative but divergent identifications were offered by some of these investigators.

Fitch and Anders (1963) argued that the organized elements were silicate mineral grains, opaque mineral particles, hydrocarbon globules, coacervates, Fox (1961) spheroids, sulfur droplets, pollens, and starch grains or spores, or other unknown terrestrial contaminants. Deflandre (1962) stated that the organized elements are unspecified terrestrial contaminants or artifacts (except those which are embedded in minerals). Urey (1962*a*) reviewed the available information and suggested that the organized elements may indicate, but they cannot yet be regarded as conclusive proof for the existence of extraterrestrial life.

Other experimental information which may suggest the presence of extra-

terrestrial biological processes includes the finding of what seems to be biochemical compounds in carbonaceous meteorites. Nagy *et al.* (1961*a*), and Meinschein *et al.* (1963), reported isolation of complex, saturated, and aromatic hydrocarbons, respectively. Anders (1962) and Krejci-Graf (1962) criticized these findings and the interpretations. However, a rebuttal has been offered (Nagy *et al.*, 1962). Calvin (1961) and Briggs (1961) reported the finding of compounds which might be cytosine or purines, respectively. It should be noted that bituminous organic matter was isolated from the Orgueil meteorite by Cloëz (1864), only a few weeks after the meteorite fell. This fact suggests that a sizeable portion of the meteoritic organic matter is likely to be of extraterrestrial origin.

Mineralogical and petrographical studies have shown that the parent body (ies) of carbonaceous chondrites was capable of supporting a form of life. It seems that liquid water was present and that this low-to-moderate temperature, aqueous environment was slightly alkaline and somewhat reducing. Extrapolation of the parent body environment from the known mineral assemblage in terms of phase equilibria data in *p*H-redox systems has been published by Nagy *et al.* (1961*b*), and in more detail (1963). Similar conclusions, arrived at by independent studies, have been advanced by DuFresne and Anders (1962). Petrographical observations by Nagy and Claus (1963) showed that the meteorite parent body had been subjected at one time to mechanical stresses that produced fractures which were later filled with magnesium sulfate. The textural patterns of the Orgueil and Ivuna carbonaceous chondrites resemble certain terrestrial rocks, such as pyroclastic rocks, deposited in water from fragmental volcanic debris. They also resemble silicate rocks altered by hydrothermal solutions. These petrographical studies have also shown that the interior of the meteorites does not contain evidence for high temperature effects acquired during the fall through the Earth's atmosphere. Consequently, organic microstructures and unorganized biochemical type compounds could have arrived in the meteorites without destruction by heat. The study of petrographical thin sections also suggests that the average pore size of the Orgueil and Ivuna meteorites is too small ($<1\ \mu$) to permit the entrance of most airborne terrestrial contaminants.

The micropaleontological examinations, the biochemical analyses, and the mineralogical and petrographical measurements strongly suggest that biological activity was active at one time on the meteorite parent body. A full evaluation of the origin of the organized elements must involve a consideration of fine morphological structures, the applicability of biological stainings and other microchemical methods.

Two suggestions had been advanced to explain the origin of the organized elements, provided that they will prove to be indigenous microfossils in the carbonaceous chondrites. Bernal (1962) suggested that life may have evolved along similar lines at various places in the Universe. Urey (1962*b*) suggested that the organized elements are terrestrial forms that contaminated the moon from Earth during early geological times. According to Urey's concept, biological matter and water may have been transferred to the moon, which was at that time closer to earth, by the impact of meteorites into terrestrial bodies of water. The carbonaceous meteorites are thought to come from the moon.

The Usefulness of the Microscopical Evaluation of Morphological Criteria

Organisms consist of highly organized organic matter. Consequently, they have a characteristic and specific chemical composition which reveals itself in specific morphology. Most morphological features serve the specific life functions of the organisms.

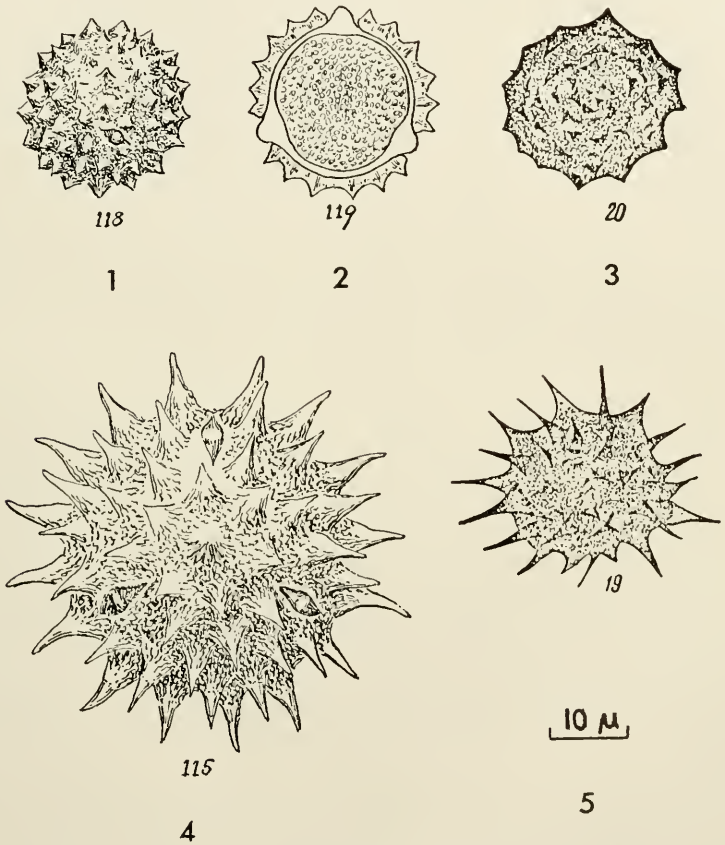
Morphological features develop through 2 basic processes: hereditary processes transmitted through genes from parent to offspring, and environmental influences affecting the individual. The first process results in genotypical morphological features; the second leads to phenotypical morphologies. Genotypical features are constant within a narrow limit (Dobshansky, 1951), whereas the phenotypical features are apt to show wide variations. Identifications based upon morphology must be restricted to genotypical features (Cholnoky, 1960). This means, of course, that a particular species cannot be identified through the examination of a single individual. A series of specimens must be examined to define the limits of phenotypical variation. The genotypical and the phenotypical morphological features are functional. However, genotypical morphology reflects hereditary needs (phylogenetic adaptation), whereas phenotypical features represent individual needs bearing on environment (ontogenetic adaptation; Goldschmidt, 1940).

The relationship between function and morphology is apparent among plants of higher and lower orders. For example, two species of the flowering plant genus *Ambrosia*, *A. elatior* and *A. artemisiaefolia*, live in habitats which are exposed to different degrees of sunshine and contain different amounts of moisture. The latter species, *A. artemisiaefolia*, lives in a semidesert environment. Consequently, the size of the foliage is smaller than that of the former species, *A. elatior*, and scleral elements are abundant in the leaves to provide mechanical support during periods of severe loss of turgor.

The *Ambrosia* pollen, *i.e.*, ragweed pollen, shows a characteristic genotypical morphology, a solid, spinose exo-exine. Clearly, the spines represent a genotypical feature because they must develop from the tapetal layer of the pollen sack. The pollen grains, during their ontogenesis, are not directly exposed to environmental influences. The spines are formed by apposition. For the same reason the spines of the ragweed pollen are solid rather than hollow. The solid intine can be penetrated by 3 pores only. The spines may facilitate the transportation of the pollen grains. The characteristic tricolpate structure is always observable upon proper focusing of the microscope (Erdtman, 1952, 1957; Faegri and Iversen, 1950; Jonas, 1952; Hyde and Adams, 1958). See FIGURE 1, pollen grain of *Ambrosia trifida*; FIGURE 2 the same in optical section; FIGURE 3, *Hystrichosphaeridium* sp. from the Upper Cambrian; FIGURE 4, pollen grain of *Dahlia pinnata*; FIGURE 5, *Hystrichosphaeridium* from the Upper Cambrian. (FIGURES 1, 2, and 4 were taken from Wodehouse, 1942, and correspond to his numbers; 118, 119, and 115, respectively; FIGURES 3 and 5 were taken from Timofeev, 1956, and correspond to his numbers 20 and 19, respectively.)

There are similar looking species of unicellular, aquatic plants. For example, *Hystrichosphaeridium* Deflandre is covered with spines. These spines, however, serve a different function, develop through different embryological

processes and are constructed differently (Deflandre, 1936; Timofeev, 1956; Evitt, 1961*a*, 1962). The spines of real *Hystrichospherids* grow out from the outer layer of the cell wall through intussusception. These hollow spines help the organisms to float in water (Schiller, 1933–37). Both pollen and *Hystrichosphere* spines help to protect the species. On casual observation ragweed pollen grains and certain *Hystrichospheres* may look alike (*cf.* FIGURES 1 and 3,



FIGURES 1 to 5. *1*, *2*, and *4*, pollen grains; *3* and *5*, *Hystrichospheres*.

and 4 and 5). Very careful microscopical examination is required by experienced microscopists to distinguish the hollow *Hystrichospheridium* spines from the solid ragweed pollen spines. This example may emphasize that detailed and careful observations are necessary for the identification of all microscopical plants and parts of plants.

Fitch and Anders (1963) questioned the validity of using fine morphological criteria in the identification of microorganisms. They claimed that structural features less than 1 μ in size are difficult to observe and they suggested that the resultant identification must be subjective. Yet the science of systematic

microbiology and micropaleontology provides numerous examples of the successful use of fine morphological characteristics (involving features less than 1 μ in size) in the identification of protobionta.

The blue-green algal genus *Oscillatoria* has 160 morphologically distinct taxa, 28 of which are less than 1 μ in diameter but they are still amenable to morphological characterization (Hollerbach *et al.*, 1953). As early as 1871, optical microscopy was sufficiently advanced to allow Pfitzer to establish two new genera *Neidium* and *Anomoeoneis*, that were formerly included in *Navicula*, by observing morphological features less than 0.3 μ in size. The recent work of Hayflick (1962) established that primary, atypical pneumonia in humans is caused by a *pleuropneumonia-like organism* (PPLO), less than 0.3 μ in size. The same detailed morphological observation is also used in characterization of microfossils. Recently, Evitt (1961*b* and 1962) has shown that the group of *Hystrichospheres* (Precambrain to Recent) consists of polyphyletic members. This finding was again based upon the observation of fine morphology, which involved the examination of the number of processes, spines, and the plate structures. Most biologists agree that the microscopical examination of fine morphological features (1 μ or less in size) is not only possible but it is a common practice in systematic zoology and botany.

Modern biological microscopes, if properly used by experienced investigators, can resolve objects as small as 0.2 μ in diameter. The theoretical limit of resolution is 0.10 μ. Clearly, it is quite possible to observe morphological features in the 0.3 to 0.5 μ range. Fitch and Anders argued that one of the organized elements embedded in mineral matter in one of the thin sections of Nagy *et al.* (1962) cannot be characterized because the spines are approximately 0.3 μ long and the resolution of a microscope which they believed to be equipped with a regular, high dry objective is 0.3 μ. This argument is in error because the spines on this organized element were observed with a $\times 70$ oil immersion objective, with a numerical aperture of 1.15, which gave a lower limit of resolution of 0.22 μ. The transparency of the embedding mineral (magnesium sulfate), its lack of color in the thin section and the lack of significant differences in refractive indices in this portion of the microscopical preparation prevented any serious interference from attaining the necessary resolution.

Fitch and Anders (1963) proposed a set of criteria to establish that certain objects are microfossils. Their criteria are essentially the customary definition of life. They suggested that to be able to prove that the organized elements are indigenous and extraterrestrial microfossils one must show (1) that they have characteristic morphologies, (2) show some evidence of propagation, and (3) show signs of metabolic processes.

Their first point needs no further discussion. One may reply to their second requirement by noting that adjoining organized elements have been observed embedded in minerals. This raises the possibility of either copulation or division (FIGURE 6*a*). Similar objects (but solitarily) were often found in the mineral matrix (FIGURE 6*b*). A less direct indication may be derived from the possible presence of deoxyribonucleic acid- (DNA)-type material, which will be discussed in another chapter. Finally, the presence of what may be bio-

genic compounds, such as certain hydrocarbons, cytosine and purines, should have already provided an answer to their last postulated requirement.

The Applicability of Biological Stains to the Investigation of Meteorite Samples

The use of biological stains for demonstrating cellular structures is indispensable in microscopical biology. During the preceding 2 decades a better understanding of the chemical nature of dyes and the chemical reactions involved, has made it possible to use the color developed as a specific indicator for the presence of a certain compound. With the development of color indices and standardization of the marketed dyes (Conn, 1953) many of the unpredictable results or uncertainties originating from the varied staining procedures have been eliminated. At present there are still some major gaps in the knowledge of the reaction mechanisms of several dyes and their exact specificity in several cases is not known; however, one is able to use them with a certain degree of confidence if one fulfills the following three criteria. (1) Use a whole array of structurally different dyes on the same substrate. (2) Use adequate amounts of controls. (3) Rely more on stains which chemically react with the substrate (or before application are in colorless form) than on those which are merely adsorptive in nature.

Slides were prepared essentially in 2 ways. (1) The sample was either crushed or dispersed on the slide in double distilled water, was covered, and the aqueous staining solution was, during the period of observation, constantly sucked through the preparation. (2) The meteorite and soil samples were crushed between 2 slides the surfaces of which were coated with fresh egg albumin. The other materials like pollen or starch grains, etc. were dusted over the albumin covered slides with a fine brush. The slides prepared in this way were then subjected to the staining procedures, washed with tridistilled water, dehydrated, mounted in balsam and coverslipped. In cases of the eosin and hematoxylin-eosin staining instead of eggwhite, collodium was used for adhesion. The staining with ninhydrin was performed in small test tubes, and the material was transferred to glycerin and examined in it. During the staining procedures special care was taken not to contaminate or crosscontaminate the preparations, therefore the stains were freshly made up with tridistilled water and the different specimens were stained in separate sets of coplin jars.

As can be seen from TABLE 1, 19 widely differing biological stains were used, of which only 1, *i.e.*, safranin, is considered to be a true adsorptive stain (the nature of dyeing with the eosin stains, like Azure II, and Dienes PPLO blue stain is still debated). Of the 19 stains only 1, Sudan IV, gave negative results with the organized elements. All of the other stains were found to be positive. One has to emphasize, however, that only a portion of the organized elements stained with the different stains and the proportion of the stained to the unstained particles varied from stain to stain. One has also to admit that several of the dyes used stained not only the organized elements but also a portion of the mineral material. However, one could easily distinguish by

TABLE 1

LIST OF STAINING EXPERIMENTS

Stain or the staining procedure applied	Carbonaceous meteorites						Noncarbonaceous stony meteorites	
	Orgueil 1.		Orgueil 2.		Ivuna		Murray	Holbrook	Bruderheim
	Organized elements	Mineral matter	Organized elements	Mineral matter	Organized elements	Mineral matter	Mineral matter	Mineral matter	Mineral matter
Acidic carmine	Purple	Lilac	Purple	Lilac	Purple	Lilac	No staining	No staining	No staining
Azure II	Blue	Faint bluish	—*	—	Blue	Faint bluish	Faint bluish	Faint bluish	Faint bluish
Chlor-zinc-iodide	Yellowish brown	No staining	Yellowish brown	No staining	Yellowish brown	No staining	No staining	No staining	No staining
Dienes' PPLO blue stain	Blue or lilac	Faint bluish	Blue or lilac	Faint bluish	Blue or lilac	Faint bluish	No staining	No staining	No staining
Eosin	Pink	Lilac	—	—	Pink	Lilac	No staining	No staining	—
Feulgen staining	Pink	No staining or slightly greenish	Pink	No staining or slightly greenish	Pink	No staining or slightly greenish	No staining	No staining	No staining
Gridley staining	Magenta or orange	Lilac	Magenta or orange	Lilac	Magenta or orange	Lilac	No staining	No staining	No staining
Haematoxylin-eosin	Lilac or pink	Lilac	Lilac or pink	Lilac	Lilac or pink	Lilac	Faint bluish	Faint bluish	Faint bluish
Janus green B.	In inside blue reticulum	Faint bluish	—	—	In inside blue reticulum	Faint bluish	Faint bluish	No staining	—
Lugol's solution	Ochraceous	No staining	Ochraceous	No staining	Ochraceous	No staining	No staining	No staining	No staining
Metanil yellow	Yellow	No staining	Yellow	No staining	Yellow	No staining	No staining	No staining	No staining
Methylene blue	Lilac	Faint bluish	Lilac	Faint bluish	Blue	Faint bluish	Faint bluish	No staining	No staining
Neutral red	Pink	Pink	—	—	Pink	Pink	Faint pinkish	No staining	—
Nielblau sulphate	Blue	Faint bluish	—	—	Blue	Faint bluish	Faint bluish	No staining	—
Ninhydrin	Lilac	No staining	—	—	Lilac	No staining	No staining	No staining	No staining
Periodic acid-Schiff	Magenta	Lilac	Magenta	Lilac	Magenta	Lilac	No staining	No staining	No staining
Safranine	Dirty orange	Pink	—	—	Dirty orange	Pink	Pink	—	No staining
Sudan IV	No staining	No staining	No staining	No staining	No staining	No staining	No staining	No staining	No staining
Toluidine blue	Pink	Faint bluish	Pink	Faint bluish	Pink	Faint bluish	No staining	No staining	No staining

Stain or the staining procedure applied	Environmental controls						Recent biological control materials					
	Rock outcrop from the neighborhood of Orgueil		Soil from the neighborhood of Orgueil		Dust from the American Museum of Natural History		Starches					
	Organisms	Mineral matter	Organisms	Mineral matter	Organisms	Debris	Arrow root	Cassava	Corn	Potato	Rice	Wheat
Acidic carmine	Nuclei purple	No staining	Nuclei purple	Pink	Nuclei purple	Pink	—	—	—	—	—	—
Azure II	—	—	—	—	—	—	—	—	—	—	—	—
Chlor-zinc-iodide	Blue or brown	No staining	Blue or brown	No staining	Blue or brown	Blue or brown	Brown	Brown	Brown	Brown	Brown	Brown
Dienes' PPLO blue stain	Lilac	Faint bluish	Lilac	Blue	Lilac	Blue	—	—	—	—	—	—
Eosin	Pink	No staining	Pink	Lilac	Pink	Lilac	—	—	—	—	—	—
Feulgen staining	Nuclei pink	No staining	Nuclei pink	No staining	Nuclei pink	Slightly greenish	No staining	Faint bluish or no staining	No staining	No staining	No staining	No staining
Gridley staining	Magenta or lilac	Lilac	Magenta or lilac	Lilac	Magenta or lilac	Lilac	Magenta	Magenta	Magenta	Magenta	Magenta	Magenta
Haematoxylin-eosin	Lilac or pink	No staining	Lilac or pink	Blue	Lilac or pink	Blue	—	—	—	—	—	—
Janus green B.	In inside blue dots	No staining	In inside blue dots	Faint bluish	In inside blue dots	No staining	—	—	—	—	—	—
Lugol's solution	Ochraceous	No staining	Ochraceous	No staining	Ochraceous	No staining	Blue	Blue	Blue	Blue	Blue	Blue
Metanil yellow	Yellow	No staining	Yellow	No staining	Yellow	No staining	No staining	No staining	No staining	No staining	No staining	No staining
Methylene blue	Blue	Faint bluish	Blue	Faint bluish	Blue	Faint bluish	—	—	—	—	—	—
Neutral red	—	—	—	—	—	—	—	—	—	—	—	—
Nielblau sulphate	—	—	—	—	—	—	—	—	—	—	—	—
Ninhydrin	Lilac	No staining	Lilac	No staining	Lilac	No staining	—	—	—	—	—	—
Periodic acid-Schiff	Magenta	Lilac	Magenta	Lilac	Magenta	Lilac	Magenta	Magenta	Magenta	Magenta	Magenta	Magenta
Safranine	—	—	—	—	Dirty orange	Pink	—	—	—	—	—	—
Sudan IV	In inside red dots	No staining	In inside some red dots	No staining	In inside red dots	No staining	No staining	No staining	No staining	No staining	No staining	No staining
Toluidine blue	Pink or lilac	Faint bluish	Pink	Lilac or blue	Pink or lilac	Lilac or blue	Blue	Blue	Blue	Blue	Blue	Blue

TABLE 1—*Continued*

Stain or the staining procedure applied	Recent biological control materials							Typical color of stain for major substrate(s)
	Pollen grains							
	Birch	Maple	Oak	Poplar	High ragweed	Low ragweed	Timothy	
Acidic carmine	Nuclei purple	Nuclei purple	Nuclei purple	Nuclei purple	Nuclei purple	Nuclei purple	Nuclei purple	Nuclei purple
Azure II	—	—	—	—	—	—	—	Microorganisms: faint blue
Chlor-zinc-iodide	Wall blue, inside brownish	Wall blue, inside brownish	Wall blue, inside brownish	Wall blue, inside brownish	Wall blue, inside brownish	Wall blue, inside brownish	Wall blue, inside brownish	Cellulose: blue, chitin: brown, pectines: ochraceous to yellow†
Dienes' PPLO blue stain	—	—	—	—	—	—	—	Living bacteria: blue, hyphae: dark lilac
Eosin	—	—	—	—	—	—	—	Tissue: pink
Feulgen staining	Wall pale bluish, nuclei pink	Wall pale bluish, nuclei pink	Wall pale bluish, nuclei pink	Wall pale bluish, nuclei pink	Wall pale bluish, nuclei pink	Wall pale bluish, nuclei pink	Wall pale bluish, nuclei pink	Nuclei: purple to pink.
Gridley staining	Wall bluish, inside orange	Wall bluish, inside orange	Wall bluish, inside orange	Wall bluish, inside orange	Wall bluish, inside orange	Wall bluish, inside orange	Wall bluish, inside orange	Spores and hyphae: blue to purple, tissue: yellow
Haematoxylin-eosin	—	—	—	—	—	—	—	Nuclei: blue, tissue: red‡
Janus green B.	—	—	—	—	—	—	—	Mithochondria: blue§
Lugol's solution	Ochraceous	Ochraceous	Ochraceous	Ochraceous	Ochraceous	Ochraceous	Ochraceous	Starch: blue
Metanil yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Proteins: yellow
Methylene blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Microorganisms: blue§
Neutral red	Red	Red	Red	Red	Red	Red	Red	Microorganisms: red§
Nielblau sulphate	—	—	—	—	—	—	—	Microorganisms: blue§
Ninhydrin	—	—	—	—	—	—	—	Aminoacids, proteins: blue to purple
Periodic acid-Schiff	Wall lilac, inside pink	Wall lilac, inside pink	Wall lilac, inside pink	Wall lilac, inside pink	Wall lilac, inside pink	Wall lilac, inside pink	Wall lilac, inside pink	Polysaccharids: magenta
Safranine	Wall red, inside orange	Wall red, inside orange	Wall red, inside orange	Wall red, inside orange	Wall red, inside orange	Wall red, inside orange	Wall red, inside orange	Cell walls, mucus: red
Sudan IV	No staining	No staining	No staining	No staining	No staining	No staining	No staining	Lipoids: red
Toluidine blue	Wall purple, inside blue	Wall purple, inside blue	Wall purple, inside blue	Wall purple, inside blue	Wall purple, inside blue	Wall purple, inside blue	Wall purple, inside blue	Acid polysaccharids: blue, basic: red.

* — = not tested.

† Stain probably was not washed out adequately in meteorite series.

‡ The stain in the meteorite series was not washed out satisfactorily.

§ Vital or supravital stain.

the intensity or shade of the color the organized elements from the minerals. The staining of the soil samples gave similar results. One may make the following comments regarding the stains.

The use of chlor-zinc-iodide in the taxonomy of the *Oscillatoriaceae* (Cyanophyta) is mandatory. It is one of the best cellulose reagents and the systematics of the sheathed genera of the above mentioned family is based upon the positivity or negativity of this reaction, *i.e.*, whether the sheath turns blue or does not stain at all. The 2 other color reactions ascribed to this stain are, however, somewhat less specific. It is accepted that chitinous substances turn brown, whereas pectic compounds show a yellowish brown coloration. The presence of proteinaceous moiety disturbs this reaction, as proteins will also assume a yellowish brown color. There is, however, some difference between the color given by pectins and that produced by proteins. To differentiate between these 2 colors requires either color charts or materials for comparison. In the case of the organized elements, chlor-zinc-iodide invariably gave a yellowish brown color in the walls, characteristic for pectic substances. Although it would be premature to conclude on the basis of this color reaction that the organized elements possess walls made up by pectins, one is able to rule out the possibility that they are recent pollen or spore contaminants because then they should either become blue (cellulose) or dark brown (chitin) in their walls. Types 1, 2, 3, and 4 have been seen reacting with this stain.

The blue stain of Dienes was developed for the dyeing of pleuropneumonia-like organisms (PPLO) as a substitute for the more complicated Giemsa staining (Dienes, 1939). It is an alcoholic solution of methylene blue and Azure II. Viable PPLO or bacteria will stain deep blue with the stain but will later become faint due to decolorization of the methylene blue, whereas dying or dead bacteria stain pink or do not stain at all. Fungus hyphae or spores usually stain very dark blue; cellulose elements, however, stain lilac. This stain was selected not so much to study its effect on the organized elements as to enable us to recognize terrestrial contaminants. However, in the samples under study, no viable bacteria were seen; fungus hyphae were absent and only a single gonotokont was observed. The organized elements turned either bluish or lilac by the stain but the majority did not stain at all. Types 1, 2, and 3 have been seen taking up the stain. The mineral matrix in the Orgueil or Ivuna meteorites and in the soil samples turned light bluish.

One of the most surprising results was obtained with the Feulgen stain. This staining technique was developed for the selective staining of nuclei and chromosomes. It involves the use of the Schiff reagent (leuko-basic-fuchsin) and its reaction with the aldehydes obtained by the acid hydrolysis (HCl) of deoxyribonucleic acids. The staining is considered to be extremely sensitive and very specific. Since its first description, in 1924, by Feulgen, there has been published voluminous literature dealing with the questions of sensitivity and of specificity of the technique (Pearse, 1960). Several modifications were proposed and, at present, the Feulgen staining has become one of the most reliable and one of the most widely used techniques for the demonstration of DNA in cells, and for the study of nuclear movements during cell division. There are other substances besides DNA that are, however, known to give a

positive Feulgen reaction. These are the plasmalogens (acetal phosphatides), (Schubert, personal communication, N.Y.U.). These latter compounds, however, are not very likely to occur in either the meteorites or in the soils as they are quite unstable and are known to be present only in the central nervous system and in the muscles of animals. Another, as yet unidentified substance yielding a positive Feulgen reaction is the binding material among the cells of some of the species of the green algal genus *Oedogonium* (Woes-Tschermak, personal communication, Vienna, Austria). This material according to our observations seems to possess a faint pinkish color even in the unstained, living algae, if viewed in the microscope in dimmed oblique light and it may be a compound similar to that described by Palik (1928) in *Hydrodictyon* (Chlorophyta) and named as erythropectin because of its pink color. In the case of the *Oedogonia* the acid hydrolysis seems only to strengthen the pink color already present and in reality we may not be dealing with a positive Feulgen reaction. This question, however, deserves further investigation.

By using the Feulgen technique on our samples it was found that a considerable number of the organized elements of type 1 and type 2, stained homogeneously pink with this stain, whereas the mineral material remained unstained or took a faint greenish color. It was most instructive to see the results of the staining on the different controls; organisms present in the soil or dust samples did not stain, except for their nuclei, which turned red. The mineral and possible organic material in these samples, similarly to the material in the Orgueil and Ivuna meteorites, either remained unstained or took a pale greenish color. The same color developed also in the walls of the pollen grains, whereas their nuclei stained pink (see FIGURE 7, *a* to *e*). Pollen grains as a whole never turned pink after the use of this staining method. In 1937, Shuita investigated the nucleic divisions of pollen grains and found the Feulgen staining (by virtue of its complete noninterference with any other cellular element of the grains except their chromatins), was the most suitable stain for such type of studies.

Another interesting observation was made regarding the starches. Because we could not find any literature data dealing with the effect of Feulgen staining on starch grains it seems to be worthwhile to deal with this problem at some length. Starch (amylum) occurs in the phylogeny of plants comparatively early. It is present in the green algae and it remains characteristic for the main line of plant evolution up to the Monocotyledoneae. Starch is always an intracellular product and in most of the green algae and in the leaves of higher plants it is formed in the chloroplasts. In the green algae usually a separate organell, the pyrenoid, is in the center of the grains, other grains may, however, directly be deposited in the stroma of the plastids or even in the cytoplasm (Czurda, 1928). Fritsch writes (1949, p. 67): "The grains . . . appear to grow by apposition of layers on all sides, and their polyhedral form (giving the entire group the shape of a shell) is a result of the fact that free deposition can take place on the external surface." In cases of grains directly developing in the stroma or in the few cases in which they arise in the cytoplasm their shape becomes more or less spherical and the layering takes a concentric shape. Similarly, in any other starch producing plant the grains consist of an inner,

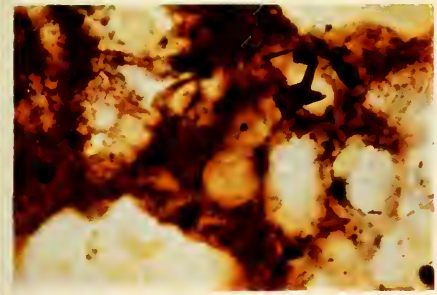


Figure 6a Petrographic thin section of the Orgueil meteorite showing two, adjoining organized elements. Magnification 590 ×.

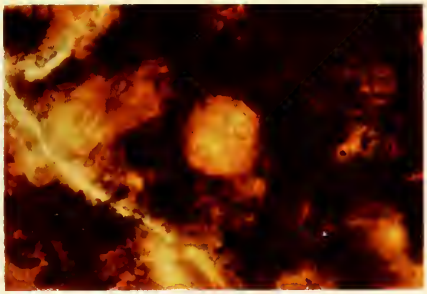


Figure 6b Petrographic thin section of the Orgueil meteorite showing a singular object, similar to those in Fig. 6a. Magnification 590×.

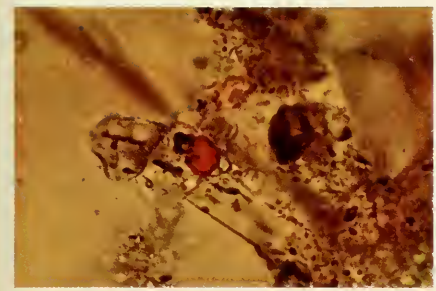


Figure 7a Type 1 organized element after Feulgen staining. Magnification 1000 ×.

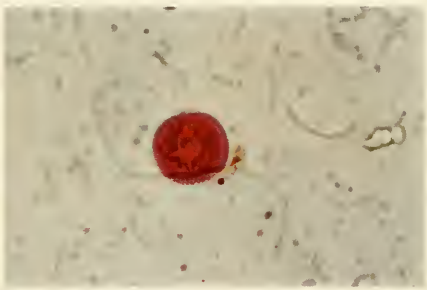


Figure 7b A type 2 organized element after Feulgen staining. Magnification 770 ×.

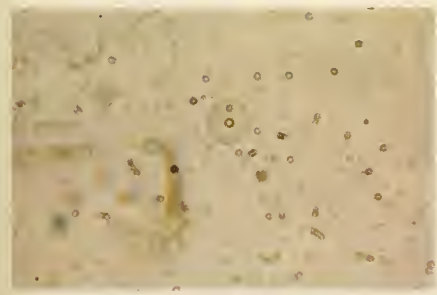


Figure 7c Cassava starch grain after Feulgen staining. Magnification 590 ×.

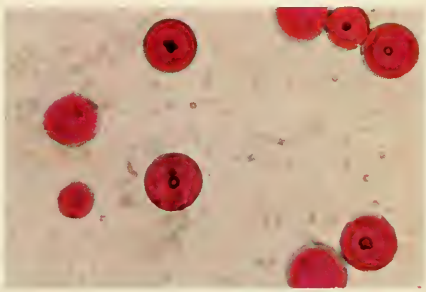


Figure 7d Cassava starch grain after periodic acid Schiff / PAS / staining. Note that Feulgen staining, when administered according to standardized techniques, does not stain starch whereas PAS does. Magnification 590 ×.

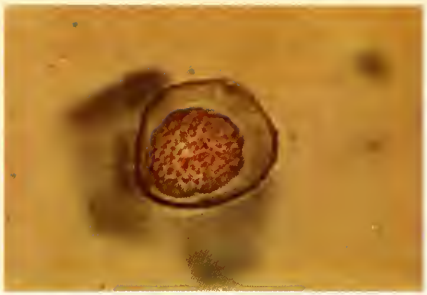


Figure 7e Elm pollen grain after Feulgen staining. Note that only the nuclei are stained. Magnification 1000 ×.

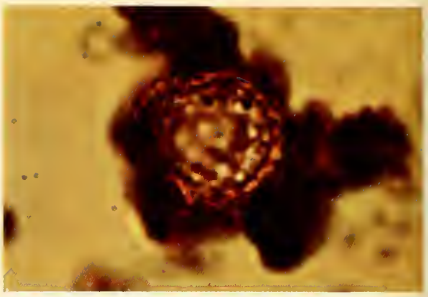


Figure 8a A type 2 organized element in optical cross section from a powdered preparation of the Orgueil meteorite. Magnification 1000 ×.

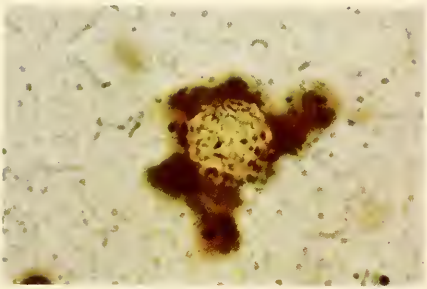


Figure 8b The same organized element photographed by focusing on its top. Note that the protrusions have dark centers indicating hollow tubes. Magnification 770 ×.

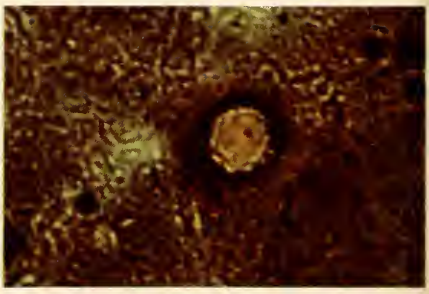


Figure 8c Cross section of an organized element similar to that shown in Figs. 8a and 8b. This object occurs in a petrographic thin section of the Ivuna meteorite. Magnification 770 ×.

Figure 8d Ambrosia pollen grain after Gridley staining in optical cross section showing solid protrusions. Magnification 590 ×.

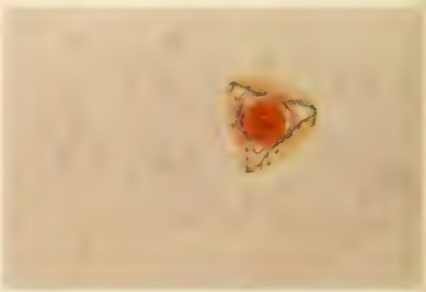


Figure 8e The same preparation showing the characteristic tricolpate, triporate structure of the Ambrosia pollen grain. Magnification 590 ×.

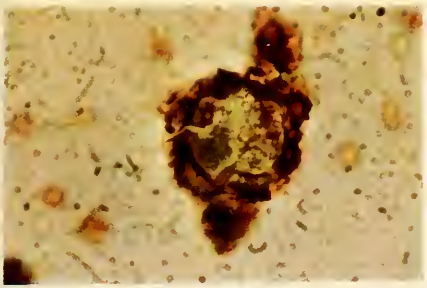


Figure 9a Organized element resembling a Dinoflagellate cyst focused to show the ridges and canals of its surface. Magnification 770×.

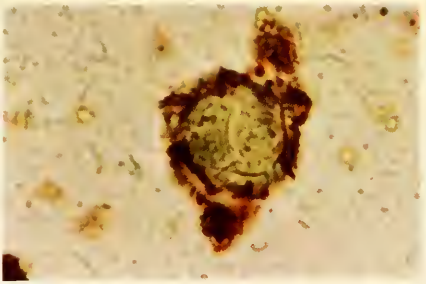


Figure 9b Same object focused on the surface appendages. Magnification 770×.

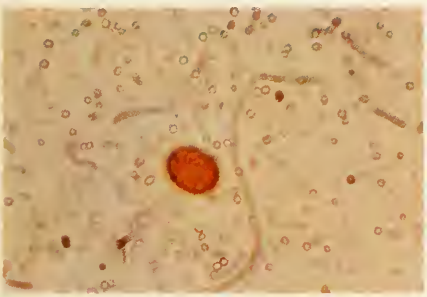


Figure 10a A type 2 organized element from the Orgueil meteorite. Magnification 590×.

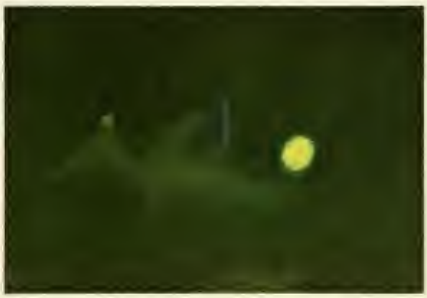


Figure 10b A similar object from the Ivuna meteorite shown in UV light. Ribs appear in the interior of the organized element. Magnification 400×.

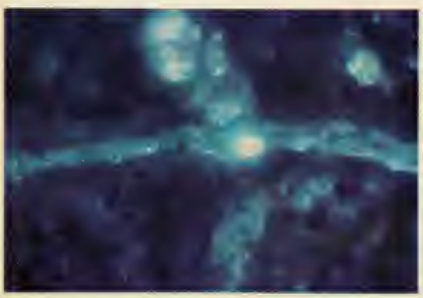


Figure 10c Petrographic thin section of the Ivuna meteorite seen in UV light, showing an organized element in a magnesium sulfate vein. Magnification 400×.

Figure 10d The same organized element in UV light after the blueish colored fluorescense of the mineral material had been eliminated by an appropriate set of filters. Magnification 400×.

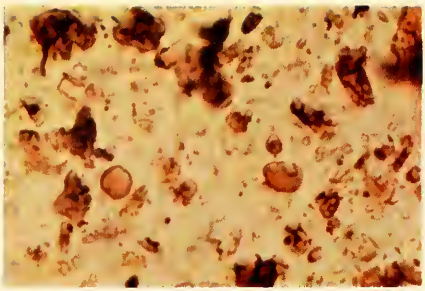


Figure 11a Two type 1 organized elements and mineral grains in a powdered sample of the Orgueil meteorite. Magnification 590 ×.

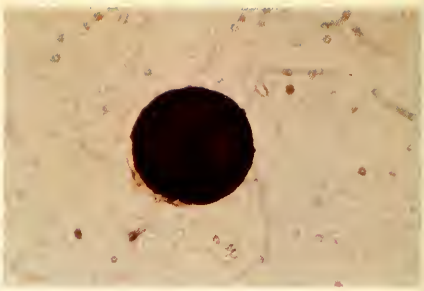


Figure 11b An opaque, magnetic particle from the Orgueil meteorite. Magnification 590 ×.

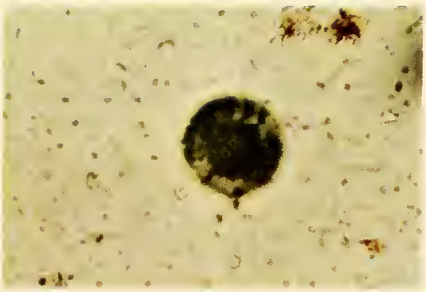


Figure 12a An organized element resembling the features of a Thecamoeba, focused on the sculptured top of the object. Magnification 770 ×.

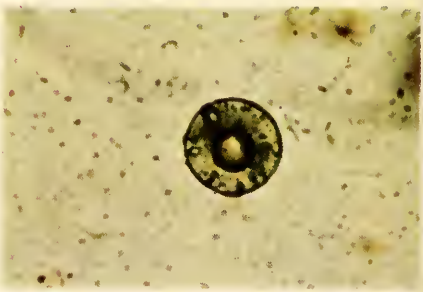


Figure 12b Same object focused on the spines of its lower surface. Note that in the hollow interior a bubble of / possibly / air occurs. Magnification 770 ×.

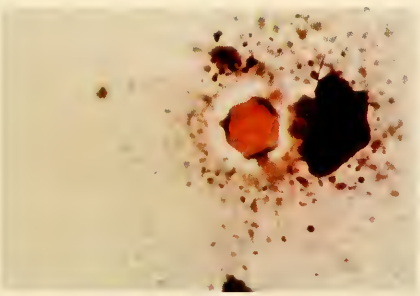


Figure 13a A type 5 organized element after Gridley staining. Focused to show the three tubular protrusions of the body. Magnification 590 ×.

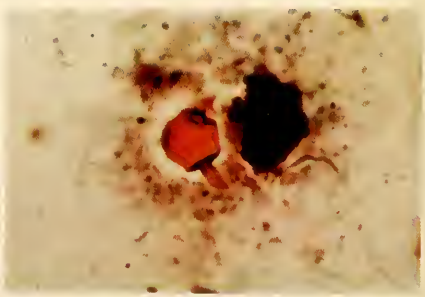


Figure 13b Same object focused on two of the protrusions only. Magnification 590 ×.

variedly shaped "nucleus" or hilum and the layered starch is around it. The mcoposition of the "hilum" is unknown. It seems that it is proteinaceous in nature. The sole exception from this is represented by Rhodophyta, in which the so-called Floridean starch develops always in the cytoplasms, is unlayered and is lacking a "nucleus." This compound, however, is closer to glycogen in its composition as it stains brown instead of blue with iodine. The shape of starch grains, their mode of layering and the form of their "nuclei" is constant and species specific, thus, it is a true genotypical feature which gives a good basis to establish relationships between different plants. The pictures of the starch grains in the endospermia of varied Gramineae, the so-called amylograms, were successfully used in the elucidation of several important microsystematic problems (Soó, 1953).

None of the 6 starches showed a positive reaction after Feulgen staining. Actually, the grains became so translucent that it took considerable time to recognize them in regular transmitted light. There were several dark blue dots on the slides, however, which in phase contrast were found to be the "nuclei" of the grains; they were invariably surrounded with a translucent envelope, showing concentric rings and corresponding in size and shape to the grains before treatment. In the case of the Cassava starch (grains of *Manihot utilissima*) the envelope occasionally turned faint blue on exposure to the Feulgen stain. It seems that the starch grains are inhomogeneous in their chemical composition and the circular striations are caused by a very thin layer of a matter different from amylum. The envelope covering the grains must be of the same material as those that cause the circles. That we were dealing with true structural elements that upon the action of Feulgen staining occasionally turned faint blue could be determined by investigating ruptured or broken grains. In these cases fringes of the envelope and of the circles could be observed on the edges of the ruptures. No other staining and not even the unstained materials gave similar results. The possibility exists that the starch was at least partially hydrolyzed by the HCl and that this procedure made conditions favorable for the detection of the envelope. The staining pattern of the starch "hila" and that of the envelope after the Feulgen stain do not allow us, as yet, to conclude anything about their nature. We may, however, conjecture, per analogiam, to the green algae that the starch "nuclei" may be proteinaceous in nature.

It is clear from these experimental results that the claim of Fitch and Anders' (1963) that one of the type 2 organized elements (stained with Feulgen staining and showing a diffuse pink coloration) is a starch grain is unfounded. These authors demonstrate that the Schiff reagent is a nonspecific carbohydrate stain, which is even capable of reacting with several inorganic materials, including clay minerals. They show pictures of deep magenta colored starch grains, dyed with the Schiff reagent and conclude that as Feulgen staining uses the Schiff reagent, it is nonspecific, hence the organized element in question is most probably a starch grain. This conclusion is in error as shown by FIGURES 7*a*–7*d*. FIGURE 7*a* shows a type 1; FIGURE 7*b* a type 2 organized element after Feulgen staining. In FIGURE 7*c* is shown the Cassava starch with Feulgen and in FIGURE 7*d* with PAS staining.

Similarly, the assumption of Gregory (1962) that the organized element in question may either be a "Hülle" cell of *Aspergillus nidulans* or the pollen of a *Juniperus* sp. or *Taxus baccata* can be rejected on the basis of the staining alone, even without taking into account the basic morphological differences. It has already been mentioned that with Feulgen staining only the nuclei of the pollen grains stain and not the body as a whole.

Interpreting the results of the diffuse Feulgen staining of the type 1 and type 2 organized elements presents a different question. Bacteria and blue-green algae are known to contain chromidial apparatus instead of a compact nucleus. It is customary to refer to the central body or centroplasm of the Cyanophyta as nuclear equivalents, containing diffused nucleic material. Indeed, Feulgen staining in such organisms will result in a diffuse pink coloration of the protoplasm. One may thus think that some of the organized elements indeed contain diffused nucleic material in their interior. The present evidence, however, is not sufficient to substantiate such a supposition. We can only state that, according to one of the most specific biological staining techniques developed, there is an indication that nucleic acids are present in the interiors of some of the type 1 and type 2 organized elements. It is, however, equally possible that an as yet unknown material is present in these organized elements, which will react with the Feulgen reagent to produce the pink color.

The Gridley staining was primarily developed (1953) as "A Stain for Fungi in Tissue Sections." It is the combination of Gömöri's aldehyde-fuchsin stain and the Hotchkiss-McManus technique. As Gridley states (p. 303): "The problem of positive tissue elements with the periodic acid-Schiff reaction was eliminated by hydrolyzing in chromic acid instead of periodic acid." For counter staining Metanil yellow is used. Fungal conidia and hyphae are stained deep blue to purple by this method while other tissue elements, mostly the proteins, stain yellow with the counter stain. The results of this technique on our samples were interesting for 3 reasons. (1) Some of the organized elements of type 1, type 2, and type 3, stained lilac with the stain, whereas the mineral matrix of the Orgueil and Ivuna meteorite in many instances took a dirty purple color. A similar staining pattern of the minerals was observed in the soil samples, in which, however, some algae stained rose and fungi became blue. It was, therefore, surprising to see that the minerals of the Murray meteorite did not stain with this stain. The starch grains took a vivid magenta color. (2) Type 5 organized elements stained orange with the Gridley staining and the surrounding halo turned yellow from the counter stain. The meaning of this reaction is obscure. (3) The forms described by Staplin (1962) as *Coelestites sexangulatus* from the Orgueil meteorite stained yellow, probably from the Metanil yellow. These forms have originally a yellowish-orange shade, however, after the exposure to the Gridley staining the change in their color was striking. On the slides prepared from the meteorites not a single spore or fungus hypha could be detected. In the preparation from Holbrook 1 specimen of *Nitzschia acicularis* (a Diatom) was seen.

A surprising result was obtained by the application of a watery solution of Janus green B. Many of the type 1 and a few of type 2 organized elements developed a blue-stained reticulate structure in their inside. This stain is a

vital or supravital stain generally used for the demonstration of mitochondria. It is considered to be more or less specific for ribonucleic acids. The reticulum that developed in the organized elements was similar to that obtained after their treatment with 6 *N* HCl. The meaning of this staining pattern is obscure.

Metanil yellow alone, in a watery solution was applied to rule out the possibility that the observed yellow coloration of Staplin's form after Gridley staining was due either to the hydrolysis with chromic acid or to the Schiff reagent. As was the case in the Gridley stained preparations, after treatment with the simple watery preparations, Staplin's *Coelestites* turned into a striking yellow. A few of type 1 organized elements also became yellow. With our present knowledge, we cannot satisfactorily interpret these results.

Neutral red, usually used in biology for its nontoxic character as a vital or supravital stain, stained some of type 1, 2, and 3 organized elements a homogeneous red.

Ninhydrin, this most sensitive amino-acid or protein reagent, stained lilac several type 1 and 2 organized elements; however, it gave a purple coating to the mineral debris in the carbonaceous meteorite and soil samples. The ordinary stony meteorites gave negative results.

Periodic acid-Schiff (PAS) reagent seemed to be the least adequate for differential staining. Being a general carbohydrate stain, it dyed magenta color the pollen and starch grains and algal cells. Several of the organized elements of types 1, 2, and 3 also took this stain and developed a color similar to those of the algal cells or starch or pollen grains. The minerals of the Orgueil and Ivuna meteorites and those of the soil samples also took up the stain. There was a significant difference between the shades of the organized elements and the starch or pollen grains on one hand and that of the minerals on the other; the latter having a more "dirty" magenta color. Furthermore, some type 1 organized elements remained totally unstained among well stained mineral aggregates. It was interesting to see that the minerals of the Murray meteorite did not, or only very occasionally, stain with PAS and that the staining of both the Holbrook and Bruderheim meteorites was negative. The quality and intensity of the color of the stained organized elements resembled closely the color of the controls. One may speculate that some kind of chemical similarity may exist between the organized elements and the controls that contain carbohydrates. Because of the nonspecificity of this reaction, however, it seems advisable not to attempt to reach any premature conclusion in this matter.

It was previously mentioned that Sudan IV was the only stain which left unaffected the organized elements, although it stained vivid red the oil droplets of the terricole Diatoms.

Toluidine blue gave a blue or pink color with some types 1, 2, and 3 organized elements. In several cases the minerals of the carbonaceous meteorites and of the soil samples also turned blue. Metachromasia, however, was not observed with the mineral particles. Samples of Orgueil, after being treated with boiling HF for 10 minutes, left acid resistant pellicles of the types 1, and 2 organized elements, which after staining with toluidine blue showed signs of

metachromasia. It would, however, be somewhat premature to conclude from this staining pattern that there are acidic or basic polysaccharides in the acid resistant pellicles of the organized elements.

One may draw the following conclusions from the staining experiments.

(1) The specificity of some of these stains is not known. However, it seems unlikely, that 18 of the 19 stains used, gave positive results by chance. To evaluate the meaning of a single staining reaction often seems to be impossible. One cannot argue that a sample is of biogenic origin on the basis of a single staining. However, if a whole array of different stains are applied, which are widely differing in their chemical composition and in their specificity, one can point out biogenic material.

(2) The use of a great variety of stains (some of them specifically developed for the scanning of certain microorganisms, like Dienes blue-stain for PPLO or the Gridley staining for fungi) facilitated the recognition and, thus, the elimination of earthly contaminants in the meteorite samples. As only small meteorite fragments or powdered material could be used for these studies, the question of contamination could be settled only on the basis of elimination. But by the use of the numerous stains and the relatively great number of controls (including soil from the impact area, and dust from the museum) one could recognize and exclude the common contaminants.

(3) An examination of the soil and dust samples has shown that microorganisms stained differently from the mineral constituents, *i.e.*, the latter did not stain at all or took a different color. These and the starch and pollen controls have confirmed the specificity of the Feulgen reaction.

(4) It has been pointed out that not all of the organized elements stained. A gradation in the staining was observed with almost every stain (with the possible exception of safranine). One reasonable explanation for this phenomenon may be that different degrees of mineralization are present in the organized elements. In terrestrial bitumens microfossils are often differentially mineralized (Andreanszky, 1954).

Physical and Chemical Observations on the Organized Elements

Fluorescence in ultraviolet light. When preparations of the 4 carbonaceous meteorites were examined with a fluorescent microscope a number of particles became readily visible. Most of these particles fluoresced with a greenish-yellow light when excited by ultraviolet radiation and when Corning 7-59 + Wratten 2B filters were used. A Zeiss fluorescent microscope was applied in these studies. Less frequently, particles fluoresced with a green, pink or red color upon excitation by ultraviolet light. An examination of the thin sections and of the crushed samples has shown that some of the mineral constituents (possibly those that were coated with bituminous matter) seem to have fluoresced with a bluish-white color. The fluorescence of the organized elements, in our opinion, can be readily distinguished from the fluorescence of the mineral matter. It is, of course, possible to select the right combination of filters to exclude the bluish-white fluorescence from the yellowish-green or pink fluorescent light. Several of the type 1 organized elements were found to fluoresce with greenish-yellow light, contrary to the argument of Fitch and Anders (1963). Fitch and Anders also claimed that pink colored fluorescence

of organized elements is not a true fluorescence. They suggested that birefringent particles may appear red when viewed with ultraviolet light in the fluorescent microscope. The reason for this could be that the usual filters transmit a portion of the red part of the spectrum. However, the particles which were described previously were not birefringent when examined in a polarizing microscope and they were photographed with an additional set of filters (Corning 7-59 + Wratten 2B + Zeiss 064 + 061) that permitted mainly blue light to enter the microscope. Consequently, the argument of Fitch and Anders is not valid in this case.

The microscopical assembly used for the fluorescence studies enabled one to view objects in UV darkfield illumination, as well as in regular transmitted light. In this way it was possible to select organized elements of distinct morphologies for the fluorescence studies. It is true that a few irregularly shaped particles also fluoresced in greenish-yellow light. These particles could be easily fragments of organized elements, broken during the crushing of the meteorite samples. On the other hand, the majority of the irregularly shaped particles fluoresced with a different color than the organized elements; they emitted bluish-white light and they were probably mineral particles (or particles coated with bituminous matter).

Fluorescent microscopy is also useful to demonstrate certain morphological features which are not readily visible in transmitted light. Mitochondriatic granules become visible in the UV microscope when they emit fluorescent light with or without fluorochromation (Drawert and Metzner, 1956). Similarly, fluorescent microscopy was found to be useful to visualize certain morphological features that were not visible when the organized elements were examined in regular, transmitted light or with phase contrast microscopy. FIGURE 10*b* shows an organized element that fluoresced with greenish-yellow light. Centripetal ribs were found to be present around the walls. This morphological feature is rather unusual, because only a few Diatom species are known to show centripetal ribs (Hustedt, 1930). This feature is not identical with the internal septae of *Coccolithophorideae*, *Silicoflagellatae*, *Foraminiferae*, and certain Diatoms such as *Naviculaceae* etc., the septae of which extend much farther into the cell. The general habit, as seen in transmitted light, FIGURE 10*a*, resembles a *Trachelomonas*, a genus of aquatic protophyta, except of the location of the pore. However, a *Trachelomonas* does not have centripetal ribs. The presence of the centripetal ribs are of particular interest regarding the argument of Fitch and Anders about pollen grains. The exo-exine of pollen grains show centrifugal thickenings but they never show centripetal ribs. Eames and MacDaniels (1947) state on p. 49 in their "Introduction to Plant Anatomy" that: "The external wall layers and surface projections of spores and pollen grains are formed in part by tapetal fluid or mother cell cytoplasm." (Therefore, only centrifugal thickenings can occur on walls of pollen grains.) It seems unlikely, that the particles shown in FIGURE 10, *a* and *b*, are pollen grains or spores. It is known that spores of fungi do not fluoresce. Höfler and Pecksieder write (1942, p. 117): "Angesichts der weiten Verbreitung primär Fluoreszenz im Gewebe der Pilzkörper berührte uns die Beobachtung um so auffälliger, dass die *Sporen der Hutpilze im UV-Licht nicht fluoreszierten, vielmehr meist völlig unsichtbar waren*."

The abundance of the organized elements. The number of the organized elements (per milligram) in the Orgueil, Alais, Ivuna, and Tonk meteorites has been reported previously (Nagy *et al.*, 1962). The numbers were arrived at by counting all types of organized elements or such fragments thereof, which appeared to be larger than 50 per cent of 1 particle. The type 1 organized elements of Claus and Nagy (1961) are the most abundant; they comprise approximately 80 to 90 per cent of all microstructures. This type has also the simplest morphology. Consequently, certain investigators were unable to distinguish this type of organized elements from mineral particles or were of the opinion that the morphological criteria are not sufficient to distinguish them from mineral particles. If one chooses to exclude the type 1 organized element one will arrive at a count that is substantially lower than that given by Claus and Nagy (1961). It should be noted, however, that some biologists, on critical examination, were inclined to include this type 1 particle among the organized elements (Papp, 1963; Cholnoky, 1963; Skuja, 1962; Palik, 1963). Also, as Urey (1962*a*) pointed out, one only needs to have *some* biogenic and indigenous microstructures in a meteorite to ascertain the existence of extraterrestrial life. The total count of organized elements, including type 1 (1300 to 1700 per mg.) shows good agreement with counts of microplanktons in fossil marine populations (1200 per mg.), as reported by Kolbe (1952) and with the counts of stainable organic microstructures in the Mokoia meteorite (1000 to 1700 per mg.) described by Briggs and Kitto (1962).

Solubility in acids. The effect of acids and organic solvents on the organized elements has been reported previously (Nagy *et al.*, 1962). It is necessary, however, to comment again on this subject because Fitch and Anders (1963) claimed to have dissolved 97 per cent of an Orgueil meteorite sample by heating it for 17 hours at 60° C. in HF and for 18 hours in 6 *N* HCl at 25° C. The remaining residue was reported to be an aggregation of "finely granular, black to brown material virtually devoid of any structure." Milder treatments in concentrated HF (Urey, 1962*a*; Staplin, 1962; and Nagy *et al.*, 1962) and 6 *N* HCl resulted in a residue which contained several transparent and acid resistant pellicles. Organized elements, including type 1, retained their characteristic morphologies upon exposure to 6 *N* HCl at room temperature for varying periods of time. The type 1 organized elements were not destroyed when boiled in concentrated HF for 15 minutes. The statement made by Fitch and Anders ". . . since they disappear after treatment with HF, we believe they are most likely grains of silicate minerals although they are classified as organized elements by Nagy and coworkers" seems to be in error. In spite of the rather severe treatment they used, Fitch and Anders were still able to find some transparent and highly organized structures of undoubtedly biogenic nature.

Problems of contamination. Several claims were made in the literature to the effect that the organized elements are terrestrial contaminants (Fitch and Anders, 1963; Deflandre, 1962; Gregory, 1962; and Pearson, 1962). Contamination is, of course, a serious problem and it cannot be fully excluded at the present time. However, it should be borne in mind that no trained microbiologist or micropaleontologist who has actually *worked* with an Orgueil sam-

ple, for any length of time, has yet (at the time of this writing) positively identified the organized elements as known terrestrial species. Certain comments, that to us seem to be somewhat vague, such as Deflandre's (1962) statement: "Positive identifications in this case are unnecessary and superfluous" cannot be taken too seriously. Deflandre, to our knowledge, has never examined a carbonaceous meteorite. Similarly, the criticisms of Gregory and Pearson cannot be accepted as strong evidence against the extraterrestrial nature of the organized elements because these authors made their identifications from a set of photographs and sketches that were reproduced rather poorly in a scientific journal. (Gregory saw some of the microscopical preparations after he submitted his paper to the press.) Gregory and Pearson identified the same organized element as 2 different terrestrial contaminants. On one occasion a single organized element, when it was briefly shown in a microscope to 18 microbiologists, was "identified" as 18 different species of protobionta or organic artifacts.

Fitch and Anders (1963) claim that only a few of their particles, which according to them are mere terrestrial contaminants, survive the combined HF, HCl treatment. We found that these particles, when we examined their preparations under the microscope, showed morphological features that were dissimilar to common airborne contaminants (Wodehouse, 1942, 1945; Gregory, 1961). They appeared to us identical to some of the forms that we found in our preparations. As a matter of fact, we found one of these forms ourselves in Fitch and Anders' preparations, in their presence, during their visit to our laboratories.

Fitch and Anders claim that some of these particles are ragweed pollen. However, according to their own measurements, given in their report, some of those particles seem to be too small to be Ambrosia pollen. It is clear that one must both critically evaluate the fine morphology and make accurate measurements of size to establish a particle as a known terrestrial contaminant. Furthermore, Fitch's and Anders' contention that some of the organized elements in our preparation are ragweed pollen is also untenable because it is based upon the comparison of photographs of rather low resolution which do not permit the evaluation of fine morphological criteria. In FIGURE 8*c* are shown some ragweed pollen grains, to demonstrate the solid spines of the exo-exine. FIGURE 8*d* is an optical cross-section of the same. In FIGURE 8*b* is shown the type 2 organized element (note the hollow protrusions). In FIGURE 8*c* is shown a similar object embedded in minerals. The identification of another organized element (FIGURE 7*b*) as either a starch grain or a recent *Juniperus* pollen is also in error because the structure of starch grains shows concentrical layering. Juniper pollens are much larger than the object in question, they do not have papillae and have rugate exo-exines (Erdtman, 1957).

Additional sources of possible terrestrial contaminations have been examined recently. Soil samples and outcrop samples have been collected in the vicinity of the villages of Orgueil and Nohic in Southern France near the location where the meteorite fell. It has been suggested (Bourrelly, 1962) that soil and rock samples from the impact area be examined to evaluate the degree of contamination from the local environment. Bourrelly noted that the culti-

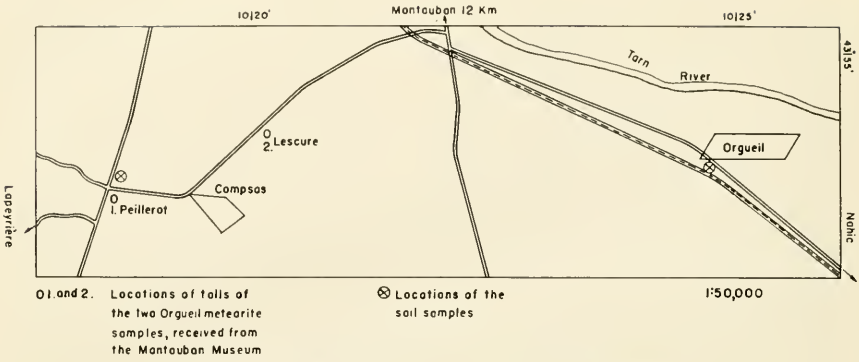


FIGURE 14. Map of the Orgueil and Campsas area.

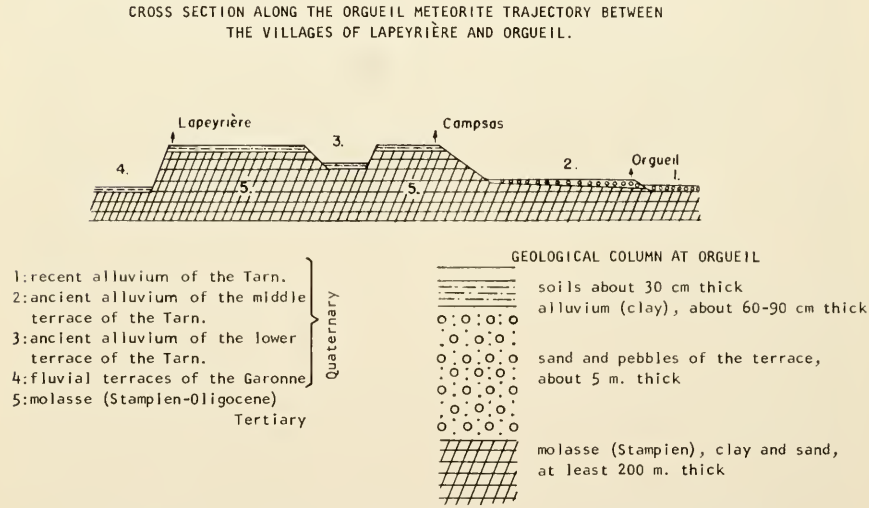


FIGURE 15. Geological cross-section in the vicinity of Orgueil, in France.

vation of land in this part of France is basically the same today as it was at the time of the fall of the Orgueil meteorite. One may expect, therefore, that a microbial population similar to that of 1864 is present in the soil. In FIGURE 14 are shown the locations of the falls (on May 14, 1864) of the stones and the locations where soil samples were collected on March 29, 1962. In FIGURE 15 a geological cross-section of the area is shown. The sedimentary strata that underlies the alluvium in the Orgueil-Nohic area consists of Tertiary formations extending to a depth of at least 600 feet. Staplin (1962) suggested that a few Cretaceous microfossil contaminants might have been included in the Orgueil meteorite from the soil in the impact area. Our studies, based upon the recent field work of Henri Coustau, revealed no Cretaceous outcrops near Orgueil and

Nohic. Staplin (1962) was undecided about the Cretaceous identity of the forms in question. It seems that these forms are not Cretaceous contaminants, after all.

Microbiological and micropaleontological examination of the soil and rock outcrop samples revealed no forms that were morphologically identical to the organized element in the Orgueil meteorite. The species of microorganisms that had been identified from the Orgueil soil samples are listed in TABLE 2. These samples still contained a considerable amount of their original water content when they arrived at our laboratories, thus several forms could be studied while still alive. The soil and rock samples were treated identically to the meteorite samples, including the biological staining techniques. We concluded that the organized elements in the Orgueil meteorite are not identical with the organisms and microfossils that were collected on March 29, 1962, by Henri Coustau, in the soils and rocks of the impact area.

Another source of contamination may be the microorganisms in the air. When a meteorite enters the earth's atmosphere it "breathes in" air because of the reduced pressure in its interior. It is conceivable that some organisms may be sucked in at such time (although the average pore size of the Orgueil meteorite is estimated to be less than 1 μ). In order to gather some information about this possibility, particles collected in the atmosphere have been examined. The airborne particle samples were received through the courtesy of C. W. Phillips, U.S. Army Chemical Corps, Fort Detrick, Md. They were collected on precleaned microscope slides at the elevation of the collection. It is known (Proctor and Parker, 1942) that at the height of between 10 and 30,000 feet mainly bacteria exist. An examination of the slides revealed no organisms that were morphologically identical to the organized elements in the carbonaceous meteorites. There are a number of reports in the literature (Hyde and Adams, 1958) on airborne pollen grains and spores; and a few reports on algae in the air (Schlichting, 1961). It seems that the organized elements of the meteorites do not correspond to known airborne contaminants.

Other possible sources of contaminations, such as chemicals used, including the water, have been evaluated previously (Nagy *et al.*, 1962).

Finally, 2 samples of the Orgueil meteorite were recently obtained from the Montauban Museum (through the courtesy of A. Cavaille). These samples have been in Montauban, France, which is near Orgueil, continuously since approximately 2 weeks after the fall of the meteorite. The samples were kept under glass jars; however, they were not stored in a sterile environment.

A microscopical examination of the Montauban samples revealed identical organized elements (except type 5) to those from other museums. It is very difficult to believe that 6 samples of the Orgueil meteorite (from the American Museum of Natural History in New York, the U.S. National Museum in Washington, D.C., from the British Museum, from the Museé d'Histoire Naturelle, Paris and the 2 from Montauban) would have been contaminated by identical microorganisms in storage. The organized element that has been claimed to be a ragweed pollen by Fitch and Anders, was also found to be present in the Montauban sample. Ragweed (*Ambrosia*) is a native American plant. It was introduced to Europe only in the early part of the twentieth century and it is still not a common plant there (Soó, 1953).

TABLE 2

BIOLOGICAL MATERIAL FOUND IN SOIL AND SEDIMENTARY ROCK SAMPLES NEAR THE VILLAGE OF ORGUEIL

Name of species	Soil sample A, surface	Soil sample B, from 40 cm. depth	Rock sample from quarry
Achnanthes cf. *microcephala*	+	+	
Actinomycetes 2 different spp.	+	+	
Adelomycetes spore 5 different spp.	+	+	
Amoeba 3 different spp.	+		
Amphora ovalis		+	
Ankistrodesmus falcatus	+		
Arcella sp.	+		
Aspergillus sp.	+		
Botryococcus sp.	+	+	
Chlorella vulgaris	+		
Chlorococcum humicolum	+	+	+
Chroococcus turgidus	+		
Chrysophyta cysta, 2 types		+	+
Ciliata 4 different spp.	+	+	
Closterium ehrenbergii	+		
Cocconeis spp. 2 types		+	+
Cosmarium sp.		+	
Cosmarium granulatum	+		
Cymbella ventricosa	+		
Cymbella prostrata		+	
Difflugia cf. pyriformis	+		
Dinobryon sertularia			+
Euglena deses		+	
Euglena sp. cysta		+	
Fragilaria pinnata	+	+	
Gomphonema olivaceum	+		
Kephyrion sp.			+
Melosira varians		+	
Navicula rhinchocephala	+		
Navicula hungarica var. *capitata*	+		
Navicula pupula		+	
Nematoda 2 different spp.	+	+	
Nitzschia acicularis	+	+	
Nitzschia frustulum			+
Nitzschia sigmoidea			+
Nostoc fusiforme	+		
Oocystis pusilla		+	
Oscillatoria animalis	+	+	
Oscillatoria terebriformis	+		
Oscillatoria tenuis	+		
Pediastrum boryanum var. *granulatum*	+		
Peridinium sp. (fragment)			+
Phormidium autumnale	+	+	
Phormidium foveolarum	+		
Pinnularia viridis	+		
Pleurococcus naegelii	+		
Pollen grains of			
Abies sp.		+	
Achillea cf. *millefolium*	+		
Agrostis cf. *vulgaris* s. *alba*	+	+	
Alnus sp.			+
Betula sp.	+		+
Cirsium sp.	+		
Hordeum cf. *vulgare*	+	+	
Knautia arvensis	+		
Pinus sp.	+		+
Polygonum cf. *convolvulus*		+	
Prunus s. *Crategus* sp.	+		
Quercus cf. *pubescens*			+
Sagittaria sagittifolia	+		
Salix cf. *cinerea* s. *caprea*	+		
Senecio cf. *vulgaris* s. *viscosus*	+	+	
Trifolium cf. *arvense*	+	+	
Triticum aestivum	+	+	
Tussilago farfara	+		

TABLE 2—*Continued*

Name of species	Soil sample A, surface	Soil sample B, from 40 cm. depth	Rock sample from quarry
Scenedesmus acuminatus	+		
Scenedesmus biiugatus	+		
Scenedesmus obliquus		+	
Scenedesmus quadricauda var. *longispina*	+	+	
Steril mycelia 3 types	+	+	+
Stephanodiscus hantzschii		+	
Stichococcus minor	+		
Strombomonas sp.			+
Surirella ovata		+	
Synechococcus elongatus	+		
Synedra rumpens	+	+	
Synedra ulna var. *oxyrhynchus*		+	
Tetraëdron muticum	+		
Ulothrix sp.	+		
Unidentifiable arthrospores (*Nostocales?*) 3 types	+	+	
Unidentifiable conidiospores 4 types	+	+	+
Unidentifiable green algal zygotes or zygospores (*Oedogoniaceae? Conjugales??*) 5 types	+	+	+
Unidentifiable moss-protonema	+	+	
Vampyrella sp.	+		
Vaucheria cf. *sessilis*	+		
Xanthidium sp.	+		

On occasion fungi are known to grow on mineral specimens in museums. In the growth process the hyphae get attached to or penetrate into the samples. Microscopical examination of the thin section of such samples reveals the spores and hyphae. No hyphae or remnants of hyphae were yet seen in carbonaceous meteorites. This renders unlikely the possibility that the organized elements are spore contaminants from fungi that grew on the samples in the museums. Such a fungal growth would be rather unusual and could occur only in the presence of adequate moisture. Some of the mineral components of the Orgueil meteorite point out that the samples were kept in dry museum storage.

Terrestrial contaminations should have been able to enter the pores if the interiors of the meteorite are contaminated. Organized elements are embedded in minerals and in the mineral aggregates in meteorites, as was reported previously. A petrographical study of the thin sections (Nagy and Claus, 1963) led to the estimation of the average pore sizes as less than 1 μ in diameter. The size of the organized elements varies between 3 to 60 μ. Although there may be a few wider fractures going through the samples it is thought to be impossible for organisms to penetrate the dense and unfractured areas of the mineral matrix.

Microscopical preparations of the carbonaceous meteorites have now been prepared from time to time, over a period of 1 year (before, during, and after the pollination time of ragweed and other flowering plants). No correlation has yet been found between the types and numbers of the organized elements and the time of preparation of the slides. This suggests that the organized elements were not introduced into the sample when the microscopical preparations were made. Identical organized elements, such as the particle that is

claimed to be ragweed pollen by Fitch and Anders, have also been found by different investigators at different laboratories at different times.

In our opinion the probability of terrestrial contamination is a most serious problem. However, the control experiments described in this report and previously (Claus and Nagy, 1961; Nagy *et al.*, 1962) strongly indicate that the organized elements (or most of them) are not terrestrial contaminants. Yet it must always be borne in mind that even unusual contaminants may become included easily in a sample. Microscopical preparations of an Orgueil meteorite sample provided through the courtesy of Fitch and Anders for us to study, contained fragments of a *Compsopogon* filament (a not too common species of Rhodophyta), individuals of *Chlorella*, a rare species of *Nägeliella*, antennae of *Cladocerae*, sqamae of *Tilia* leaves, and emergentia of unknown origin. Although the organized elements were clearly visible, the presence of the aquatic contaminants suggested more of a sample of a Recent sediment than that of a carbonaceous meteorite.

The Diverse Morphology of Organized Elements

An examination of approximately 400 microscopical preparations of carbonaceous meteorites, and related material, has as of now revealed 30 distinct morphological types of organized elements. Other investigators (Staplin, 1962; Palik, 1962, 1963; Ross, 1963) found several other types. None of these organized elements seems to be identical to known terrestrial species, although they resemble them.

Organisms can be classified into four symmetry groups. The simplest symmetry group is the sphere, and the most advanced one is the bilateral type. (Asymmetrical categories can be derived from each of the four groups.) Trigonal symmetry is the least common among terrestrial organisms. Organized elements, however, often fall into this class. Organized elements contain examples of each of the symmetry categories (see FIGURES 6, *a* and *b*; 7, *a* and *b*; 8, *a*, *b*, and *c*; 9, *a* and *b*; 10, *a*, *b*, *c*, and *d*; 11*a*; 12, *a* and *b*; and 13, *a* and *b*).

Conclusions

Consideration of the fine morphology, physical and chemical tests, staining with biological stains, and further evaluation of contaminations suggest that the Orgueil, Ivuna, Tonk, and Alais carbonaceous meteorites contain indigenous, organic microstructures which seem to be of biogenic origin. Full proof of the indigenous and biological nature of the organized elements is still not available but the indications seem to be strong.

It has been shown that fine morphological criteria are of diagnostic value. As a matter of fact, microbiologists and morphologists are using such criteria every day in a variety of problems. It has been shown, as it is known to many investigators, that morphological features of 0.3 μ size can be observed and identified by optical microscopy. The value of morphological criteria was noted by Fournier (1962) when he stated at the First International Conference on Palynology, that a worker in biology . . . "classifies his pollen based on morphological features alone, a fact that has proven no detriment to his work."

The criticisms of Fitch and Anders have been considered and found to be unacceptable. A critical, systematic and objective evaluation of the organized

elements is essential if an accurate identification* of these particles is to be achieved.

Acknowledgments

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* Because of the obviously great significance of the organized elements, one must, of course, be unusually careful in evaluating their true identity. Our objections to the publishing of photographs of our yet unreported organized elements, taken of our preparations at our invitation but published without our permission (Anders and Fitch, *Science*, 1962, 138, 1392) is based also on the fact that publishing photographs of organized elements adjacent to Recent microbiological objects does not seem to facilitate identification because both types of these particles are three dimensional objects. Furthermore, in a scientific report, critical or otherwise, one is obliged to quote not only criticisms but also confirmations. It is important that the organized elements be treated seriously and be evaluated by investigators with sufficient experience in observing morphological features because, after all, the organized elements could be the first tangible evidence for the existence of extraterrestrial life.

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PANEL DISCUSSION

The Identity of the "Organized Elements"

H. C. Urey (Moderator; *University of California, LaJolla, Calif.*): In reviewing the events that led to this symposium, the moderator recalled that approximately one year ago at The New York Academy of Sciences a presentation was made by Nagy *et al.* regarding the finding of what might be biogenic hydrocarbons in the Orgueil meteorite. The moderator stated that although he viewed their finding with skepticism at that time, he later looked over the mass spectrometric data collected by the investigators and was sufficiently impressed to suggest that additional analyses be run, such as infrared and ultraviolet spectra. Once these suggestions had been carried out, the moderator viewed microscopic preparations obtained from the Orgueil and Ivuna meteorites that reminded him of biological matter. The moderator said he then posed the following question to himself: Suppose these were living things, how did they become imbedded in dolomite? In a subsequent published article, the moderator suggested that these "organized elements" might be earthly forms that had somehow been transferred from the earth to the moon in early geological times and later had returned to earth in carbonaceous meteorites. Reactions among scientists to this theory varied; doubts were expressed, and the moderator himself was (and still is) unsure of it. The moderator believes, however, that the study of carbonaeceous meteroties for life-like forms is not an unreasonable pursuit, particularly when one considers that the United States plans to spend some 25 billion dollars to put a man on the moon.

The moderator acknowledged, on the other hand, that he was also impressed by the arguments of Fitch *et al.* that the "organized elements" might be merely terrestrial contaminations, such as ragweed pollen. He noted, however, that investagators Nagy, Claus, Meinschein, and Hennessy have been willing to show their sample preparations freely and to solicit the opinions of others. He noted, also, that they are enthusiastic; and while it is true that enthusiasm may lead to errors, it is also true that lack of enthusiasm is not an especially strong motivation for further work.

J. D. Bernal (*Department of Physics, Birkbeck College, University of London, London, England*): This discussant suggested that the problem be defined in terms of the question, "What is it we are looking at?"; also that the problem be approached in terms of all related subjects and that carbonaceous meteorites be considered only as related to other meteorites. He raised the question of whether carbonaceous meteorites represent the beginning or the end of the development of meteorite bodies; the origin of these objects, he pointed out, is an extremely important question. In his opinion, the "organized elements" could be contaminations, "jokes of nature," or remnants of organisms. One approach to the contamination problem is to determine whether anything could have gotten into the samples since the meteorite fell on earth; another is to ascertain whether the "organized elements" resemble any known biological forms. If they are not contaminations, the burden of proof lies with Nagy *et al.*, and it is the biologists who must evaluate such proof. As for being "jokes of nature," the "organized elements" might, for example, be mineral concretions

that give the appearance of microfossils. Some of the Precambrian microfossils shown earlier in the sessions might also be such particles. Bernal stated that if one could prove that the Precambrian forms were indeed fossils, it would perhaps aid in the identification of the "organized elements." Finally, if the "organized elements" are neither contaminations nor "jokes of nature," one might then wonder whether anyone had "faked" them. Again the burden of proof would lie with those who say that the "organized elements" are truly indigenous fossils in the meteorites. If they are indigenous microfossils, what would this mean? One must consider where these objects might have originated, whether life was brought to earth on meteorites, and where life might first have arisen, on earth or on another body.

Among other considerations mentioned by Bernal was that the carbonaceous meteorites might contain between six and eight per cent of organic matter, most of which is definitely not terrestrial contamination. According to Meinschein and Nagy, the meteorite hydrocarbons are products of life; but can we really say this, or might they be abiotic matter from which life originated? Some scientists even question whether petroleum hydrocarbons are of biological origin. It was recalled that in an earlier paper in this symposium the syntheses of biochemicals from abiotic sources was described; however, no mention was made of lipids. Meinschein's and Nagy's evidence depends on lipids, but one does not known whether lipids can be produced abiotically.

Next, according to Bernal, there is the question of the mineral composition of the meteorites. Water must have been present when these minerals were synthesized. Were the serpentine minerals the decomposition products of other silicates? The discussant pointed out that one fact is known: some of the serpentine particles were euhedral. Mason postulated that the primitive meteorite aggregated from dust particles, but the carbonaceous meteorites do not fit into this picture. Sztrokay found veins filled with bituminous material in the Kaba carbonaceous meteorite, which would suggest an elaborate chemical history. All of this was very puzzling according to Bernal. One might ask why it is necessary to bother with all this when in ten years time someone will land on an asteroid and settle the question of extraterrestrial life; however one must remember that the solution to this problem depends on many people, specialists in their various fields, who must get together and coordinate their efforts since no one man can settle this problem alone.

H. C. Urey: The moderator stated that the subject of extraterrestrial life was of such great importance that it might affect scientific thinking about the moon and, in fact, about the entire solar system. He did not, however, agree with Bernal regarding the origin of meteorites. Moreover, to understand better the "organized elements," one must also question what primitive life forms would be like. Would they survive as long as present life forms do? What would be the biochemical composition of primitive life forms? Would it be the same as it is today? Would the porportions of amino acids be the same as they are today? These, he felt, were all questions which would yet have to be answered.

B. J. Cholnoky (*National Institute for Water Research, Pretoria, Republic of South Africa*): Cholnoky stated that the only important consideration is whether there are or are not life forms in carbonaceous meteorites. It matters not which

scientist is right or wrong. The problem is basically biological. To the biologist it should be of no importance, as far as identification goes, whether the "organized elements" came from meteorites, from outer space, or from somewhere else; all that should matter is whether the "organized elements" can be identified as remnants of once living matter. Cholnoky emphasized that he is a microbiologist, who has spent 51 years studying microorganisms. He is not particularly interested, he stated, in fossils in meteorites, as such, but only in life forms in general.

There were then two main points which he wanted to make: First, he expressed his surprise that physicists and chemists seemed willing to offer critical evaluations regarding the biogenicity of organic microstructures. As a biologist, he would never think himself competent to comment on purely chemical and physical problems. He suggested that physicists and chemists adopt a similar attitude regarding biological problems. Consequently, he believed that the suggestion put forth in an earlier paper by investigators from Florida *i.e.*, that protenoid coacervates may resemble living cells in appearance, must be rejected. The methods of identification of these must be judged as insufficient; any identification of strains of coacervates must be submitted to experts. Although experiments with coacervates go back to the work of deJong, and were designed to investigate vacuole formations, deJong never said anything about cell walls.

Secondly, Cholnoky commented on claims that the "organized elements" in carbonaceous meteorites were only grains of starch or pollen contaminations. He said he has seen starch grains under microscopes on innumerable occasions and could not identify the "organized elements" as starch grains. To argue a point at meetings with photographic evidence was not satisfactory, since microorganisms are three dimensional and their morphology cannot be adequately represented in two dimensional photographs.

Sidney W. Fox (*Institute for Space Biosciences, Florida State University, Tallahassee, Florida*): The discussant stated that he had heard the word Florida mentioned, so he assumed Cholnoky's first point was in reference to his work. He wondered if Cholnoky had made correct distinctions. The Florida group works with microspheres, which can be separated by centrifugation; these microspheres are more stable than the Oparin coacervate droplets. He wanted to make another point, which he had forgotten to mention at the earlier session that day. Should the micropaleontologists and meteorite investigators conclude that the "organized elements" were not fossils of micro-organisms, but preprotobionata, *i.e.*, a type of abiotic microspheres, then this would be an even more significant finding, because it would indicate the discovery of precursor organic particles from which life forms could have later evolved.

Robert Ross (*Department of Botany, British Museum of Natural History, London, England*): This discussant reported on his own studies of the Orgueil meteorite:

The Orgueil meteorite fall consisted of about 20 stones. Two of the specimens at the British Museum (Natural History) arrived there as complete stones. He had studied one of these, which had not yet been examined for "organized elements" by other workers. In straight crushed preparations, he

did not find the large number of particles that he had expected to find after reading the report by Claus and Nagy. Nevertheless, he did find a small number of particles which, if found in terrestrial samples, he would have said to be of biological origin. Two colleagues at the British Museum agreed that these particular objects looked as if they might be of biological origin.

Plans have been made to conduct more refined experiments on these organic particles. Electron microprobe analysis, X-ray microanalysis, and electron microscopy are being contemplated. Certain additional experiments have already been performed, however. In addition to the examination of straight crushed preparations, density separations were carried out with aqueous cadmium borotungstate solutions; this liquid was used instead of the organic liquids used by Nagy *et al.* It was thought that the use of inorganic liquids would eliminate some of the criticisms raised against these investigators, *i.e.*, that the "organized elements" were mere droplets of bituminous matter, dissolved and then precipitated from organic liquids.

A fragment of the Orgueil meteorite was used, and its surface was scraped off with sterilized instruments. The sample in water was then subjected to repeated and prolonged freezing and thawing in an attempt to break up the mineral matrix and to disintegrate the stone. This process was partially successful. The disintegrated material was then suspended in cadmium borotungstate solution and centrifuged. Four fractions were obtained, one of which sank in liquids of 2.4 density. In the three light fractions, representing densities of below 1.6, equal to 1.6, and between 1.6 and 2.4, a number of the Type I "organized elements" of Claus and Nagy were found. In these density range fractions, furthermore, there were also other objects, which resembled collapsed spore membranes. Finally, two unusual forms were found in the lightest fraction. Each of these objects consisted of a hollow tube, approximately 25μ long and 1$\frac{1}{2}\mu$ wide. The tubes contained an infilling $\frac{3}{4}\mu$ wide, that had a refractive index different from the tube walls and quite different from the Canada balsam in which these objects were mounted. The fillings were probably air bubbles. One end of each of these tubes blended smoothly into what looked like a torn piece of membrane, approximately 10μ wide. The overall appearance of these forms, the tubes and the torn membranes together, approximated a mushroom shape. These forms had been associated with the meteorite matrix; the sterile procedures used suggested that they were not contaminations acquired during the study, but were part of the Orgueil meteorite. The forms reminded Ross somewhat of the fossil hystrichospheres that Papp had described earlier during the sessions. They might be parts torn from such an organism. He concluded they were of biogenical origin. Claus and Nagy had shown that the objects they found take up biological stains and resist acids. These crude tests suggested that they consisted of carbon compounds. All this evidence, he believed, adds up to a strong indication, but not proof, that there are indigenous remains of living organisms in the Orgueil meteorite.

H. C. Urey: He expressed the opinion that Ross's findings were quite impressive. He thought, however, that one might still wonder about what happens to living matter when it is fossilized for four and a half billion years; also, what would the very earliest forms of life look like? He stated that it would be

significant if one could find many objects with only narrow variations in their morphology, since the same types of organisms should not vary widely in morphology.

George Claus (*Department of Microbiology, New York University Medical Center, New York, N.Y.*): Claus stated that Type I of the "organized elements" is by far the most common. Morphological variations, as well as size distribution of organisms, follow a Gausian distribution curve. "Organized elements" follow the same pattern.

F. W. Fitch (*Department of Pathology, University of Chicago, Chicago, Ill.*): According to Fitch, many different kinds of particles described in Orgueil meteorite preparations have been called "organized elements." In his opinion, one deals with a heterogeneous population of objects which can be divided into two general classes—particles having a simple appearance and particles having highly structured morphology. The rather featureless objects are numerous but seem to have no specific properties indicating biological origin. Particles having complex morphology are quite rare and some may have a biological origin. However, there is no proof that they are not terrestrial contaminants. Fitch wondered what would be adequate criteria for identifying the "organized elements" as extraterrestrial forms having biological origin. He did not believe that morphology alone was adequate evidence. There are at least 250,000 plant species on the earth. It is impossible for any individual to be familiar with more than a fraction of these and to identify isolated plant fragments. There were no experts specializing in the study of pollen and of fungi at this meeting, according to Fitch; therefore evaluation of the objects at this meeting must necessarily be incomplete.

Bartholomew Nagy (*Department of Chemistry, Fordham University, New York, N. Y.*): Nagy stated that because there were no experts on pollen at the meeting, he and Claus took their microscopic preparations to a meeting they attended during the previous week of the First International Congress of Palynology at Tucson, Arizona. At this meeting there were approximately 300 experts on pollen from 22 different countries. The slides were exhibited in public, and anyone who wished to examine them under the microscope could do so. Approximately 80 specialists did so and to his knowledge, no one definitely identified the "organized elements" as recent pollen contaminations. Since, however, Anders *et al.* have argued that the "organized elements" were ragweed pollen grains and starch grains, he thought it might be interesting to recall Erdtman's comments on the "organized elements." Erdtman is a Swedish pollen expert. His first impression was that the "organized elements" were indeed pollens; however, after more careful examination, he concluded that this was incorrect because they were similar to hystrichospheres, a pelagic form of protobionta.

H. C. Urey: Urey noted that enthusiastic people can make mistakes, but a mistake should not stop anyone. If one cannot identify these objects, one should consult others. Photographs of objects projected on the screen do not settle the question.

Rainer Berger (*Lockheed California Company, Burbank, Calif.*): Berger agreed that undoubtedly more experimentation is needed. As Cholnoky pointed out, biochemical tests could be inconclusive when applied to fossils.

For example, people buried in Pompeii in ash from the eruption of Vesuvius apparently have no carbon left, because the tissues have been fully replaced by mineral matter. Nobody doubts that they are remnants of people, yet biochemical tests on them would give negative results.

Berger went on to say that one does not know what happens to meteorites during their passage through the atmosphere. There is a question as to whether air or air-borne pollen is sucked in. He wondered if it might be possible that the pollen could become imbedded in the meteorite and become fossilized during museum storage. He also wondered how long it takes to fossilize organisms.

Warren Meinschein (*Esso Research and Engineering Company, Linden, N. J.*): Meinschein's opinion was that it requires a long time to fossilize organisms and it certainly requires water.

R. Berger: Berger wondered if there was enough water for this to occur in the museum.

D. J. Hennessy (*Department of Chemistry, Fordham University, New York, N. Y.*): According to Hennessy, the issue at the present time was whether these "organized elements" were terrestrial or extraterrestrial. Since Orcel in the Paris Museum has large, single pieces of the Orgueil meteorite, perhaps he could be persuaded to permit drilling into one with a sterile drill to obtain a sample from the interior.

Edward Anders (*The Enrico Fermi Institute for Nuclear Studies, University of Chicago, Chicago, Ill.*): Anders suggested that it was utterly misleading to speak of "organized elements" as if they were a single, well-defined family of particles with certain generic properties. Instead, it appeared that the organized elements fell into two sharply distinct classes. Particles of the first class have a striking morphology, and most of them are probably biogenic. However, they are quite rare, even in Nagy's samples, and they have not been seen in the Orgueil samples studied at Chicago. Most of them show a strong resemblance to common airborne contaminants, such as pollen grains, fly ash, etc., and it seems likely that most of them are in fact terrestrial contaminants. Particles of the second class are probably indigenous to the meteorite. But they seem to lack all other properties suggestive of a biological origin: their morphology is nondescript, and resembles that of mineral grains; they do not take biological stains, or take them atypically; they do not fluoresce in ultraviolet light; they dissolve in acids; and they have nearly the same density as the mineral grains in the meteorite. In view of these findings, Fitch and Anders believed that two questions needed to be settled before all others. For the particles of the first class, what is the evidence that they are not terrestrial contaminants? And for the particles of the second class, what is the evidence that they are not in fact mineral grains?

[*Note added by the discussant in proof.* Most of the evidence obtained since the meeting has favored the view that the majority of the organized elements are either contaminants or mineral grains. The spiny Type II elements ("hystrichospherids"), alleged by us to be ragweed pollen grains, have in fact been identified as ragweed pollen by several pollen experts. The Type V element ("dinoflagellate"), discovered by Claus and Nagy on a Gridley-stained slide of Orgueil was shown to resemble Gridley-stained ragweed pollen (Fitch

and Anders, Science, 1963, in press). The particles of simple morphology, which we said resembled mineral grains, do indeed have the chemical composition of limonite (hydrated ferric oxide), according to electron microprobe analyses by Nagy, Fredriksson, Claus, Anderson, Urey, and Percy (Nature, 1963, in press), and they dissolve in acids without leaving a structured organic residue (Anders and Fitch, Science, 1962, **138:** 1392). The case for their biological origin now rests entirely on their featureless morphology.]

H. C. Urey: Ross's findings impressed the moderator. Ross had worked with a complete stone which had probably been heated during passage through the atmosphere. The moderator wondered whether there had been any signs of contamination on Ross's sample, and whether it had been marked with paint or mounted on wax.

R. Ross: The samples of the Orgueil meteorite in the British Museum were kept in a box with a glass cover on it. There were no precautions taken during the years to keep them sterile in storage. He had used, however, sterilized instruments to work with the samples. He had also scraped away exposed surfaces of the meteorite with sterile instruments before taking a sample, and before placing the samples into water and subjecting them to freezing and thawing.

P. Morrison (*Department of Physics, Cornell University, Ithaca, N. Y.*): Morrison stated that he would like to see a count of the relative distribution of the "organized elements."

G. Claus: According to Claus, "organized elements" were present in Orgueil on the average of 1700 per milligram. Type I was by far the most common and on one side Type II represented approximately five per cent of the total count. Claus noted, regarding the staining, that there are many "organized elements" which do not take stains.

H. C. Urey: This was a puzzle to Urey, and he wondered if the simpler ones could be artifacts and the more complicated ones, contaminations.

Paul Tasch (*Department of Geology, University of Wichita, Wichita, Kansas*): Tasch observed that, exclusive of magnetic particles, the "organized objects" found in some carbonaceous chondrites fall into three classes: (1) terrestrial contaminants in addition to those already cited by Claus and Nagy; (2) proteinoid microspheres of Fox or organic-chemical analogues of Morrison; and (3) indigenous microfossils. He believed that allowance for (1) and (2) had been made, but (3) still remained to be explained. The discussant had observed three distinct objects in Claus and Nagy's thin sections. Two of these were on display at the International Palynological Conference at Tucson, Arizona, and one was presented by Claus in his talk. The discussant's first impression of one of these objects embedded in salt was that it resembled a chrysophyte; another object, named *Daidaphore Berzelii*, had a hystrichospherid-like organization. A third object appeared to have antapical horns and a girdle, thus suggesting a dinoflagellate.

Ross, according to Tasch, had reported how he isolated a distinct object from a carbonaceous meteorite, and had indicated his conviction that it was not a contaminant, but resembled a process of hystrichosphere.

Tasch also pointed out that F. L. Staplin of Imperial Oil, Ltd. processed a sample of the Orgueil meteorite and wrote a report, soon to be published, which

the discussant had already seen in a preprint. Staplin found, after palynological processing of the sample, a group of micro-objects in the final residue. In his judgment, these were the closest in affinities to a hystrichosphere-leiosphere assemblage. Among the objects, he also found some chrysophytes.

Now, continued Tasch, these three observations, in addition to those of Claus, all seemed to be consistent.

Tasch suggested that to advance this discussion the problem of contaminations be bypassed and the objects found in the meteorites be accepted as indigenous. What would then follow? Pelagic protists closely resembling terrestrial types must have lived on the parent body; once this is admitted, then it follows that there were water bodies in which they lived. In addition, there must also have been a supply of phosphorous, nitrogen, and other nutrient substances. That, according to Tasch, would be as far as paleobiology can take it. It would then be necessary for Urey, Bernal, and others to explain where it is possible for such water bodies to exist.

A. Papp (*Department of Paleontology, University of Vienna, Vienna, Austria*): Papp emphasized that the basic rule of the natural sciences is that an experiment must be repeatable before one can accept the findings as valid. Research on the "organized elements" started only a short while ago, yet the experiments had already been successfully repeated, and independently, by Ross in England, Staplin in Canada, and Skuja in Sweden. Now the Anders group came up with negative results. A most important question would be whether any one else has found the "organized elements" besides Nagy *et al.*; the answer to this is yes. They were found in England, in Canada, in Sweden and, according to Papp, one would venture to say they were also found in Chicago.

There is then the question as to the differences in yield; the answer to this would be that the greatest number of objects were found by those who did most of the work. Papp illustrated this by pointing out that in two kilograms of sedimentary rock one would find more microfossils than in only one gram of rock. It would seem that the problem of differences regarding the number of "organized elements" would be related to the relative amounts of time spent on the problem by the different investigators.

With respect to attempts to identify and to classify these forms, Papp concluded that it was unimportant whether they look somewhat like dinoflagellates or something else. They are something different, and it would be impossible to include them in terrestrial systems. One could only compare them with terrestrial forms and state that they resemble certain terrestrial species, and even with this, one would be saying very much.

In summary, Papp believed that the "organized elements" had been proven to be organic and that their organized nature had been confirmed independently in four countries. He was impressed that "organized elements" were not identical to, but only resembled terrestrial organisms and, therefore, he considered that the question of their origin was closed.

H. C. Urey: He and his associates in LaJolla could not find anything in their sample of Orgueil. They then sent their samples to Nagy and the "organized elements" were encircled on the slides and returned to them. They still could not find one. Finally, however, a technician did find an "organized element."

A. Papp: Six months previously one of Papp's colleagues, a mineralogist in

Vienna, had said that the "organized elements" were mineral spherolites. Papp stated that this only showed that one needs extensive training in microbiology to recognize these forms.

H. C. Urey: Urey mentioned that Volcani, the microbiologist at LaJolla, was not discouraged.

C. M. Palmer (*Division of Water Supply and Pollution Control, Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio*): He stated that it should be mentioned that Claus was capable of observing and finding structural details that other people often overlooked; *i.e.*, details on filamentous algae which had never been seen, although the forms had been known for more than 100 years.

Pierre Bourrelly (*Department of Cryptogamic Botany, Musee d'Histoire Naturelle, Paris, France*): Bourrelly saw the microscopic preparations. He believed that the "organized elements" were definitely organisms. They did not look much like hystrichospheres, and they did not seem to be contaminants. He was puzzled because they resembled terrestrial forms; he thought they should exhibit greater differences.

R. Berger: He recalled that Papp had implied that the terrestrial evolutionary sequence might have occurred elsewhere. There might be an equivalent biochemistry which would lead to similar organisms.

J. D. Bernal: He expressed the opinion that the carbonaceous meteorites could not be of terrestrial origin because of their unusual mineral content. If they were not terrestrial, he questioned what the origin might be. Only Earth and Mars are capable of holding water; therefore, if the "organized elements" arose elsewhere than on earth, one would be forced to choose between two possibilities: that the same biochemistry is prescribed for every origin of life and, therefore, life always follows the same trend; or that all life forms originated from the same ultimate life source. In other words, one is faced with these questions: did life originate in several places as a result of the same biochemical mechanism, or did life evolve only once and then spread to different places? He added that four billion years might not be a sufficient amount of time for biochemical evolution on earth.

H. C. Urey: According to Dr. Urey, life might have transferred to the moon from earth.

P. Tasch: He was one who considered the moon transfer theory a serious possibility, though a difficult one to accept.

H. Dombrowski (*Department of Balneology, Justus-Liebig University Giessen, Germany*): Dombrowski stated that there is an analogy between the problem of "organized elements" and his work obtaining living bacteria from salt deposits. Chemists have known for a long time that salts contained less than 0.01 per cent of nitrogen. The origin of this nitrogen could not be explained until biologists started to work on salt samples. Now it is known that this nitrogen content is associated with the bacteria embedded in the salt.

Brian Mason (*Department of Mineralogy, The American Musuem of Natural History, New York, N. Y.*): Mason thought that it would be very difficult not to contaminate a meteorite in a museum. The American Museum of Natural History acquired its Orgueil sample in 1901, but it is not known what happened to it before this date. The sample was kept in an open box. It should be

mentioned that magnesium sulfate in the meteorite in New York is $MgSO_4 \cdot 4H_2O$; in Chicago it is $MgSO_4 \cdot 7H_2O$. This would only show how the environment can effect the "organized elements."

J. D. Bernal: He mentioned that the Orgueil sample in the Bombay Museum in India fell to a fine dust because of the humidity.

W. G. Meinschein: The hydrocarbon distribution in Orgueil resembled biogenic hydrocarbons in Recent marine sediments. Mass spectra showed that these hydrocarbons could not have been contaminated by accident, much less by intent. The hydrocarbon concentrations were above Recent sediment concentration levels.

B. Mason: This discussant was of the opinion that the organic matter in Orgueil was clearly indigenous, but that one must prove that it was not formed by inorganic processes.

W. G. Meinschein: It was emphasized by Meinschein that 23 saturated hydrocarbon compounds had been identified. The parent sterol hydrocarbons were present and the aromatic hydrocarbons were those which are found in Recent sediments. Clearly, he believed, they were of biogenic origin.

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