HIGH DILUTION EFFECTS:
Physical and Biochemical Basis

By Nirmal C. Sukul and Anirban Sukul

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Dedicated to

Dr. B.N. Chakravarty who has explored and realized some of the immense therapeutic potential of Homeopathy through years of his clinical observation.

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PREFACE

Our present day concept of pharmacology is restricted to the interaction between drug molecules and biomolecules. Any interaction beyond molecules and that too with the specificity of molecules is considered a myth in the arena of science. Since high dilution of drugs, which cross the Avogadro number, do not contain drug molecules they are not supposed to cause any action on a biological system. But high dilution effects have become the common experience of homeopathic physicians and their millions of patients all over the world. Besides clinical evidences, there is now a large body of experimental evidences which confirm that drug action beyond molecules on an organism is a reality. A drug leaves something, a phantom entity, in its high dilution. What is the physical feature of this phantom? How do the phantoms of different drugs differ from each other? How do they act on an organism? Do they always need a living system for producing an action or behave just as a chemical reacting with another chemical? The present book is an attempt to find answers to all these questions. The approach is scientific and based on the existing knowledge of science.

Drugs at ultra high dilution have been used in the novel therapeutic system known as Homeopathy for about a couple of centuries. The system declined in the middle of the 20^{th} century because of keen competition from the conventional medicine. It has regained its past glory to some extent during the last couple of decades mainly because its drugs are effective, relatively inexpensive, non-invasive with respect to drug delivery and have little undesirable side reactions. Unravelling the mysteries of this important therapeutic system offers a challenge not only to biologists but also to physicists and chemists as well. Although the business potential of homeopathic medicines is not as much lucrative at present as the conventional medicine, scientific research on homeopathy would certainly open up new avenues to the ultimate benefit and welfare of the mankind.

The book has four chapters besides introduction. In the 1^{st} chapter sources of homeopathic drugs, their preparation techniques, and preservation have been described. The 2nd chapter deals with the clinical evidences obtained in support of the high dilution effects on human patients. In the same chapter evidences recorded from laboratory experiments on animals and plants have been described. Experimental evidences from *in vitro* and *ex vivo* tests have also been included here. The 3rd chapter describes the physical characteristics of drugs at ultra high dilutions as evidenced by nuclear magnetic resonance (NMR) spectra, infrared (IR) spectra, electronic spectra and fluorescence spectra of some selected potentized drugs. Since water is thought to carry the information of drug molecules or particles at high dilutions, the structure and dynamics of liquid water as the medium of homeopathic potencies have been discussed here. In the 4th chapter the possible mechanism of action of high dilutions in the living system has been discussed in terms of

interaction between structured water molecules of a potency and its primary biomolecular target in an organism. The association of water structure with the primordial life forms during the emergence of life on this planet has been discussed at the end. We would consider our work worthwhile if it could develop interest in scientists and practising physicians and engage them in serious research to open the black box of Homeopathy and explore its vast therapeutic potential.

We thank our research scholars like Paramita Sarkar, Souvik Ghosh, Ashis De and Sudeshna Ghosh for conducting all the experimental works. We thank Dr. B.N. Chakravarty for providing financial support to our latest investigations. We also thank the Head of the department of Zoology, Visva-Bharati University for providing necessary laboratory facilities for conducting experimental work on Homeopathy.

Santiniketan
September 2003

N.C. Sukul
A. Sukul

INTRODUCTION

One of the fundamental principles of pharmacology is the dose response. The higher is the dose the stronger or more intense is the effect. If the dose is very low there would be no beneficial effect. Again, if the dose is very high there would be toxic effect. Many drugs at high doses are toxic; even therapeutic doses of many drugs have side reactions. Thus in order to minimize or eliminate toxicity or side reactions, drugs should be used at a very low dose or at an extremely high dilution. But how to retain efficacy of a drug at a high dilution? We know that in Homeopathy drugs have been used at extremely high dilutions, dilutions that very often cross the Avogadro number. There are at present a large body of reliable evidences that show that these drugs at ultra high dilutions are effective. This topic would be discussed in details in chapter II.

We would discuss here the basic principles of Homeopathy, and in Chapter I the methods of preparation of high dilutions of drugs. This would help in understanding the physico-chemical basis of drugs at high dilutions and the mechanism of action of these drugs in the living system. In the book **'high dilutions'** mean dilutions of drugs used in homeopathic pharmacy. The details of these dilutions are described in Chapter I.

Principles of Homeopathy

Samuel Hahnemann (1755-1843), a German Physician, introduced this therapeutic system in his book *'Organon of the Rational System of Medicine'* in 1810. The principles of Homeopathy laid down in the book were further elaborated and modified in subsequent editions of the book, published in 1819, 1824, 1829 and 1833 under the title *Organon of Medicine.* Hahnenmann described his basic ideas of Homeopathy before the publication of *'Organon of Medicine'* in two articles, 'Essay of a new Principle' and 'Medicine of Experience', published in 1796 and 1805, respectively. This novel idea of Homeopathy first occurred to him in 1790 when he was translating Cullen's *Materia Medica.* He took a small dose of cinchona (Peruvian bark) and experienced symptoms of intermittent fever like malaria. In a series of experiments conducted during 1790 to 1810, Hahnemann observed that some substances derived from plants, animals and minerals could produce specific sets of symptoms in healthy people. The same substances, in minute doses, showed therapeutic effect on patients showing similar sets of symptoms. By 1820 Hahnemann published the detailed symtomatology of more than 90 substances in six editions of his book *Materia Medica Pura*. Homeopathy flourished in Europe and the United States in the latter part of the 19th century (Jacobs and Moskowitz, 1996).

Homeopathic *materia medica* and *repertory* were further enriched by the contribution from James Tyler Kent (1849-1916), E.A. Farrington (1847-1885), C. Von Boenninghausen (1785-1864), and others. The sixth edition of *Organon* was published in 1921, long after the death of Hahnemann.

Homeopathy is based on the principle *Similia Similibus Curentur* or like cures the like. A substance, that produces in a healthy individual a pathological condition simulating the symptoms of a disease, would be the right remedy of that disease. A homeopathic physician should collect all the subjective and objective symptoms from a patient, compare them with the symptoms of a drug mentioned in the *Materia Medica* and then apply the remedy to the patient at a very low dose. The symptoms of a right remedy should exactly agree with the totality of symptoms shown by an individual patient. A patient of typhoid or malarial fever may show the specific symptoms of a particular disease. But an individual patient would show many more symptoms besides the specific symptoms of the disease. Homeopathy takes care of all the symptoms of a patient taken together. Thus, in Homeopathy there is no fixed medicine for a particular disease. The basic constitution of the patient is important in Homeopathy. A single simple drug should be administered at a time on a patient. A patient should be treated with a very minute dose of the remedy.

Homeopathy has a holistic approach towards treatment. It takes into account all the mental and physical conditions of a patient, his or her way of life, habit, cravings for any food/drink, tolerance to cold or warmth, sexual life etc.

Born in early nineteenth century Homeopathy has its own concept about the etiology of a disease. It believes that a spiritual power or vital force animates the healthy organism and keeps it in a harmonious order. During illness the vital force is overwhelmed by a disease force. An artificial disease force, applied through the use of the right remedy, annihilates the original affection and restores health. Sometimes patients do not respond to well selected remedies due to their inherent conditions called miasms. The concept of miasms introduced by Hahnemann has been discussed in Chapter IV.

At the time of Hahnemann and also half-a-century after his death the idea of vital force or vitalism was prevalent among scientists. Early philosophers believed that living organisms, though composed of inanimate molecules, are radically different from non-living matter and are endowed with a divine life force, called vitalism. In the 1850s, Louis Pasteur concluded that fermentation of sugar into alcohol by yeast was catalysed by “ferments”. He postulated that these ferments were inseparable from living yeast cells, a view of vitalism. In 1897 Edward Buchner discovered that yeast extracts could ferment sugar to alcohol, proving that fermentation was promoted by molecules that continued to function when removed from cells. Frederic W.K. Kuhne called these molecules as enzymes (Nelson and Cox, 2000)

CHAPTER I

PREPARATION OF HIGH DILUTIONS OF DRUGS

As mentioned under introduction, high dilutions of drugs are used in Homeopathy. At present there are more than 3000 homeopathic drugs.

1.1. Sources of drugs

Homeopathic drugs are produced from natural substances such as plant materials, animal sources, minerals, diseased parts of man etc. They are also produced from the diluent medium of aqueous ethanol exposed to ionizing radiation. Remedies of plant and animal sources are named according to the binomial nomenclature introduced by Linnaeus (1753).

1.1.1. Plant materials

Plants constitute more than 60% of homeopathic remedies. Different parts of plants such as roots, barks, stems, leaves, flowers, fruits and seeds are used in homeopathic medicine. Roots of annual herbs are taken out when they yield ripe fruits. In case of perennial plants roots should be lifted in the spring in the 2nd or 3rd year. Examples are *Ipecacuanna* and *Bryonia* (Cook, 1988). Fully grown and uninfected leaves are collected after sunset before the flowering season. Examples are *Rhus toxicodendron* and *Ocimum sanctum.* Flowers are collected when they begin to open. *Cina* serves as an example. Here the flowering tops of the plant *Artemisia* sp. are used. Barks are collected from young plants. *Cinchona* serves as an example.

Bulbs and corms are modified underground stems. They are collected when they are fully grown. Bulbs of *Allium cepa* and *Allium sativa* are used. Corm of *Colchicum autumnale* is the source of the drug. Dried seeds provide source of drugs such as *Nux vomica* and *Ignatia. Lycopodium* is produced from spores of the plant *Lycopodium clavatum, Opium* from the latex of the capsules of the poppy plant, *Papaver somniferum* and *Carbo vegetabilis* from the charcoal. Whole plants are also used for the preparation of drugs like *Aconitum napellus, Calendula officinalis, Chamomilla, Phytolacca decandra* (Cook, 1988).

1.1.2. Animal substances

Animal products constitute 20% of homeopathic remedies and are collected from whole animals or their parts. *Apis mellifica* is prepared from the whole honey bee, *Cantharis* from the dried powder of the beetle, *Cantharis vasicata*, *Sepia officinalis* from the ink gland of the cuttle fish, *Lachesis* from the venom of the bush master snake and *Tarantula hispanica* from the poisonous Spanish spider (Cook, 1988). *Lac caninum* is prepared from dog's milk and *Calcarea carbonica* from oyster shell.

1.1.3. Minerals and chemicals

Homeopathic remedies are produced from minerals like gold (*Aurum metallicum*), iron (*Ferrum metallicum*), copper (*Cuprum metallicum*), silver (*Argentum metallicum*), zinc (*Zincum metallicum*) etc. Remedies are prepared from inorganic compounds like sodium chloride (*Natrum muriaticum*), mercuric chloride (*Mercurius corrosivu*), magnesium carbonate (*Magnesia carbonica*), arsenous oxide (*Arsenicum album*), sulphuric acid (*Acid sulphuric*), calcium sulphide (*Hepar sulphuris*), potassium bichromate (*Kalium bichromicum*) etc. They are prepared from organic compounds like picric acid (*Acid picric*), acetic acid (*Acetic acid*), lactic acid (*Acid lactic*), nitroglycerine or glycerol trinitrate (*Glonoin*), cane sugar or sucrose (*Saccharum officinale*), glycerol (*Glycerinum*), chloroform (*Chloroformum*), strychnine (*Strychninum*) etc. They may be prepared from rocks (*Hecla lava*) or spring water (*Sanicula*).

1.1.4. Other biological materials

Drugs produced from diseased tissues are called nosodes. Examples are *Tuberculinum* from T.B. infected tissue, *Medorrhinum* from gonorrhoeal discharges, *Bacillinum* from tubercle bacillus, *Psorinum* from scabies pustules etc. Sarcodes are prepared from the extracts of fresh organs or glands removed from cattle, pigs or sheep. Examples are *Pancreas, Lung, Kidney* etc. Bowel nosodes are derived from cultures of intestinal bacteria present in human faeces. Examples are *Morgan pure, Proteus, Bacillus* No.7 etc.

1.2. Proving of drugs

Originally, those substances were tested on healthy human volunteers of both sexes. A person is asked to consume one substance at a time and the symptoms produced by the substance are recorded. The process is known as proving of the drug. According to Hahnemann the idea of proving first occurred to Albrecht von Haller (English Translation of *Organon of Medicine* 6th edn, 1921). The initial dose of the drug depends on its toxic properties. The dose may be gradually increased during proving which may span from a few days to several days. Sometimes, the crude drugs do not show the same symptoms as are elicited with the highly diluted and potentized forms of those drugs. For this, the potentized forms of a drug, usually the thirtieth potency, is given to a volunteer, one dose daily on empty stomach, for several days. We would discuss what is meant by a potency in Homeopathy in the following paragraphs. The amount of the potentized drug may be increased if the smaller amount produces a very mild effect. There may be individual variation in response to a particular drug given for a fixed period. In order to rectify the situation a number of healthy provers is given the same drug and the symptoms found in them are recorded. Well-documented symptoms of drugs, collected in this way, constitute the basis of the *Homeopathic Materia Medica*. One may question the veracity of the *Materia Medica*. But evidences from modern toxicology and experiences of many homeopathic practitioners are mostly in agreement with the *Materia Medica* (Fisher, 1981; Sukul, 1997). Attempts should be made to verify the symptoms in a scientific

way using modern technology. Reproving of *Belladonna* confirmed the results of earlier proving and recorded some additional symptoms (Bellows, 1906).

Many problems are involved in proving of drugs. Potential provers may not be all perfectly healthy and their responses to a particular drug or to a particular dose of the drug are likely to vary. Royal (1991) has discussed about the problems and protocols of proving in a paper.

Some recent studies on provings were conducted in a systematic way (Walach, 1997, Goodyear *et al.*, 1998). Dantas and Fisher (1998) reviewed the works on provings and suggested procedures for qualitative improvement of this kind of study.

1.3. Preparation of mother tinctures

Three types of media are used for the preparation of homeopathic drugs and their dilutions. These are aqueous ethanol, usually 90% ethanol, water and lactose or sugar of milk. Plant and animal products are extracted at room temperature with aqueous ethanol. Salts are usually dissolved in distilled water in the proportion of 1:9 or 1:100 depending on their toxicity. There are nine classes of drugs used in Homeopathy and the proportions of the drug and the vehicle differ according to the class. In case of metals precipitates are used for potentization. Substances soluble in aqueous ethanol are extracted with this medium. These are called mother tinctures. Many mother tinctures, particularly the non-toxic ones, are administered at material doses directly on patients. However, the doses are very small, usually 10 to 20 drops and are administered with a little water. This part of Homeopathy is quite similar to other systems of Medicine in vogue. Mother tinctures are denoted by M.T. or with a suffix θ. Final alcohol strengths of mother tinctures may be 33 1/3%, 50% or 80-90% (v/v), depending on the water content of the starting material (Cook, 1988).

1.4. Potentization of drugs

The uniqueness of Homeopathy lies in the use of extremely high dilutions of drugs which go beyond the Avogadro number. These dilutions are prepared in a specific way and are called potencies in Homeopathy. The process is called potentization or dynamization. The process of dynamization was elaborated in the fifth edition of *Organon* published in 1833 and also in a later work of Hahnemann, Chronic *Diseases* published in 1838.

Succussion

Mother tinctures are diluted with aqueous ethanol in succession. The mother tincture is mixed with its diluent medium (aqueous ethanol) in two proportions by volume, 1:99 and 1:9. Potencies prepared in 1:99 and 1:9 proportions are called centesimal and decimal, respectively. The centesimal scale was introduced by Hahnemann and the decimal scale by Constantine Hering during the lifetime of Hahnemann.

The mother tincture is diluted with 90% ethanol in any one of the two proportions in a round vial. According to the *American Homeopathic Pharmacopoeia* 87% alcohol should be used for the dilution of a mother tincture (Anonymous, 1920). The vial is filled upto two-thirds of its space with the diluted drug, tightly corked and then shaken by 10 powerful downward strokes of the arm. The potency prepared thereby is the 1st potency of the drug. This process of mechanical agitation is called succussion. All subsequent potencies are prepared by further diluting each potency in the same proportion used initially and giving the mixture 10 downward strokes. Thus the dilution of a drug in the first centesimal potency is 10^{-2}. Centesimal potencies in regular use are 3, 6, 30, 200, 1000, 10000 or higher. They are written in simple numerals with or without a suffix c such as *Nux vomica* 30 or 30c. Decimal potencies are expressed by the suffix x such as *Ferrum Phos* 3x or 6x. Normal uses of decimal potencies are 1x, 2x, 3x, 6x and 12x.

The Hahnemann method of dilution is time consuming and involves a large number of containers for preparing a potency. A quicker and more practical method introduced by Korsakov in 1832 requires a single container for the preparation of high potencies of a drug. The container is simply emptied and refilled with the diluent medium in each step of successive dilution. The emptied vial still contains some residual amount of the preceding potency for further dilution.

The dilution and mechanical agitation can be done by hand or by a machine. Manual preparation cannot be uniform in strength every time a vial is shaken. Machine is needed for the preparation of higher potencies like CM (diluted one hundred thousand times) and MM (diluted one million times). Hahnemann followed the duodecimal system, which was in vogue in his time, in choosing the potency numbers as 6, 12, 24 etc. Higher potencies like 200, 1M, CM, introduced later are based on the metric or decimal system in units. Besides decimal and centesimal series, based on serial dilution of 1:10 and 1:100, respectively, there exists the millesimal series which is based on the serial dilution of 1:1000 and is denoted with the suffix m (Cook, 1988).

Towards the end of his life Hahnemann introduced a new series called the LM potencies. This has been mentioned in the 6th edition of *Organon*. Here the substance is triturated to the 3C level with lactine. One hundredth part of this potency is diluted with 500 parts of 20% alcohol. One part of this is diluted with alcohol 1:100 and succussed 100 times. One drop of this is used to moisten 500 small sugar globules to prepare the 1st potency called LMI. One globule of LMI is mixed with 100 parts of alcohol and the mixture is succussed 100 times to prepare LMII. In this way potencies upto LMXXX can be prepared. The approximate dilution in each step here is 1:50000.

Trituration

Solid drugs which are insoluble in a liquid medium such as aqueous ethanol or water are at first attenuated with a solid medium lactose upto the 3rd potency, and then diluted with aqueous ethanol for further potentization. In this case one part by weight of a drug is mixed in a porcelain mortar with one-third of 99 parts, or 33

parts by weight of lactose. The mixture is triturated with a porcelain pestle for 6 minutes and then scrapped with a porcelain spatula for 4 minutes. The process is repeated once and then half (33 parts by wt) of the remaining lactose is added. The mixture is triturated and scrapped in a similar manner. The last part of lactose is added and the mixture is triturated and scrapped in a similar way. This is the first centesimal potency of the drug prepared by trituration. Subsequent potencies of the drug are prepared by adding one part of the preceding potency to one-third of 99 parts by wt of lactose and the whole process is repeated in the same way. In the decimal scale, the method is the same with the proportions of the drug and lactose being 1:3 in the initial step. Lactose would be added in two more succeeding steps, each time 3 parts by wt. Thus in the 1st decimal potency the proportions of drug and lactose would be 1:9. The importance of trituration in liberating medicinal properties of drugs has been discussed in Chapter III.

1.4.1. Conversion of solid potencies into liquid ones

One part by wt of the 3rd potency of a drug prepared by trituration in lactose medium is mixed with 50 parts by wt of distilled water and the mixture is shaken or stirred. Fifty parts by wt of ethanol are added to the aqueous mixture so as to fill the vial upto two-thirds of its space. The vial is stoppered and shaken by 10 downward strokes. This is the 4th centesimal potency of the drug. Subsequent potencies are prepared by mixing one part by volume of the 4th potency with 99 parts by volume of 90% ethanol and subjecting the mixture to 10 succussions. In case of a decimal potency one part by wt of 6 x trituration of a drug is mixed with 50 parts by wt of distilled water in a vial. Fifty parts by wt of ethanol are added to the mixture which is succussed 10 times. This is 8 x potency of the drug. One part by wt of this potency is mixed with 9 parts by wt of 90% ethanol and the mixture is succussed 10 times to produce the 9 x potency of the drug. All subsequent potencies in the decimal scale are prepared in this way. The methods used by pharmacists are described in the *Homeopathic Pharmacopoeia* (Anonymous, 1920, 1962). Latest homeopathic pharmacopoeias are mentioned under 1.5.

1.5. Preservation of remedies and pharmaceutical forms

Homeopathic potencies are available in liquid form and in sucrose globules (Anonymous, 1962). Globules are prepared from pure cane sugar which should be white. They are of different sizes denoted by numbers ranging from 8 to 80. The number specifying a size is determined by laying 10 globules in close contact with each other in a line. The length of the line in millimeter gives the number for that size. A vial is filled upto two-third of its space with the globules of a particular size. A few drops of a liquid potency are poured into the vial to just moisten all the globules. The vial is corked, shaken and rolled so that all the globules are uniformly moistened by the liquid potency. The cork is loosened and the vial is turned upside down to allow excess liquid drain out. After keeping the vial in the inverted position for 9/10 hrs it is turned upright, well-corked and kept in a cool dry place away from

light. Liquid drugs would remain in fine capillaries of sugar globules. Liquid potencies in dilute ethanol are unsuitable for the preparation of medicated globules. Because high water content would make the globules break and coalesce with each other. The preparation becomes pasty. In the first volume of his book, *Chronic Diseases*, Hahnemann advised to moisten the globules with drops of a liquid potency for 1 minute in a deep porcelain bowel. The contents of the bowel are then emptied on a piece of dry filter paper so that excess liquid is absorbed and the globules become dry soon. Dry globules are then kept in a vial. Ethanol being highly volatile, liquid potencies are prone to rapid evaporation. Medicated globules on the other hand are known to retain their properties for many years. Sugar globules can be kept in an oven at 100ºC for 10hrs before medication. This would remove all moisture from the globules.

Homeopathic medicines should be kept away from direct sunlight, any other radiation, high magnetic field and volatile odorous substances such as camphor. Liquid potencies are supplied in amber glass dropper bottles. Standard procedures for the manufacture of different homeopathic remedies and their preservation have been described in homeopathic pharmacopoeia. There are homeopathic pharmacopoeia of India, 2nd edn, 1966 (Manager of publications, Ministry of Health, Government of India), homeopathic pharmacopoeia of the United States, 1976 (The American Institute of Homeopathy, Washington DC), British homeopathic pharmacopoeia, 2nd edn, 1999 (British Association of Homeopathic Manufacturers), German Homeopathic Pharmacopoeia, 2001 (Deutescher Apother Verlag, Balogh International Inc.), European Homeopathic pharmacopoeia, 4th edn, 2002 (Directorate for the quality of medicines of the Council of Europe) etc. The International Pharmaceutical Federation (FIP), the Hague, the Netherlands, has laid down international guidelines for good pharmacy practice at its council meeting in Tokyo on 5 September, 1993. These standards can be followed by different pharmaceutical organizations and governments. Licenses are issued to manufacturing companies which are required to adopt good manufacturing practices (GMP) and good laboratory practices (GLP). They must have in-process quality control of medicines of their own.

There is no fixed rule concerning dilution, succussion and trituration. In fact, Hahnemann himself recommended different dilutions and number of strokes to prepare a potency (Royal, 1991). Vortexing and sonication in lieu of manual strokes also produce effective potencies (Sukul, 1997). We have recently observed that successive dilution alone without any mechanical agitation produces an effective potency (Sukul *et al.,* 2001).

Summary

Drugs used in Homeopathy have their sources from plants, animals, minerals, diseased parts of man and irradiated aqueous ethanol. The ethanol extract of a drug is mixed with aqueous ethanol, usually 90% in the proportion of 1:99 or 1:9 and the mixture is shaken by 10 powerful downward strokes to prepare the first centesimal or decimal potency. Subsequent potencies are prepared by further dilution of the 1st

potency with aqueous ethanol and succussion of the mixture. Korsakovian method uses a single vial which is emptied and refilled with aqueous ethanol during the process of potentization of a drug. The residual amount left in the vial is used for the next dilution. In the LM potencies introduced by Hahnemann, the dilution is 1:50000. In the millesimal series, denoted with the suffix m, the dilution is 1:1000.

Drugs insoluble in aqueous ethanol or water are at first attenuated with lactose by trituration upto the 3rd potency, and then diluted with aqueous ethanol for further potentization. Sucrose globules are soaked with liquid potentized drugs and are stored in closed vials. Vortexing and sonication of a dilution in lieu of manual strokes also produce effective potencies. Successive dilution alone without any mechanical agitation makes an effective potency. Homeopathic medicines are produced according to the methods laid down in homeopathic pharmacopoeias. Homeopathic drug manufacturers are required to adopt good laboratory practices (GLP) and good manufacturing practices (GMP) in order to maintain high quality of their products.

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CHAPTER II

EVIDENCES FOR HIGH DILUTION EFFECTS

Homeopathic practitioners have long observed the therapeutic effect of potentized drugs. Most of these observations were not properly recorded. Some well-documented records are, however, available. Most conclusive evidences come from the results of experiments conducted on both animals and plants. Some evidences, both clinical as well as experimental, have been reported in an earlier book (Sukul, 1997).

2.1. Clinical evidences.

There is usually no fixed medicine for a particular disease in Homeopathy. Medicines are selected on the basis of symptoms of individual patients. This comes in the way of collecting clinical evidences in support of Homeopathy. Homeopathy would simply show that diseases, whatever their name is, are cured or ameliorated by potentized medicines. If we look for evidences from this angle we would certainly have plenty of clinical evidences in favour Homeopathy.

While describing the Materia Medica of some selected medicines, Nash (1913) presented many interesting cases of successful treatment of patients with potentized medicines from his own experience. He also mentioned the exact potencies used, the number of times they were administrated, and in some cases the pathological aspect of his patients. Ghatak (1927), a renowned homeopath of yester years, nicely presented more than 17 cases of treatment with Homeopathy. In every case the change of symptoms in patients in response to treatment with appropriate remedies was recorded chronologically. Some selected examples are given below.

Case (i) : Tumour on the cervix of uterus of a 34 year old patient having 3 children. Symptoms : piles sore, pricking pain, bleeding and oozing of fluid, aggravation in the rainy season and amelioration by heat, fever every evening, no taste in mouth, constant pain in lower abdomen ameliorated by warmth, wants to commit suicide during excessive abdominal pain, wants open air, pain increases at night, pus in ear with foul smell, vertigo frequent, more on tilting head on the right side. It is not known whether the tumour is benign or malignant.

Treatment : *Aurum met* 1000, *Thuja* 1000, *Aurum met* 10,000, *Aurum met* CM, *Thuja* 1000. Treatment continued over a period of about 9 months and the patient was relieved of all her symptoms.

***Case (ii)* :** 21-year-old male, mild remittant fever in the evening, violent sneezing, coryza in the morning and evening, feels cold, wants to cover the whole body, does not like to take bath, feels something inside throat, feels burning sensation all over body sometimes during fever but does not like to remove cover, no thirst, dry mouth,

history of malarial fever 5 years back. The case was suspected to be kala-azar or leishmaniasis but never diagnosed by pathological tests.

Treatment : *Sabadilla* 200, 1000 and *Arsenicum iodatum* 200. Treatment continued for about 3 months with total alleviation of all the symptoms.

Case (iii) : 37-year-old male, fistula close to anus, operated once, but watery yellowish pus coming out from a small hole surrounded by hard elevated area; sore. It started from a boil 10 years back; following that many boils appeared on the back; pain in fistula more after stool, liquid or solid; very irritable in nature; perspiration with foul smell in palm and feet; frequent urination; cannot tolerate cold but also feels uncomfortable in hot season.

Treatment : *Acid Nitric* 200, 1000, *Hepar sulphur* 200, *Acid Nitric* 10M, 50 M; burning of palm and feet reported at this time; *Sulphur* 1000, *Acid Nitric* CM. Treatment continued over a period of about 14 months and the patient was cured.

Case (iv) : 36-year-old male, bronchial asthma, chronic, suffering for 12 years, sits in stooping posture with sweating on forehead during spasm, wants fanning, expectoration of thick mucus in morning, burning of palm and feet, better by cold application, irritable, history of pustular eruption on hands and knees due to scabbies, history of piles.

Treatment : *Carbo veg* 200, *Sulphur* 30, 200, *Carbo* veg 500, *Natrum sulph* 1000, *Acid Nitric* 1000. Treatment continued for 7 months and the patient was relieved of his symptoms.

All the 4 cases were not properly diagnosed and treated between 1915 and 1922. All these patients received allopathic treatment before they came for homeopathy. Usually, it takes more than a year to cure chronic diseases with homeopathic medicines. However, the medicines give rapid results when acute diseases are treated. In course of my (NCS) practice of homeopathic medicine for the last 35 years I observed very prompt action when I treated such acute cases as headache, diarrhoea, influenza, severe colic, vomiting, any type of pain anywhere in the body, status epilepticus, common cold, fever, haemoptysis, ulcer due to injury or burning, stomatitis, bleeding from piles, haematuria due to urethritis or cystitis, hiccough, vertigo, measles etc. In all these cases I noticed rapid alleviation of symptoms by potentized drugs within a short time from a few minutes to 7 or 8 days.

Dr. B.N. Chakravarty, Hony. Physician to the President of India and the Governor of West Bengal, has kindly provided, on request, some interesting case records for inclusion in this book.

Case (i) : Japanese viral encephalitis of a 28-year-old woman. Symptoms: unconscious for 19 days, left-sided hemiplegia, constant convulsive motion on the right side of the body, loss of all superficial and deep reflexes. Prior to the onset of this disease, the patient was reported to have almost regular dreams of being murdered by a group of people. Then she developed headache, fever and unconscious state.

Treatment: *Lachesis* 200, *Lachesis* 1M through inhalation. The patient recovered in 3 weeks (1987).

Case (ii) : A 25-year-old female patient with idiopathic spontaneous hypoglycemia. Symptoms : recurrent hypoglycemic shock, blood sugar 18-20 mg/100ml during the episode, hot patient, excessive craving for sweets, short necked, fanciful dreams. Earlier she had recurrent tonsilitis and skin disease treated by antibiotics.

Treatment : *Sulphur* 30 in 2 doses. Improvement in a week (1983).

Case (iii) : A 14-year-old girl suffering from hepatitis B infection. Symptoms : Coma, lying like a log of wood.

Treatment : *Zincum met* 200 by inhalation every 3 hrs. Recovering in one month (1990).

Case (iv) : A 15-year-old girl had cerebral haemorrhage due to head injury. Symptoms: Comatose, superficial and deep reflexes lost, aphasia, lying like a log of wood, very little fidgety feet.

Treatment : *Zincum met* 200 given in repeated doses by inhalation. The patient recovered in one year (1994).

Case (v) : A 35-year-old female had primary infertility with secondary amenorrhea, ceasation of ovarian function and uterine fibroid tumour on the posterior wall at the junction of upper one-third and lower two-third of the uterus. Symptoms: Amenorrhea for the last two years, obase, sweaty palms and soles, craving for eggs, irregular menstruation earlier.

Treatment : *Calcarea carb* 200 repeated 5 times followed by *Thuja oc* 200 repeated 3 times. The patient improved in two years (1994).

Case (vi) : A 75-year-old man suffering from stokes Adams Syndrome (complete heart block). Symptoms : Irregular heart beat with omission every 3/4 beats, sunken eyes, unconsciousness with intermittent convulsion.

Treatment : *Digitalis* 30 repeated every 2 min 5 times. The condition improved in one hour (1994).

All the six cases very clearly indicate that homeopathic potencies can cure or ameliorate very serious cases in a relatively short time provided the selection of the remedy is right. One can note here very precise selection of a single remedy for an ailment.

Two cases of another successful physician (Dr. Rathin Chakravarty) is given below.

Case (i) : A 35-year-old female suffering from bilateral fibroadenoma. Symptoms: Thirstless, highly emotional and of soft temperament, hot patient.

Treatment: *Pulsatilla* 200 given at 4 doses twice daily. The drug was repeated for 2 consecutive months. Improvement occurred in 3 months (2000).

Case (ii) : A 25-year-old female suffering from uterine fibroid tumour. Symptoms: Pain in abdomen, dismenorrhea, menorrhagia, fastidious, likes milk and traveling.

Treatment : *Tuberculinum bovinum* 200 given at 4 doses. Improvement occurred in 2 months (2002).

These two cases show that right remedies can effectively tackle uterine tumours in a rather short time.

Clinical investigations involving a large number of patients treated with a homeopathic medicine were conducted and the results were reported to be positive (Reilly *et al.,* 1986; Ferley *et al.,* 1989; Fisher *et al.,* 1989). Kleijnen *et al.,* (1991) reviewed 96 published reports of 107 controlled clinical trials using homeopathic and related drugs. Methodologies adopted in some of the trials were not good enough to make a definite conclusion. However, the authors believe that the results showed a positive trend. Recent studies of metaanalysis of clinical trials with homeopathic drugs show a difference between drugs and placebo controls (Linde *et al.*, 1997; Cucherat *et al*., 2000).

Many important references concerning research in Homeopathy have been compiled by Fisher (1991). Homeopathic Research Reports published by Foundation for Homeopathic Education & Research, California USA (1989, 1990) contains very brief summary of important works on Homeopathy.

An audit was conducted of 829 consecutive patients suffering from a chronic illness like eczema, rheumatoid arthritis, unstable angina, ulcerative colitis, manic depressive psychosis, biliary cirrhosis, asthma etc. Of these, 61% showed sustained improvement with homeopathic treatment (Sevar, 2000). Conventional treatment had either failed, plateaued in effect or was contraindicated by adverse effects in those cases. In another study involving 30 doctors of conventional medicine homeopathy was compared with conventional medicine with respect to effectiveness in a primary outcomes criterion of cure or major improvement. Four hundred fiftysix patients were observed for (1) upper respiratory tract complaints including allergies, (2) lower respiratory tract complaints including allergies, or (3) ear complaints. Patients showing positive response constituted 82.6% for homeopathy and 68% for conventional medicine (Riley *et al.,* 2001). Efficacy of homeopathic drugs may not be evident on a mass scale due to miasmatic blockages in some individuals.

In clinical investigation proper assessment of the effectiveness of homeopathic potencies should be made. For this, adequate placebo controls should be maintained, and modern diagnostic procedures such as blood chemistry, X-ray, ultrasonography, CT scan, MRI, ECG, EEG etc. should be adopted. Results with clinical investigation may show marked individual variation simply because of miasms. We would discuss about miasms in Chapter IV.

2.2. Experimental evidences from animals

Animals have been used as models for the demonstration of action of potentized homeopathic medicines. If homeopathic medicines act on human patients they must act on animals. In allopathic medicine, animals particularly rats, mice, hamsters,

guineapigs, rabbits and monkeys have long been used for trial and development of new drugs. Many laboratory animals have long been known to serve as good animal models for such parasitic diseases as leishmaniasis, malaria, trypanosomiasis, filariasis, echinococcosis etc. Good animal models are available for other diseases like cancer, hyperglycemia, blood pressure, epilepsy etc. Animal models have helped in understanding the development of a disease at the tissue and molecular level. They also help in understanding the mechanism of absorption, distribution, biochemical transformation and excretion of drugs. Dosages and side reactions of drugs are determined through animal models.

Many traditional and 'folk' remedies used for human and animal illness over centuries have been tested on animals in recent times to verify their efficacy and find out their side reactions, if any. Active principles in effective plant and animal products have been identified through experimentation on animals. Many allopathic drugs in current use have been discovered by massive empirical screening on animal models. Once a compound passes animal tests, it is put to human trial. After successful human trial the drug comes to the market for general use. In homeopathy the system is just the reverse. The medicines have been used for human illness for about a couple of centuries. They have been initially proved on man and then introduced to Homeopathy. They are now put to trial on animals just to prove their efficacy and understand their mode of action. Since potentized drugs are highly diluted and used in minute doses, no restriction on human consumption of homeopathic drugs has ever been imposed.

2.2.1. Biological effects on mammals and birds

Homeopathic medicines have long been used for the treatment of diseases in domestic animals. Thus *Caulophyllum*, when applied on a herd of pigs, prevented still birth in the animals (Day, 1984). Microdoses of *Arsenic* facilitated excretion of this metal through urine and stool of rats (Cazin *et al.,* 1987). *Zincum* 12x effected histamine release from stimulated mast cells of rats (Harisch and Kretschmer, 1988). *Silicea* 6c and 10c stimulated release of platelet-activating factor from peritoneal macrophages in mice (Davenas *et al.,* 1987). Low doses of interferon showed immunomodulatory activity of B-cell and T-cell system in mice (Daurat *et al.,* 1988). High dilutions of endogenous substances such as thymulin, a hormone from thymus, and bursin from the bursa of Fabricius of chickens, produced immunomodulation in mice and bursectomised chickens, respectively (Bastide and Boudard, 1998). High dilutions of *Silicea* accelerated healing of perforations of mouse ear (Oberbaum *et al.,* 1998). High dilutions of caffeine and adenine prevented teratogenic effect induced by those drugs on mice (Taddei-Ferretti and Cotugno, 1998). Burlakova (1998) observed high dilution effects of several biologically active substances at organismic, cellular and macromolecular level of both plants and animals. She concluded that the effect could not be linked to any definite substance structure or biological organization level.

a) Catalepsy model

We developed a non-sacrifice animal model by which we could demonstrate the biological effects of potentized drugs such as *Agaricus muscarius, Graphites, Cannabis indica* and *Gelsemium* in 30th and 200th potencies (Sukul *et al.,* 1986). These drugs enhanced restraint-induced catalepsy in rats. Catalepsy is a transitory state of immobility in which the animals fail to correct the imposed posture. It can be induced by restraint on movement or by drugs. It is a central nervous system phenomenon involving serveral neurotransmitter systems (Sanberg, 1980, Hartgraves and Kelly, 1984; Klemm 1983, 1985; Sukul *et al.,* 1988, Zarrindast and Habibi-Moini 1991). A number of homeopathic drugs has been listed in Kent's *Repertory of Homeopathic Materia Medica* under the rubric 'catalepsy' (Kent 1911). We improved the technique and observed that potentized *Agaricus* could significantly influence catalepsy in albino mice induced by neurotransmitter drugs. Thus *Agaricus* 30 suppressed mouse catalepsy induced by dopamine (DA) receptor blocker haloperidol (5 mg/kg i.p.). However, *Agaricus* promoted catalepsy when given with DA agonist apomorphine (5 mg/kg) and D_2 agoinst bromocriptine (5 mg/kg). While apomorphine does not produce catalepsy by itself, bromocriptine produces a very mild catalepsy (Sukul and Klemm, 1988). This is a good model, but care should be taken that animals are not stressed before starting an experiment. The results show that homeopathic potencies can interact with neurotransmitter drugs.

Oral route effective

We further observed that *Agaricus* 30 could reverse haloperidol catalepsy only when it was administered through the oral route (Sukul, 1995). *Agaricus 30* also suppressed mouse catalepsy induced by adrenergic agonists such as phenylephrinie, an alpha 1 agonist (2 mg/kg i.p.) and clonidine, an alpha 2 agonist (1 mg/kg i.p.). Here also *Agaricus* was effective only through the oral route and not through the intraperitoneal route (Ghosh *et al.,* 1997). The results suggest that receptors in oral mucosa play a role in mediating the effect of homeopathic potencies.

Dose response

If *Agaricus* 30 is diluted with 90% ethanol without any mechanical agitation it could still be effective in reducing haloperidol catalepsy upto the dilution 1:20000. Higher dilutions like 1:40000 and 1:50000 did not produce any anticataleptic effect. However, the dilution 1:50000 could be made effective if it was subjected to agitations, in this case, by sonication. The anticataleptic effect was observed when *Agaricus* was administered before and immediately after haloperidol (Sukul *et al.,* 1996). This shows that there is a limit to dilution of a homeopathic potency and that mechanical agitation plays a role in maintaining the efficacy of a diluted drug. In a recent study we have observed that mere successive dilution without mechanical agitation could produce an effective homeopathic potency. But here the dilution is

maintained at 1:100 in each successive steps. So, higher dilution requires mechanical agitation.

b) Righting reflex model

Besides the catalepsy model we also developed other animal models using the effect of ethanol. We know that drunk persons move to and fro due to loss of righting reflex. Righting reflexes maintain the normal erect posture of an animal through a series of responses which are integrated mostly in the mid brain (Ganong, 1989). Ethanol can induce loss of righting reflex in mice which can be measured in terms of sleep time (Draski *et al.,* 1992, Reeve *et al.,* 1992). Because of the loss of righting reflex due to alcohol the animals cannot move but lie motionless as if they are sleeping. *Nux vomica* is known to be effective against the effects of alcoholism (Kent, 1911; Boericke, 1927). We (Sukul *et al.,* 1999) observed that *Nux* 30, given through the oral route, reduced significantly ethanol-induced sleep time in albino mice. Mice were given 25% ethanol i.p. at 4 g/kg body weight six hours after treatment with *Nux* 30. This simple model can be easily tested in any laboratory.

Aqueous ethanol, the effective medium

We prepared *Nux vom* 30 in our laboratory from the ethanolic extract of the ground seeds of *Strychnos nuxvomica* L. But here instead of succussion by hand we sonicated each dilution at 20 kHz for 30 sec. We used 90% ethanol for successive dilution of *Nux* θ. Besides 90% ethanol, we prepared *Nux* 30 using other diluent media such as pure ethanol and pure water. We observed that neither pure ethanol nor pure water made an effective potency of *Nux* showing antihypnotic effect in albino mice (Sukul *et al.,* 1999a). This experiment shows that aqueous ethanol is the suitable medium for the preparation of an effective homeopathic potency. The potentized medicines were stored in the laboratory for about 6 months before testing on animals. Pure water preparations might have lost their efficacy during storage. This experiment needs to be repeated with other models. The results are important and linked to the physical basis of a potency.

2.2.2. Biological effects on amphibians

The common laboratory animal toad has long been used for biological and pharmacological experiments. We conducted experiments on toads to develop suitable animal models for demonstrating the biological effects of homeopathic potencies.

a) Young toads

In another experiment young toads, *Bufo melanostictus* weighing 0.03 - 0.22 g, were tested for ethanol-induced loss of righting reflex with *Nux* 30. Here *Nux* 30 was prepared with 90% ethanol, pure ethanol, pure water and lactose. The lactose

preparation was done by trituration. Controls for the liquid potencies were prepared in the same way in 30 successive dilution and sonication without using the mother tincture of *Nux* at the initial level. Control for the solid potency was prepared by trituration of lactose without *Nux* θ at any stage. The drug or control was diluted with distilled water and the toadlets were exposed to the mixture by immersion for 5 min. The treatment was repeated twice everyday for 3 consecutive days. On the 4th day they were exposed to 209 mM ethanol solution by partial immersion. Every 10 min the toadlets were transiently removed from the ethanol solution and placed in a supine position on a dry flat surface. Failure to right within 60 sec was considered loss of righting reflex. Toadlets treated with *Nux* 30 prepared with 90% ethanol took significantly longer time to lose righting reflex than those treated with the control solution (Figure 1). *Nux* 30 prepared with other media showed no difference from the control with respect to the loss of righting reflex in toadlets. This shows once again that aqueous ethanol is the suitable medium for the preparation of a homeopathic potency (Sukul *et al.,* 1997). The potentized medicines were stored for some months before use.

b) Adult toads

We conducted another experiment using adult toads, *Bufo melanostictus*. Here *Nux* 200 and 1000 were administered orally on freshly collected adult toads (30 in each group). Six hours later they were injected i.p with 25% ethanol at a dose of 8g/kg body weight. The duration of sleep time was measured as in the case of mice. Toads treated with either of the two potencies of *Nux* regained righting reflex more quickly than those of the control group. This shows that adult toads can serve as good animal models for demonstrating the biological effect of a homeopathic potency (Sukul *et al.,* 2000 bc). Thus we see that potentized *Nux vomica* produces antialcoholic effect both in amphibians as well as in mammals.

2.2.3. Effects on chronic alcoholism

Chronic alcoholism is a major health problem all over the world and Homeopathy has some effective remedies to tackle the problem. We tested one of these remedies, *Nux vomica* on rats for demonstrating its antialcoholic effects.

a) Ethanol intake

Rats of the Charles Foster strain were allowed to swim in a 20 cm deep plastic tub. They swam actively, paused for sometime and continued swimming. The period of immobilization with the head seldom protruded over the surface of water was recorded for each rat. The duration of immobilization is directly proportional to the depressive like state. The highly active rats are characterized by a period shorter than 130 sec and the lowly active ones by the period longer than 300 sec. It is the lowly active rats that are potential alcoholics (Paul *et al.,* 1992). These rats were selected for tests involving ethanol consumption. In a random population of rats the

consumption of ethanol would vary widely. So the potential alcoholics among the rat population would be suitable for experiments on alcohol consumption. The selected rats were given 20% ethanol for 10 days and then both 20% ethanol and water in a two-choice bottle test. The rats were then treated orally with *Nux vom* 30 and *Nux vom* 1000. While *Nux vom* 1000 was prepared by usual succussion, *Nux vom* 30 was prepared by sonication. One batch was given *Nux vom* 30 through the intraperitoneal route. One batch served as the untreated control. While *Nux* 1000 was given one dose everyday, *Nux* 30 was given one dose every 15 days. Treatment and daily assessment of ethanol and water consumption were continued for one month. Both *Nux* 30 and *Nux* 1000 reduced ethanol consumption in rats significantly. However, *Nux* 30 given through the intraperitoneal route did not show any difference in ethanol consumption from the control. *Nux* 30 (oral) was more efefctive than *Nux* 1000 (Figure 2). This shows that sonication makes a more powerful potency than manual succussion. This experiment once again shows that oral route is the effective route for a homeopathic potency. It is thought that oral receptors mediate the action of a homeopathic potency (Sukul *et al.,* 1998). In another experiment we observed that both *Nux vom* 30 and *Nux vom* mother tincture reduced alcohol consumption in rats significantly (Sukul *et al.,* 2000a).

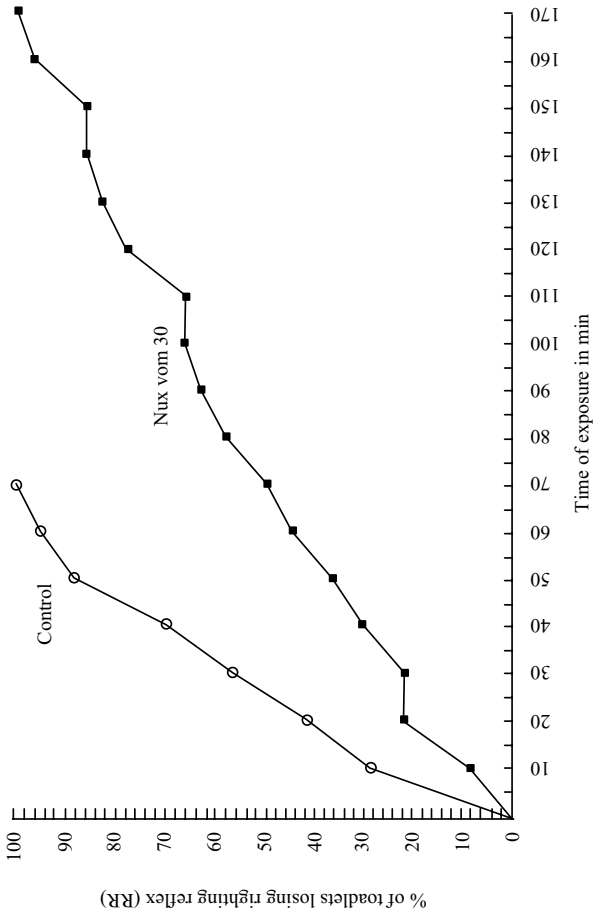

Figure 1. *Loss of RR during anaesthesia with 209 mM ethanol was significantly delayed from 10 min onwards ($p<0.005$, by x^2 test) with young toads treated with Nux vomica 30 CH prepared with 90% ethanol by sonication as compared to the control. Control (o) treated (■), n=60 in both the test and the control. (Reproduced, with permission, from Sukul et al. : Hydrated ethanol, the effective medium for a homeopathic potency as tested by a new toad model. Indian J Landscape Syst Ecol Stud 1997; 20:155)*

b) Ethanol-induced neuropathy

Chronic alcoholism may lead to autonomic disfunction in alcoholics with peripheral neuropathy. Degenerative changes in the vagus and sympathetic nerves were reported in patients with alcoholic neuropathy. Prolonged consumption of white rum (40% ethanol) produced severe degenerative lesions in the adrenergic nerve plexuses

of rat atrio-ventricular valves (Ferreira *et al.,* 1975). We have made an experiment to see whether *Nux vom* 30, prepared by sonication, could reverse the peripheral neuropathy in alcoholic rats. In this experiment rats were given normal diet and 20% ethanol as the only drink for 2 months. Then they were treated with *Nux* 30, one dose every 15th day for 4 months. During treatment the rats were kept on a two-choice bottle, one containing 20% ethanol and another tap water. The daily consumption of ethanol and water was recorded for each rat for the entire period of 6 months. Besides the treated and untreated batches of alcoholic rats, there was one batch without ethanol. All the rats were sacrificed after 6 months and their atrio-ventricular valves were examined for any degenerative changes in the adrenergic nerve plexuses. The latter showed significant degeneration in alcoholic untreated rats. Treatment with *Nux* 30 not only reduced ethanol consumption in rats but also brought about considerable regeneration of most of the nerve terminals. This experiment shows that a homoepathic potency can produce visible anatomical changes in treated animals (Sukul *et al.,* 1998).

2.2.4. Effects on parasitic diseases

An early report shows that cholera epidemics in Europe were effectively tackled by homeopathic medicines (Leary, 1994). The work refers to the epidemic in 1854. The results may not be reliable because modern diagnostic techniques were not available at that time and adequate placebo controls were not kept. *Abrotanum* D1 was tested on 27 dogs and 26 cats infected with intestinal nematodes in a Veterinary clinic of Germany and the results were positive (Krause, 1993).

Besides diseases due to metabolic and immunological diorders or injuries, and toxic substances, diseases caused by parasites or pathogens are also effectively treated by homeopathic medicines. Some homeopathic drugs showed antiviral effect against chicken embryo virus (Singh and Gupta, 1985). Trichinosis or trichinellosis is a disease caused by the nematode parasite, *Trichinella spiralis* in man. Adult worms live in intestinal mucosa, but the juveniles migrate to muscles and get encysted there. The worm also infects rats, mice, bears, pigs etc. Human infection results mostly from consumption of poorly cooked pork containing encysted juveniles. There is no effective remedy for the elimination of *T. spiralis* from hosts. Thaiabendazol at 3g/day for 5 days may alleviate some symptoms but may not eliminate encysted juveniles in muscles (Webster, 1991). We conducted an experiment on albino mice inoculated with *T. spiralis.* Hydrocortisone was given to obtain a more or less uniform infection in mice. Thirty days after inoculation, mice were treated first with *Cina* 1M for 3 days followed by *Calcarea fluor* CM in one batch. There was one more batch in which the second medicine was *Thuja* CM. Since trichinosis involves cysts in muscles *Calcarea fluor* and *Thuja* were used. *Cina* was used initially because the exciting cause for the cyst was a round worm. Larval densities in the muscle were determined on days 10, 17 and 24 following the last date of treatment after sacrificing 6 mice from each group. The untreated control showed 14.79% rise in larval densities relative to the pre-treatment level. Treatments significantly reduced juvenile densities (40.97%, 62.62%) in muscles of the mice as

compared to the control. The partial elimination of juveniles from muscles has been attributed to immunologically nonspecific and specific reactions of

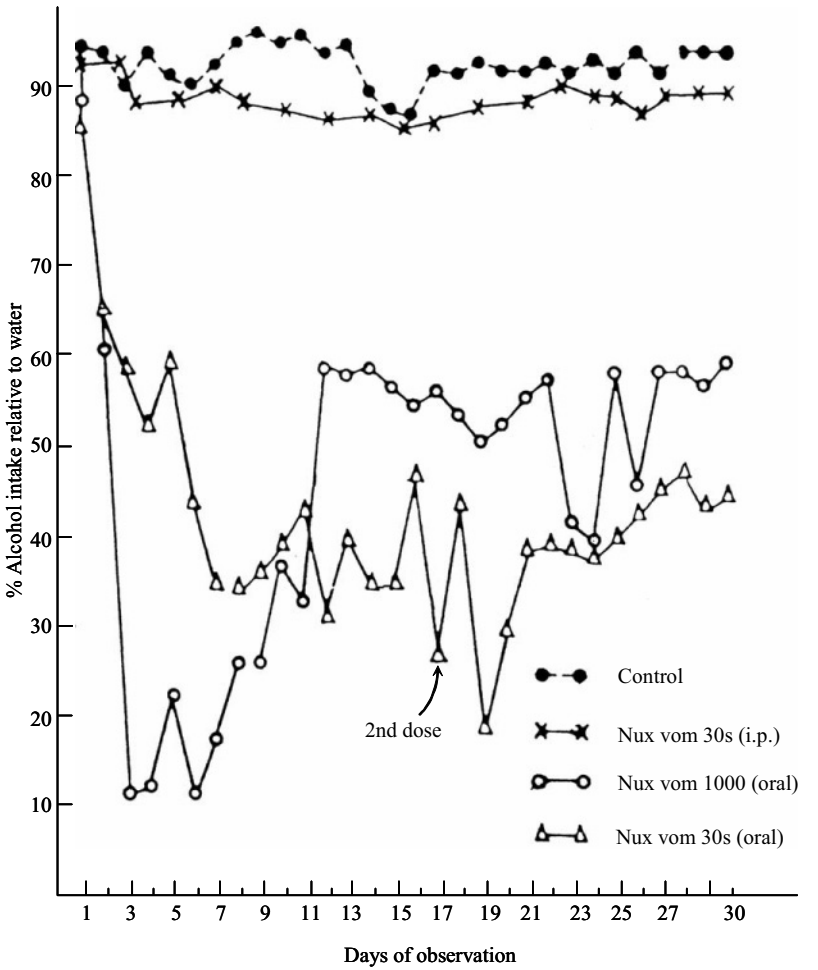

Figure 2. *Effect of potentized Nux Vomica on alcohol intake in rats. Nux vom 30 prepared by sonication(s) and given by oral route once every 15 days reduced alcohol consumption relative to water in albino rats as compared to the control. Nux vom 1000 prepared by succussion and given one dose daily also reduced alcohol consumption in rats as compared to the control. Nux vom 30s/given intra-peritoneally (i.p.) did not show any significant variation in alcohol consumption from the control. (Reproduced, with permission, from Sukul et al. : High dilution effects of Strychnos nuxvomica L on hypothalamic neurons and adrenergic nerve endings of alcoholic rats. In : High dilution effects on cells and integrated systems : C Taddei Ferretti, P Marotta [editors]. Copyright © 1998 by World Scientific Publishing Co Pte Ltd)*

the mouse initiated by the potentized drugs. The action of the drugs is thought to be mediated directly through the nervous system which in turn triggers the necessary inflammatory response to the parasitic invasion. Since the drugs were administered through the oral route, they might have acted through oral afferents and the impulses reached the cortex through nucleus tractus solitarius and thalamus. There in the cortex the message was processed and the autonomic outflow might have influenced the immune system in such a way that the juveniles of *T. spiralis* were partially destroyed through antigen dependent cell mediated cytotoxicity (ADCC). There is evidence that neural mechanisms influence immune responses and inflammation (Barnes, 1994, Roitt *et al.,* 1993, Libert, 2003; Wang *et al.,* 2003). Release of histamine from mast cells is brought about by neurotransmitters. Uninfected mice have mast cells and basophils which bear receptor-bound IgE. The binding of specific antigen to the receptor-bound IgE activates the cell. Antigen-IgE stimulation of mast cells is considered to play a role in the elimination of helminth parasites (Foreman, 1994). This may be the possible way by which potentized drugs reduce *T. spiralis* infection of mice at the muscle phase (Sukul, 1997). The work has been reproduced recently with similar results.

Dirofilaria immitis is a nematode parasite living in the aorta of dogs. Infected dogs carry microfilaria in their blood. Since *Cina* is used for worm infection we selected this remedy for treatment of canine dirofilariasis. An examination of blood of an infected dog shows large number of microfilaria. *Cina* is prepared from the extract of the flowering top of the plant *Artemisia nilagirica.* We used the mother tincture of *Cina* and its two potencies like *Cina* 200 and *Cina* 1000. Treatment of dogs (4 in each batch) continued for 30 days. The daily dose for each potency was 0.1 ml/dog. The mother tincture was allowed to evaporate and the residue administered orally at 10 mg/kg/day for the first 15 days and 20 mg/kg/day for the next 15 days. *Cina* θ, *Cina* 200 and *Cina* 1000 reduced microfilarial density in treated dogs by 78.38, 63.06 and 71.40%, respectively on the last day of treatment (Sukul *et al.,* 1999b). Filarial worms are known to cause immunosuppression (Ottesen 1980). It is possible that potentized *Cina* might have removed immunosuppression resulting in vigorous responsiveness of the host to parasite antigens thereby clearing microfilaria from the blood (Sukul *et al.,* 1999). In another experiment on microfilaraemic dogs we used *Cina* 30 prepared by succesive dilution and sonication at 20 kHz for 30 sec in each step. Treatment continued for 30 days. Treated dogs showed a marked reduction in microfilarial count to a maximum of 93% (Figure 3) (Sukul *et al.,* 2000).

2.2.5. Electropysiological Studies

In the foregoing paragraphs we have seen that biological effects of homeopathic drugs manifest themselves after a time interval which may vary from a few hours to several days. According to Sukul's (NCS) hypothesis homeopathic potencies act through the nervous system in animals. The information of drug molecules is conveyed to the brain by the oral afferents. The effect occurs through the autonomic

outflow (Sukul, 1997). Since autonomic functions are centrally controlled in the hypothalamus we can expect a change in the electrical activity of hypothalamic neurons immediately after a homeopathic drug is put on oral mucosa. An experiment was designed just to verify this idea.

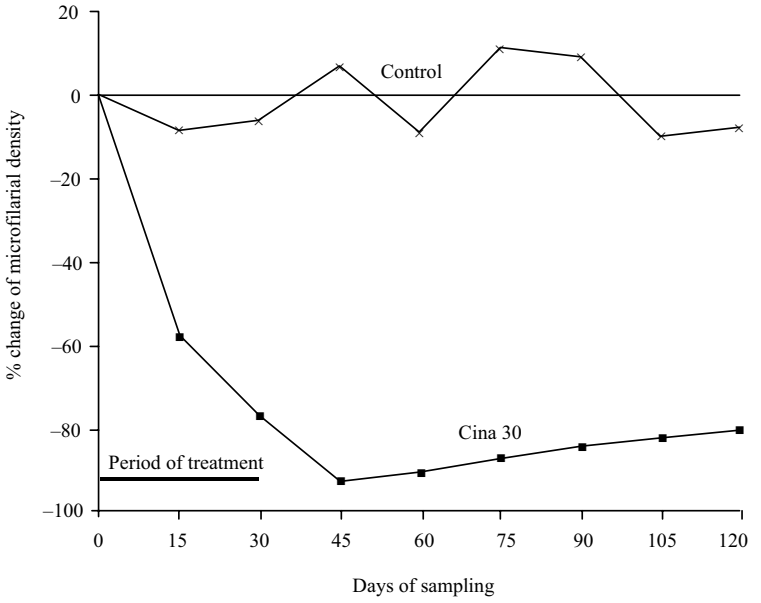

Figure 3. *Change in mean percentage of microfilarial density in untreated (X) and treated dogs (■) over a period of 120 days. Dogs (4) naturally infected with Dirofilaria immitis were treated with Cina 30, one dose every day for 30 days. Four dogs served as controls. Mf density on day 0 served as standard. The horizontal bar at the base indicates the period of treatment. Blood was sampled from dogs every 15 days. (Reproduced, with permission, from Sukul et al. : Antifilarial effect of Artemisia nilagirica at an ultra high dilution on canine dirofilarialis. In : Waste Recycling and Resource Management in the Developing World : BB Jana, RD Banerjee, B Guterstam, J Heeb [editors]. Copyright © 2000 by the University of Kalyani, India and International Engineering Society, Switzerland)*

a) Lateral hypothalamic neurons of cats

In this experiment cats were anaesthetized with urethane and fixed on a stereotaxic table. Single unit discharges were recorded from 9 cats of either sex with a tungsten microelectrode (tip 1.5 μm in diam, resistance 2-3 MΩ) from the lateral hypothalamic neurons (LHA). After each experiment the animal was perfused with saline and 10% formalin through the heart, and the recording site was examined histologically. After recording the background activities of a neuron for 10 min, the

control solution was applied and the activity was recorded for 10 min. After the application of a drug into the mouth, the activity was recorded for 15-20 min. The drug was repeated once and the recording was made for the same period. We applied three drugs, namely *Arnica montana* 30, *Hypericum* 200 and *Arsenicum album* 30. *Arnica* is normally used for an injury, *Hypercium* for a punctured wound affecting nerves and *Arsenic* for some toxic effects of drugs. The test animals suffered injuries and punctured wounds when their skull was exposed and electrodes driven into their brain. They received anaesthesia which had some toxicity. Activity was recorded from 7 LHA neurons of the right side and 3 LHA ones of the left using *Arnica* 30. The drug produced a significant reduction in firing rate in case of 5 neurons. The effect lasted from 1.5 to 7 min with a latency period varying from 30s to 60 min. In one neuron the firing rate increased within 30 sec and the effect lasted for 3 min. The 3 neurons of the left side and one from the right did not show any change in the firing rate after *Arnica* 30. *Hypericum* 200 produced a decline in firing rate and the effect lasted from 3 to 14 min with a latency period varying from 10 sec to 6 min. In one neuron a transient increase in firing rate preceded the decline. One neuron did not show any response to *Hypericum* 200. *Arsenic* 30 produced an instantaneous (latency 1 sec to 1 min) increase in firing rate in case of 5 neurons, but the effect was transitory (50 sec to 1 min) (Figure 4). In one neuron the drug produced decline in firing rate after the second application and the effect lasted for 30 sec with a latency of 1 min. One neuron did not respond to *Arsenic* 30 (Sukul *et al.,* 1991).

b) Medial frontal cortex neurons of an awake rat

A hole was trefined on the skull of a male albino rat in the area of medial frontal cortex. A glass-coated silver microelectrode (tip 3.5 μm in diam, resistance 1.5 - 2.5 MΩ), mounted on a micromanipulator, was inserted into the cortical area and sealed on the surface of the skull by a dental acrylic in such a way that the micromanipulator remained operable. Recording was started 4 days after operation when the rat behaved normally, and continued for several days. After the last session the rat brain was examined histologically for the recording site. The effect of anaesthesia could be avoided in the study on an awake rat. The rat readily took the control solution or the drug. Here *Arnica* 30 produced a decrease in firing rate in neurons and the effect lasted for more than 20 min with a latency period of 1.5 to 6 min. In one neuron the response was discernible only after the second application of the drug. *Arnica* 30 produced an increase in firing rate in one neuron within 1.5 min and the effect lasted for 2 min.

Hypericum 200 produced a decrease in firing rate in case of 5 neurons and the effect lasted from 1.5 - 14 min with a latency of 1 sec to 2 min. In two neurons there was a transient potentiation followed by inhibition. In one neuron there was an increase in firing rate within 2 min and the effect lasted for 11 min. *Arsenic* 30 produced a marked increase in firing rate within 1 sec and the effect lasted for 20 sec. In all the cases the control did not show any marked change in the firing rate (Sukul *et al.,* 1991).

In the above experiments we see that the action of a homeopathic potency starts within a few seconds to a few minutes. The same drug may produce both inhibitory

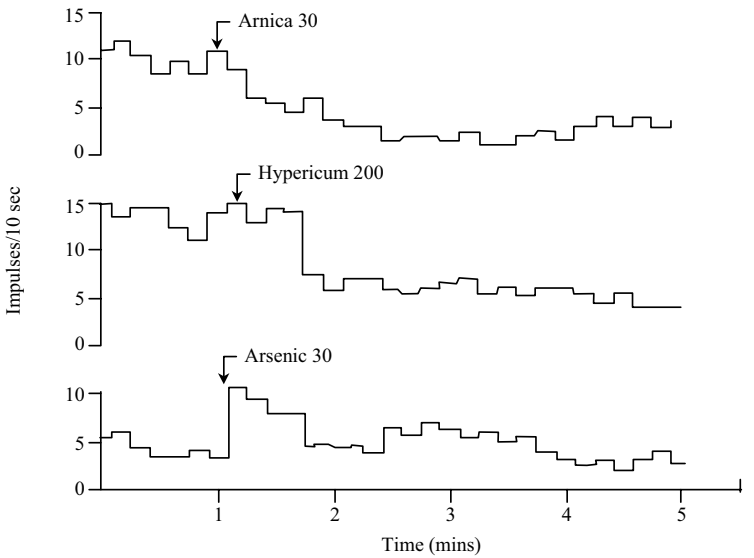

Figure 4. *Inhibitory effect of Arnica 30 and Hypericum 200, and excitatory effect of Arsenic 30 on the lateral hypothalmic area (LHA) neurons of cats. Arrows indicate the time of application of drugs on the tongue of the test animal. (Reproduced, with permission, from Sukul et al. : Neuronal activity in the lateral hypothalmus of the cat and the medial frontal cortex of the rat in response to homeopathic drugs. Indian Biologist 1991; 23 : 19)*

and excitatory effect on different neurons. Some neurons may even be totally non-responsive to a drug. It is evident that drugs are effective through oral receptors. The final therapeutic efefct of a drug depends on the interaction of different neurons in different areas of the brain.

c) Hypothalamic neuronal responses of alcoholic rats to Nux vomica

In another experiment we examined the response of hypothalamic neurons of rats to potentized *Nux vomica.* Rats used in the electrophysiological tests all belong to Charles Foster (CF) strain. A batch of 15 rats was given 20% ethanol by force drinking at 1.5 ml/kg body weight for 7 days. They were given food and water *ad lib.* After 7 days neuronal activity in the lateral hypothalamic area (LHA) of these

rats in response to 20% ethanol, distilled water, *Nux vom* 200 and *Nux vom* 1000 was recorded. Rats were anaesthetized with peraldehyde (1.28 mg/kg, i.m) and fixed on a streotaxic table. A hole was trefined on skull in the LHA according to specific stereotaxic coordinates (Konig and Klippel, 1963). A glass-coated silver electrode (tip 0.5 mm, resistance 0.002 Ω) was inserted into the LHA, sealed with dental acrylic on the skull and was connected through a head-stage pre-amplifier (AK 100, Digitimer, UK), a preamplifier and filter (Digitimer) to an oscilloscope (L&T Gould) and a 2-channel recorder (L&T). While the gross activity in the LHA was recorded in 7 rats, unit activity in the LHA neurons was done in 7 rats. In case of gross activity a spike of fixed amplitude was selected and its frequency per 20 sec was counted. In case of unit activity, impulses per 20 sec were counted. Drugs or control solutions were put on the tongue of the rats and the effect was recorded. Single unit discharges in the LHA were recorded by means of glass-coated silver microelectrode (tip 3.5 μm in diam, resistance 1.5 - 2.5 MΩ). After each experiment, the rat was perfused with saline and 10% formalin through the left ventricle of the heart and the recording site was examined histologically.

Nux vom 200 and *Nux vom* 1000 increased the neuronal activity in the LHA of the alcoholic rats. This increase in neuronal activity was more pronounced with *Nux vom* 1000 than with *Nux vom* 200. Distilled water and 20% ethanol reduced the neuronal activity in the LHA. Application of *Nux vom* θ (mother tincture) also reduced the activity. The effect of the drugs was instantaneous occurring in a few seconds. The effect lasted from a few secs to a few min (Paul *et al.,* 1992, Sukul *et al.,* 1998). In this experiment the rats were conditioned in the sense that they were continuously given ethanol. This may be the reason that the neuronal responses to drugs were more or less uniform. Thus potentized *Nux* was always excitatory and 20% ethanol, water and *Nux* θ were always inhibitory (Figure 5). It might be that neurons of alcoholic rats have assumed a specific state so that they can respond to drugs uniformly. We can assume that neurons in a diseased state undergo specific changes so that they become responsive to an appropriate remedy in a non-random way. The changes are manifest in the symptoms of the disease. Here enhanced susceptibility has been produced in rats. Electrophysiological responses appear to constitute holistic processes. A stimulus applied by homeopathic drugs elicited a magnified response in the form of electrocardiogram (ECG) and electroencephalogram (EEG) from rats and man (Torres, 2003). We also conducted another electrophysiological experiment where rats were conditioned in a different way and appropriate remedies were applied.

d) Hypothalamic neuronal responses of rats on salty diet to Natrum mur

Albino rats of the CF strain were kept on a diet containing 4% salt (sodium chloride) for 7 days. They were anaesthetized and fixed on a stereotaxic table. A glass coated silver microelectrode (tip 3.5 μm in diam, resistance 1.5 - 2.5 MΩ) was inserted into the lateral hypothalamic area and connected through a head-stage, a pre-amplifier and a filter to an oscilloscope. After recording the neuronal activity by a two-channel recorder for 5 min, *Natrum mur* 30 (*Natrum muriaticum* 30) and *Natrum*

mur 200 were applied on the tongue and the activity recorded. *Natrum mur* 30 produced a reduction in the frequency of spikes (- 51.7 ± 12%, $P<0.05$) and the effect lasted for 4.16 - 57.67 min. A second application of *Natrum mur* 30 produced further reduction of the spike frequency in all the 6 cases observed. *Natrum mur* 200 also produced inhibitory effect on the LHA neurons (Sukul *et al.,* 1992). Application of the control solution did not show any marked change in the spike frequency. Distilled water showed an excitatory effect (+160%). In rats on salty diet intensity of thirst and water intake increased with the increase in plasma osmolality which is perceived by osmoreceptors in the hypothalamus. Distilled water which quenches thirst, therefore, showed an excitatory effect on the LHA neurons. So the afferent pathway of that effect of a potentized drug or distilled water here would be as follows : oral receptors through facial, glossopharyngeal and vagus nerves, through Nucleus tractus solitarius, medial lemniscus to thalamus and from thalamus to hypothalamus. Finally, the impulses reach the associative regions of the cortex. The drug *Natrum mur,* which is prepared from salt, has been selected because it counters the ill-effects of overconsumption of salty diet.

In another experiment we gave *Natrum mur* 30, one dose daily for 18 days, to a batch of albino rats. These rats were tested in a similar manner for their sensitivity to *Natrum mur* 30 and distilled water. Since *Phosphorus* is an antidote to *Natrum mur* (Boericke, 1927), *Phosphorus* 200 was applied on the tongue of those rats in the electrophysiological experiment. *Natrum mur* 30 produced 47% reduction of spike frequency of LHA neurons of those rats. *Phosphorous* 200 also had the inhibitory effect (-53%) on the LHA neurons. Distilled water produced the excitatory effect (+83%) on those neurons (Sinhababu *et al.,*1998). Here we see that rats, given *Natrum mur* 30 for 18 days, behaved as if they were on salt diet. It appears that both salt diet and *Natrum mur* 30 caused central and peripheral reorganization of the taste and other oral sensor system of rats so that the latter became responsive to potentized *Natrum mur.* The animals were conditioned both by salt diet and *Natrum mur* 30 to respond uniformly to an appropriate remedy like *Natrum mur.* The electrophysiological experiments were conducted in our laboratory (Visva-Bharati) as well as in the laboratory of the Institute of Higher Nervous Activity, Leningrad University, USSR.

e) Effects on synaptic transmission

The results of the electrophysiological experiments indicate that hypothalamic and cortical neurons play an important part in mediating the therapeutic action of potentized homeopathic drugs. If the electrical activity of hypothalamic neurons undergoes a change due to the application of a homeopathic potency, it is quite probable that synaptic transmission in the LHA would also undergo a change. The latter change would be reflected at the level of neurotransmitters and their metabolites in the hypothalamus. In fact, these changes were detected in another experiment.

We have already noted that potentized *Agaricus muscarius* influences catalepsy in mice. It is known that neurotransmitters such as dopamine and serotonin

play a role in catalepsy. We, therefore, wanted to study whether a potency of *Agaricus muscarius* alters the level of dopamine and serotonin and their metabolites in the mice. This experiment was conducted in the laboratory of Neurosciences and the department of Medical Anatomy, Texas A & M University, USA.

Agaricus 12 was administered orally on 6 mice at the rate of 3.1 ml/kg. The same amount of blank ethanol (90%) was administered on another group of 6 mice to serve as controls. All the mice (30 - 35 g) were sacrificed by decapitation 6 hr after the administration of the drug or the control. The brain was quickly removed, kept over ice and the hypothalamus scooped out by means of a sharp needle, placed in a microcentrifuge tube, weighed, and then frozen at -70^0C. Hypothalamic mass ranged from 7 to 12 mg for the controls and 8.5 to 18 mg for the treated group. The levels of dopamine (DA), serotonin (5 - HT) and their metabolites dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindolacetic acid (5-HIAA) were determined by the HPLC using an electrochemical detector. Just prior to assay

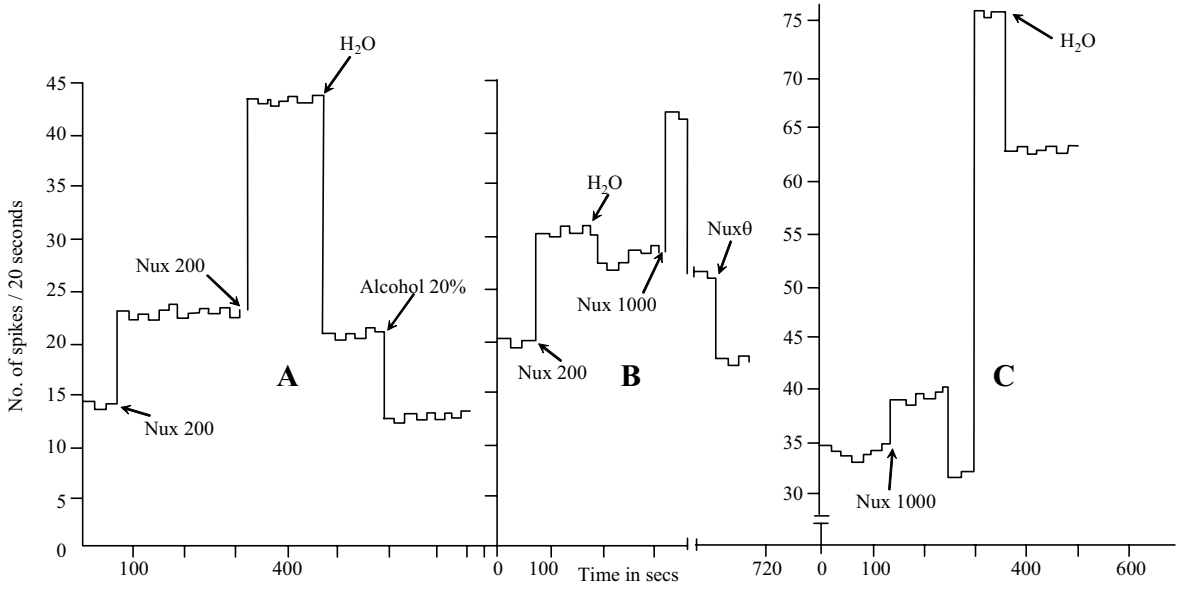

Figure 5. *Histogram showing excitatory effect of Nux vom 200 and 1000 and inhibitory effect of distilled water, Nuxθ and 20% alcohol, applied on the tongue, on the lateral hypothalamic area neurons of rats given 20% alcohol at 15 ml/kg body weight for 7 days. A, B gross and C unit activity (Reproduced, with permission, from Sukul et al : High dilution effects of Strychnos nuxvomica L on hypothalamic neurons and adrenergic nerve endings of alcoholic rats. In : High dilution effects on cells and integrated systems. C Taddei Ferretti, P Marotta [editors]. Copyright © 1998 by World Scientific Publishing Co Pvt Ltd)*

each sample was sonicated in 0.16 perchloric acid containing an internal standard. The sample was centrifuged (2 min at 13000xg) and the amine determinations were accomplished by injecting the supernatant of the centrifuged brain homogenate on to a reversed phase C-18 column. After elution of the amines with mobile phase (0.0465 g Na_2HPO_4; 2.8g citric acid; 18.6mg EDTA; 20mg sodium octyl sulfate in 900ml double distilled water with 100ml methanol), they were quantified with a glassy carbon electrode at +0.62V. The column was heated to 35^0C; flow rate of the mobile phase was 0.8ml/min. Sample peak heights of DA, DOPAC, 5HT and 5HIAA in tissues were compared to extracted standard peak heights of the same amines, that were processed in the same way for their quantitative estimation in tissues. The internal standard was used to accommodate for changes in extraction efficiency and detector sensitivity. This was done by comparing the peak height of the known amount of epinine added to a tissue sample with that of its standard. Unextracted standards were also used to rectify any differential loss in amines during extraction. Sample values were calculated as amine content per gm of wet weight of brain. The treated group revealed statistically significant ($P<0.05$) increases in DOPAC and 5HIAA, while there was no significant change in the steady-state levels of the transmitters DA and 5HT (Table 1). No significant change was observed in DOPAC/DA and 5HIAA/5HT ratios (Sukul 1990). The results suggest increased transmission in the dopaminergic and seratonergic neurons of the hypothalamus following the oral application of potentized *Agaricus.* All the experiments indicate that hypothalamus plays an important role in initiating and mediating the action of a homeopathic potency.

Table 1. Significant increase ($P<0.05$, t-test) in dihydroxyphenyl acetic acid (DOPAC) and 5-hydroxyindol acetic acid (5-HIAA) in the mouse hypothalamus following treatment of mice with Agaricus muscarius 12 through the oral route. No significant change in the level of dopamine (DA) and serotonin (5 HT) was observed. Data from Sc Cult 56:134-135 (1990) reproduced with author's permission.*

Animal group	Dopamine ng/gm	DOPAC ng/gm	5-HT ng/gm	5HIAA ng/gm	DOPAC/DA	5HIAA/HT
Controls :						
Mean	318.9	125.2	902.4	747.8	0.410	0.840
± S.E.	26.4	4.5	49.4	52.5	0.043	0.074
Agaricus :						
Mean	445.1	191.1	926.3	902.8	0.431	0.979
± S.E.	56.6	28.7	31.2	28.2	0.027	0.039
T values	– 2.02	– 2.27*	– 0.41	– 2.60*	– 0.42	– 1.67
Df = 10						

2.3. Experimental evidences from plants

Kolisko (1926 cited by Righetti, 1994) observed enhanced growth of germinated seeds with homeopathic dilutions of silver nitrate (*Argentum nitricum*). The experiment was followed up by Pelikan and Unger (1965 cited by Righetti, 1994)

using *Argentum nit* 8x and 19x. Pongratz and Endler (1994) and Pongratz *et al.* (1998) used *Argentum nit* 24x, 25x and 26x on wheat seedlings and confirmed the results obtained by earlier workers. The effect of homeopathic drugs on plants was reported by other workers (Boiron and Zervouacki, 1962; Boiron and Marin, 1971; Auquiere *et al.,* 1981, 1982; Jones and Jenkins 1981, 1983; Dutta, 1989, Bornoroni, 1992; Brizzi *et al,* 2000).

2.3.1. Effect on parasites and pathogens of plants

As in the case of man and animals homeopathic drugs can also ameliorate diseases of plants caused by parasites and pathogens. *Arsenicum album* 199c and *Kali iodide* 200c reduced fruit rot of guava and mango (Khanna and Chandra, 1977, 1978). This work, however, did not have controls. *Cina, Santonin* and *Filix* at 1 ppm concentration partially inhibited hatching of eggs of root-knot nematodes *Meloidogyne incognita* of vegetable crops (Sen and Dasgupta, 1985). The mother tinctures of the drugs were used in this experiment.

Meloidogyne incognita is a nematode parasite of vegetables occurring all over the world. It also attacks cereals, pulses, tea, coffee, fruit trees etc. Since *Cina* is effective against nematode parasites of man and animals, this drug has been selected for the treatment of plant diseases caused by nematode parasites. *M. incognita* or the root-knot nematode is a parasite in the roots of plants causing root-galls. *Cina* is produced from the ethanol extract of the flowering tops of *Artemisia* sp. *Cina* 1000c was purchased from C. Ringer, Calcutta. The drugs were produced according to the homeopathic pharmacopoeia of India (1966). Sucrose globules, soaked with the liquid drug, were mixed with sterile distilled water in the proportion of 7.2 mg globules/ml of water. The control solution was similarly prepared from sucrose globules soaked in 90% ethanol. Cowpea plants *Vigna unguiculata,* grown in pots, were inoculcated with the 2nd-stage larvae of *M. incognita*. Four days after inoculation, one of the inoculated groups was treated by foliar spray, once daily for 10 days, with *Cina* 1000c solution. Treatment was given in such a way that all the leaves of the plant were completely drenched in the solution. The other inoculated group was similarly treated with the control solution. All the plants were uprooted 30 days after inoculation. Biomass of the test plants, root-gall numbers, root-nodule numbers, nematode population in roots and rhizospheric soil and root-protein content were estimated. Treatment with *Cina* 1000c increased the plant growth and reduced nematode infection significantly (Table 2). This experiment was repeated twice (Sukul and Sukul, 1999). In another experiment *Cina* 200c and *Cina* 1000c were applied by foliar spray on tomato plants inoculated with root-knot nematodes. Here also both the potencies reduced nematode infection and improved plant growth significantly (Sukul, 1999).

Table 2 : Increase in growth and decrease in root-knot nematode (Meloidogyne incognita) infestation of cowpea plants following treatment with foliar spray of the aqueous solution of Cina 1000c. Values are means with S.E. of 10 plants of each batch. Different letters (a, b, c) against the values in a column indicate significant difference (P<0.01) by ANOVA. Data from *Environ Ecol* 17 : 269-273 (1999) with permission.

Treatment	Shoot length cm	Shoot wt g	Root length cm	Root wt g	Root nodules	Root gallls	Nematodes/ 2g root	Nematodes/ 200g soil	Root protein mg/g
Uninoculated untreated	250±9a	228± 7a	32±4a	15±2a	80±7a				12.8±0.5a
Inoculated untreated	248±10a	230±8a	20±5b	26±1b	68±5b	403±16a	158±38a	424 ± 20a	6.2±0.3b
Inoculated, treated with *Cina* 1000.	261±6b	243±6b	28±7a	20±2c	98±6c	65±10b	478±27b	122±17b	13.6±0.2a

Drug solutions applied on leaves of infected plants have no lethal effect on parasitic nematodes. These potencies are thought to have stimulated natural defense response in plants which in turn reduced nematode infection. Plants have no specialized immunocompetent cells. All the cells in a plant have the ability to recognise the self from the non-self. The results of these molecular recognition events in response to a local stimulus are also transmitted to distant regions of the plant (Bowles, 1992). Pathogenesis-related proteins (PR proteins) are known to accumulate in the leaves of potato plants following root invasion by cyst nematodes (Hammond-kosack *et al.,* 1989). Prior inoculation of tomato and pyrethrum plants with *M. incognita* or *M. hapla* induced resistance in the plants to a different species such as *M. hapla* (Ogallo and McClure, 1995). Salicylic acid increases throughout the plant after only one part of the plant is infected by a pathogen. Following this increase in salicylic acid PR proteins are expressed in the plants (Malamy *et al.,* 1990 cited by Jones, 1994). Application of salicylic acid or its analogue aspirin (acetyl salicylic acid) induces rapid expression of PR genes (Ward *et al.,* 1991 cited by Jones, 1994). It is quite probable that potentized *Cina* induced systemic resistance in plants thereby reducing infection of root-knot nematodes. Betti *et al*. (2003) observed that potentized *Arsenic trioxide* induced increased resistance in tobacco plants to tobacco mosaic virus (TMV).

Control of plant diseases by homeopathic potencies would not only eliminate pesticidal residues in treated plants but also do away with environmental pollution in the agroecosystem. For this, new *materia medica* in relation to plants should be developed by drug proving on plants and by merging plant pathology with toxicology.

2.4. Evidences from in vitro *tests*

In vitro tests were conducted on isolated organs, cell cultures or macromolecules such as proteins. High dilutions of *Apis mellifica* and histamine influenced

degranulation of human basophils (Poitevin *et al.,* 1988). High dilutions of anti-IgE antibodies promoted human basophil degranulation *in vitro* (Davenas *et al.,* 1988). The work, published in *Nature*, generated a lot of controversy among scientists. Belon *et al.* (1999) demonstrated the inhibitory effect of high dilutions of histamine on the human basophil degranulation. High dilutions of drugs showed positive effects on rat liver microsomes (Kretschmer, 1990). Van Wijk and Wiegant (1998) observed that rat hepatoma cells, pretreated with heat shock, sodium arsenite or cadmium chloride, developed a charcteristic set of heat shock proteins (hsps). When the sensitized cell populations were exposed to very low doses of each of the three types of stressors, a set of hsps characteristic of the stressor developed, thereby providing experimental evidences for the specificity of the similia principle. Cambar *et al.* (1998) reviewed the works on the protective influence of high dilutions of heavy metals on the metal-induced toxicity in cell cultures. Cristea (1998) employed a classical pharmacological technique to assess the efficacy of potentized *Belladonna.* The principal alkaloid in the *Belladonna* mother tincture is atropine which serves as an antagonist against the neurotransmitter acetylcholine (Ach). High dilutions of *Belladonna* upto the 200th potency was applied on the isolated rat duodenum mounted in an isolated organ bath. The effect of *Belladonna* potencies on the rat duodenum was bidirectional and multiphasic. Bastide and Boudard (1998) reported immunomodulating effects of high dilutions of such endogenous substances as thymulin, bursin and cytokinins on mice and birds. The experiments conducted were *in vitro*, *in vivo* and *ex vivo.*

2.4.1. Boyd's in vitro *experiment and other related experiments*

A new type of *in vitro* tests was conducted without any living cells, tissues or organs. Here a potentized homeopathic drug was considered as a distinct chemical differing in chemical reaction from its vehicle such as water or aqueous ethanol. Boyd (1941, 1942, 1946, 1954) first conducted this type of *in vitro* tests using *Mercuric chloride* 30. He prepared *Merc cor* 30 in double-distilled water and tested it on the activity of disastase which is a mixture of starch-digesting enzymes. He observed that *Merc cor* 30 accelerated the rate of hydrolysis of starch by disastase. More than 500 comparisons between the tests and the control were carried out and the difference was statistically significant at 0.001 level (Boyd 1954). The experiments were conducted with utmost care.

We repeated and extended a part of Boyd's experiments using modern techniques and instrumentation facilities. We used two different potentized drugs, namely *Mercuric chloride* 30 and *Mercuric iodide* 30, and prepared them both in double distilled water and the usual medium of 90% ethanol. The purpose was to see whether the metal ion or the halide ion could play any individual role in altering the enzyme activity. The efficacy of the two media, water and aqueous ethanol, was also tested by these experiments. It is the experience of homeopathic physicians and pharmacists that potentized homeopathic drugs prepared in aqueous ethanol keep their activity for a pretty long time. We wanted to test whether the pure aqueous preparation of a homeopathic potency could keep its activity as long as the hydrated

ethanol preparation. For this, we tested *Merc cor* 30 (*Mercurius corrosives* 30) in aqueous preparation of 3 different ages, one 4-day old, the 2nd one-month old and the third twelve-month old. The hydrolysis of soluble starch by α-amylase was measured by the standard biochemical procedure (Bernfield, 1955). Pancreatic α-amylase broke down starch yielding mainly maltose, a disaccharide of α- (1-4) glucose (Nelson and Cox, 2000). The breakdown product maltose was quantified from a standard curve prepared with the help of a Jasco spectrophotometer (model 530). There were 10 replicates for each drug and its corresponding control. *Merc cor* 30 in water (4-day old and one-month old) and in 90% ethanol media increased the enzyme activity significantly ($P<0.01$, student t-test). The 11-month old *Merc cor* 30 in aqueous preparation did not change the enzyme activity with reference to its matched control. *Merc iod* 30 in both water and aqueous ethanol media enhanced the enzyme activity significantly ($P<0.01$) as compared to their respective controls (Table 3). A notable feature in this *in vitro* study is that the blank media like water and aqueous ethanol altered the enzyme activity as compared to the control to which no blank medium was added. While water increased the enzyme activity, aqueous ethanol reduced it (Sukul *et al.,* 2002).

Table 3 : Effect of different drugs and their diluent media on the hydrolysis of starch by α-amylase ay 27^0C for a period of 15 min. Data reproduced with permission from Homeopathy 91 : 217-220 (2002). Copyright© with Elsevier.

Drugs	Amount of maltose released ± S.E. (μg)
Merc cor 30c in water (4-day old)	500 ± 14*
Merc cor 30c in water (1-month old)	430 ± 9*
Merc cor 30c in water (12-month old)	302 ± 7
Merc cor 30c in 90% ethanol	428 ± 12*
Merc iod 30c in water (fresh)	386 ± 7.5*
Merc iod 30c in 90% ethanol	302 ± 10*
Control I : enzyme + substrate + water	302 ± 6
Control II : enzyme + substrate + *Water* 30 (4-day old)	322 ± 10
Control III : enzyme + substrate + water (1-month old)	328 ± 6
Control IV : enzyme + substrate + *Water* 30 (12-month old)	322 ± 6
Control V : enzyme + substate + 90% ethanol	228 ± 5
Control VI : enzyme + substrate + *Ethanol* 30	238 ± 10

* Significant difference ($P<0.01$) from the corresponding control (II, III or IV). *Merc cor* 30 in water and in 90% ethanol differs significantly from *Merc iod* 30c in water and 90% ethanol ($P<0.01$). Control dilutions made in water (I-IV) differ from those containing alcohol (V and VI). (one-sided t-tests throughout). No significant differences between water controls (I-V) (ANOVA).

Results of *in vitro* tests with isolated organs or cell cultures suggest that potentized drugs are effective not only on the entire organisms but also on their isolated parts and even cells. *In vitro* tests with enzymes show that potentized drugs can act on chemicals without any mediation of living organisms. Here we see that both water and aqueous ethanol can serve as good media for the preparation of potentized drugs. However, water could not retain the efficacy of a potentized drug for a long

time as is the case of 11-month old *Merc cor* 30. Boyd (1954) also observed that the efficacy of *Merc cor* 30 in water deteriorated with the passage of time.

Let us examine whether the metal ion in a salt or the halide ion plays any individual role in altering the enzyme activity. In aqueous solution a salt molecule such as $HgCl_2$ or HgI_2, is dissociated into a cation (Hg^{2+}) and an anion (Cl^- or I^-) surrounded by several water molecules. The hydration stabilizes the ions (Watanabe and Iwata, 1997). Mercury ions usually inhibit the enzyme activity by binding to cysteine, histidine and threonine residues (Muller and Saenger, 1993). Two residues known to be essential in substrate binding in α-amylase are His-101 and His-299 (Qian *et al.,* 1993). In the potentized form, as in our tests with *Merc cor* 30 and *Merc iod* 30, mercury served as a promoter of the activity of α-amylase. Chloride ions are known to activate α-amylase, but the activity is not dependent on this ion (Kabuto *et al.,* 2000). Three residues involved in chloride binding are Arg-337, Arg-195 and Asn-298 (Qian *et al.,* 1993). In our experiment both *Mercuric chloride* 30 and *Mercuric iodide* 30 enhanced the enzyme activity. If idodide had played an independant role *Merc iod* 30 would have retarded the α-amylase activity. But this did not happen. It appears that the compound as a whole, rather than its individual ions, induces specific activity in the diluent medium during dynamization and the medium itself plays a role in acquiring and retaining the activity. The solution structure of the diluent medium appears to have altered the structure of the enzyme thereby changing its activity. The functionality of biological molecules is strongly influenced by their three-dimensional structures, which are primarily determined through non-covalent interactions with metal ions, hydrogen-bonding interactions and solvation (Rodgers and Armentrout, 1997). In this case H-bonding interaction and solvation by structured water of *Merc cor* 30 or *Merc iod* 30 altered the activity of α-amylase (Sukul *et al.,* 2002). The results further show that water molecules carry the specific information of drug molecules during the process of dynamization. Ethanol molecules having a large non-polar part stabilize the specific water structure acquired during the process of successive dilution and mechanical agitation.

2.4.2. In vitro *experiment on red blood cells*

Based on previous works on Homeopathy we have hypothesized that the primary target of a homeopathic potency in an organism is the water-channel protein or aquaporin (Sukul and Sukul, 2001). Aquaporins occur in all life forms and facilitate permeation of water across biological membranes. We have discussed in details about the structure and function of aquaporins and their relation to health and disease in chapter IV. There are several types of aquaporins (AQP) and one type AQP1 occurs abundantly in red blood cells of vertebrates. If the primary target of a homeopathic potency is aquaporin, application of a homeopathic potency on cell membranes would affect water flow into the cells. In order to test this hypothesis we treated red blood cells of a fresh water fish (*Clarius batrachus*) with *Mercuric chloride* 30 (*Merc cor* 30) and *Nux vomica* 30 (*Nux vom* 30) separately in a hypotonic medium. In the control red cells were treated with *Ethanol* 30. The diluent medium in all the three potencies consisted of 90% ethanol and 10% distilled water.

The hypotonic medium in which the red blood cells were incubated at 30^0C for 30 min with either a drug or a control solution was sterile distilled water. The tests were performed on blood cells collected from two groups of fishes, one given i.p. injection of ethanol at 2g/kg body weight and the other without any ethanol injection. In another experiment fishes were pre-treated *in vivo* with *Ethanol* 30 and *Nux vom* 30 separately and then given ethanol injection at 2g/kg body weight. RBC's of these fish were tested *in vitro* with the same potencies. After treatment blood samples were centrifuged, supernatant fluid part removed and erythrocyte pellets dried in a BOD incubator at 90^0C for 12 hrs. The difference between dry weight and wet weight of erythrocyte pelletes gave the weight of intracellullar water in red cells.

Erythrocytes from ethanol-injected fish permeated more water than those from normal fish. Water permeation was significantly enhanced with *Merc cor* 30 and *Nux vom* 30 as compared to the control. RBCs from fish pretreated with *Nux vom* 30 inbibed more water in *in vitro* treatments than those from fish pre-treated with *Ethanol* 30. Since aquaporins are mainly responsible for water transport through the plasma membrane of red blood cells it is thought that potentized drugs such as *Merc cor* 30 and *Nux vom* 30 acted upon these proteins and facilitated water influx into the cells (Sukul *et al.,* 2003).

Summary

Evidences in support of high dilution effects come from clinical records by practising physicians and scientists, and results of experiments conducted in laboratories. Unlike crude drugs, the therapeutic effect of a high dilution depends not on the common symptoms of a particular disease but on the totality of the symptoms of an individual patient. Thus clinical trials would yield positive results if the objective is to see whether diseases, whatever their name is, are cured by high dilutions. Case records of homeopathic physicians show that diseases such as uterine tumours, leishmaniasis, anal fistula, bronchial asthma, viral encephalitis, hepatitis, cerebral haemorrhage, heart block etc. were cured or ameliorated by high dilution of drugs. Occasional failures in clinical trials result from specific inherent condition of patients called miasms.

High dilutions of drugs have been used on human patients for a couple of centuries, and animal experimentation has been done only to confirm their therapeutic effects and study their mode of action. High dilutions have been found to produce effects on such animals as rats, mice, birds, toads and fishes. The basic principle is to create a disease in the animals and test appropriate remedies on them. Some models like catalepsy and righting reflex ones are non-sacrifice animal models which can be easily used to test the biological effects of potentized drugs. Potentized *Nux vomica* significantly reduced alcohol intake in rats and reversed to some extent

alcohol-induced degenerative changes in the adrenergic nerve plexus of atrio-ventricular valves of rats. Potentized *Cina, Calcarea fluor* and *Thuja* reduced infection of *Trichinella spiralis* in albino mice. Potentized *Cina* reduced *Dirofilaria immitis* infection in dogs. High dilutions did not kill the nematode parasites directly but worked through the immune system of the host animals.

In electrophysiological experiments potentized *Arnica, Hypericum* and *Arsenicum album,* given on the tongue, altered the rate of discharge of the lateral hypothalamic neurons in anaesthetized cats. *Arnica* 30, *Hypercium* 200 and *Arsenic* 30, administered orally, altered the firing rate of the medial frontal cortex neurons of awake rats. While potentized *Nux vomica* increased the activity of lateral hypothalamic neurons of alcoholic rats, distilled water, *Nux* θ and 20% alcohol reduced it. *Natrum mur* 30 and 200, applied on the tongue of albino rats, kept on a diet containing 4% sodium chloride, produced inhibitory effect on the lateral hypothalamic neurons. Distilled water showed an excitatory effect on those neurons. *Natrum mur* 30 and *Phosphorus* 200 produced inhibitory effect on hypothalamic neurons of rats given *Naturam mur* 30, one dose daily for 18 days. Here also distilled water produced the excitatory effect. *Agaricus muscarius* 12, administered orally on mice, increased the level of dihydroxyphenyl acetic acid (dopamine metabolite) and 5-hydroxyindol acetic acid (serotonin metabolite) in the mouse hypothalamus indicating increased transmission in the dopaminergic and serotonergic neurons there. These results indicate that hypothalamic and cortical neurons play an important role in mediating the therapeutic action of potentized drugs.

Arsenicum album 199c and *Kali iodide* 200c reduced fruit rot of guava and mango. *Cina* 1000c, applied by foliar spray, reduced root-knot disease, caused by nematode parasites, of cowpea plants. The drugs augmented the natural defense response in plants and thus reduced the parasite infection.

High dilution effects were observed in *in vitro* tests involving isolated organs, cell cultures or macromolecules such as proteins. *Mercuric chloride* 30c and *Mercuric iodide* 30c enhanced an enzyme activity *in vitro*. Unlike high dilutions in aqueous ethanol, efficacy of high dilutions in water deteriorates over time. *Mere cor* 30 and *Nux vom* 30 altered water permeation in fish erythrocytes *in vitro* thereby indicating the influence of high dilution on water channel proteins or aquaporins on plasma membranes.

CHAPTER III

PHYSICAL BASIS OF DRUGS AT HIGH DILUTION

We have already noted in the 1st chapter (1.4, 1.4.1) that potentized homeopathic drugs are prepared in aqueous ethanol, usually 90% ethanol. In practice, homeopathic physicians usually prescribe 30th potency of a drug or its higher potencies for an ailment. We have also noted that these potencies work on patients. There are also evidences from experiments on animals and plants that potentized drugs are effective. Drugs in high potencies also differ from each other with respect to their efficacy on organisms. Let us examine what physical entities are present in potentized drugs. Are the entities molecules of the concerned drug or something else? It is obvious that when the solution or suspension of a drug in a solvent is diluted with the solvent, the dilution would contain fewer molecules of the concerned drug. The mass of one mole of a drug, say *Natrum muriaticum,* specified by its chemical formula, here NaCl, is obtained by adding up the masses in atomic mass units of the atoms that appear in the formula, and expressing the result ("molecular weight") in grams. One mole of a drug is an amount that contains an Avogadro's number, i.e. 6.022×10^{23}, of specified particles (molecules or other fundamental structures) of that substance. Avogadro put forward his hypothesis in 1812. In a 1909 classical experiment Jean Baptiste Perrin determined the Avogadro number which was confirmed later by other physicists (Alberty and Silbey, 1995; Vemulapalli, 1997). Hahnemann could not have known that his potencies actually crossed the Avogadro number. If a drug is successively diluted with a solvent, say 90% ethanol, in the proportion of 1:100 to prepare the 12th potency, its dilution would be 10^{-24}. Since Avogadro's number for any drug is 6.022×10^{23} mol^{-1}, the 12th and higher potencies of a drug, obtained from one mole of the drug itself, would not contain any drug molecules. If the dilution is prepared, as usual, from an amount of substance less than one mole, the number of dilutions necessary to obtain the absence of drug particles is still less than 12.

In compliance with the Avogadro number, all higher potencies of a drug contain only the molecules of the diluent medium, i.e. water and ethanol. We have noted in the foregoing chapter that potencies, prepared with pure water, are also effective although the efficacy of the aqueous preparation declines with the passage of time. There are several hypotheses that have attempted to unravel the mystery of the physical basis of potentized drugs. All these hypotheses are based on the molecules of the diluent medium. These works have been reviewed earlier (Rubik, 1989; Jacobs and Moskowitz, 1996; Sukul, 1997). According to Barnard (1965), polymers of water appear during the process of succussion. These polymers are thought to assume specific configuration in a potency depending on the chemical nature of the concerned drug. Callinan (1986) proposed that vibrational energy of water molecules is raised much above the ground state by succussion. The cumulative effects of this energy may influence the clustering of water molecules

and finally lead to stable cluster configuration. According to Sharma (1984, 1986), resonant promotion of the lone pair of electrons of the -OH groups of diluent molecules contributes to the physical basis of a potentized drug. Different diluent media (water, alcohol, lactose), used in potentizing drugs, have in common the -OH groups in their molecules. An oxygen atom has four equivalent valency orbitals, two of which are occupied by bond pair and the rest by a lone pair of electrons. During succussion or trituration the outermost electron shell of the drug molecule comes close to the outermost electron shells of the diluent molecules repeatedly. This induces resonant promotion of the lone pair of electrons of the -OH groups of the diluent molecules to energy levels equal to those of chemically active electrons in the drug molecules. The process imprints chemical specificity of drug molecules on the molecules of the diluent medium or vehicle. According to Dutta (1979, 1994), light isotopes of drug molecules are formed during the process of dynamization of a drug. These isotopes have chemical properties similar to those of the drug but negligible mass. These isotopes continue to exist in the medium when the original drug molecules disappear during the process of successive dilution. As for example, positronium, which is made up of an electron, the elementary negative charge, and a positron the elementary positive charge, is the light isotope of hydrogen. Light isotopes of helium and lithium have been reported. These isotopes are thought to carry the message of original drug molecules and produce the necessary biological effects. Light isotopes increase in number proportionately with the incerasing degree of dynamization due to autocatalytic effect. The higher is a potency, the more numerous are the light isotopes in the medium. This makes the higher potencies of a drug produce stronger and more prolonged effect on a patient.

Water molecules may form clusters or clathrates around drug molecules or particles (Wei *et al.,* 1991). Anagnostatos *et al.,* (1991, 1993) put forward a hypothesis that the drug molecules in the centres of clathrates are dislodged due to the force generated by succussion. The vacant clathrates remain and are immediately surrounded by water molecules. The dislodged drug molecules are also surrounded by water molecules. All these clathrates are thought to carry the message of drug molecules when the latter disappear in course of successive dilution and succussion.

Experiments have been conducted on the diluent media to find out the physical basis of potentized drugs. The data on the spectroscopic analyses of homeopathic potencies are presented below.

3.1. NMR spectroscopy

Before describing the application of Nuclear magnetic resonance (NMR) spectroscopy to potentized homeopathic drugs we would first discuss the basic principles of NMR spectroscopy. This spectroscopy is a powerful tool providing structural information about molecules. Like UV-visible and infra red spectrometry, NMR spectrometry is also a form of absorption spectrometry. Nuclei of some isotopes possess a mechanical spin and the total angular momentum depends on the nuclear spin, or spin number 1. The numerical value of I is related to the mass number and the atomic number and may be 0, ½, 1 etc. The medium of homeopathic

potencies is usually water or a mixture of water and ethanol. Obviously, the medium is made up of molecules of hydrogen (proton), oxygen and carbon. The proton (H) and deuterium (^{2}H) have a spin number of ½ and 1, respectively. Ordinary carbon ^{12}C, and oxygen ^{16}O have I = 0 and are non-magnetic. Each nucleus in which the value of I is greater than 0 has a characteristic magnetic moment. Since an electric charge is associated with an atomic nucleus, the spinning nucleus produces a magnetic field with its axis in line with the axis of the spin. The magnetic moments of nuclei are oriented in random directions in a sample of H_2O. If the sample is placed in a uniform magnetic field Ho, the nuclei will tend to align themselves along the field. But because the nucleus is spinning, its axis would not be parallel or antiparallel to the applied magnetic field. There would be a finite angle between the spin axis and Ho with the result of a circular motion of spin axis around Ho. This motion of the nucleus is called precession (Figure 6). The motion is comparable to a spinning top whose axis makes a second rotation different from that of the earth's gravitational field at the time of slowing down of the speed of spinning of the top. The frequency of precession is most important in NMR.

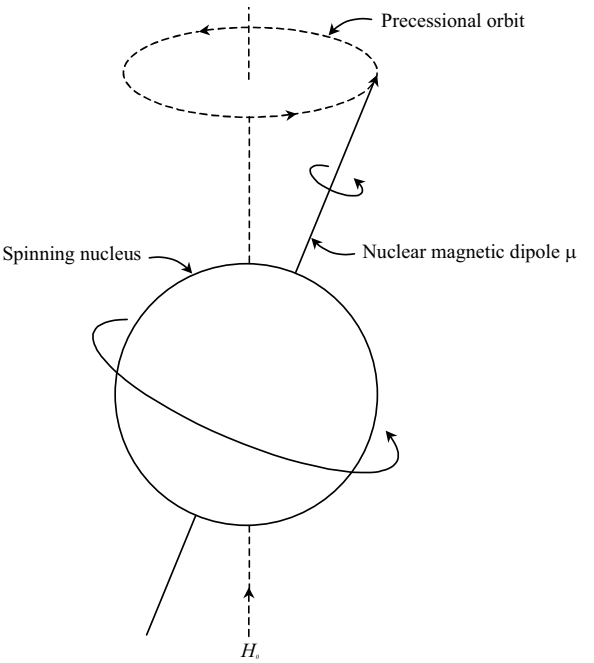

Figure 6. *Precession of a nucleus under an applied magnetic field H_o*

In terms of quantum mechanics, the spin number I determines the number of orientations a nucleus may assume in an external uniform magnetic field according to the formula 2I+1. Since the proton has a spin number I = ½, there would be two energy levels. The low energy orientation corresponds to that state in which the nuclear magnetic moment is aligned parallel to the external magnetic field, and the high energy orientation corresponds to that state in which the nuclear magnetic moment is antiparallel (opposed) to the external magnetic field Ho. There is, however, a slight excess population of nuclei in the lower energy state (lower spin state). It is possible to induce transitions between these two orientations of the nuclei. The precessional frequency of a spinning nucleus is exactly equal to the frequency of electromagnetic radiation necessary to induce a transition from one nuclear spin state to another. Electromagnetic radiation is an oscillating electric field E in space which is propagated with the velocity of light. It has an electric vector E, directed along the displacement direction of the wave. The associated magnetic field vector H lies perpendicular to the electric vector and perpendicular to the direction of propagation. The radio frequency electromagnetic energy is applied in such a way that its magnetic component H_1 is at right angles to the axis of the main external magnetic field Ho and is rotating with the precessing proton. When the frequency of the rotating magnetic component is equal to that of the precessing nucleus they are said to be in resonance, and the absorption or emission of energy by the nucleus can occur. It is the excess of muclei in the lower energy state that gives rise to net absorption of energy in the radio-frequency region. At the point of resonance the absorbed energy is at a maximum, and the precessing nuclei are tilted away from alignment with Ho toward the horizontal plane (Figure 7). The magnetic component thus generated in that plane can be detected. The resonance can be produced by keeping Ho constant and changing the frequency of the rotating coil with its axis at right angles to the main magnetic field Ho. Or, the oscillator frequency is kept constant and Ho is varied over a narrow range.

As the excess population of nuclei in the lower energy are raised to the high energy state by absorption of energy, the intensity of absorption signal diminishes and finally disappears. The phenomenon is known as saturation in which the populations of muclei in the two spin states become equal. However, there exists a mechanism by which the nucleus in the higher energy state can lose energy to its environment and thus returns to its lower energy state. The mechanism is called a spin-lattice or longitudinal relaxation (T_1). It involves transfer of energy from the nucleus from its high-energy state to the lattice. The term lattice refers to the framework of molecules (say drug and solvent or diluent) containing the precessing nuclei. All these molecules have transational, rotational and vibrational motions and have magnetic properties. Thus, a variety of small magnetic fields are present in the molecular lattice. A particular small magnetic field, properly oriented in the lattice, can induce a transition in a particular precessing nucleus from a higher to a lower energy state. The spin-lattice relaxation, therefore, maintains an excess of nuclei in the lower energy state, a condition necessary for the observation of NMR phenomenon. Another type of relaxation process known as spin-spin or transverse relaxation (T_2) involves transfer of energy from one high energy nucleus to another.

There is no net loss of energy, but the spread of energy among the contiguous nuclei concerned results in broadening of band. This relaxation does not contribute to the maintenance of the excess population of nuclei in a lower energy state.

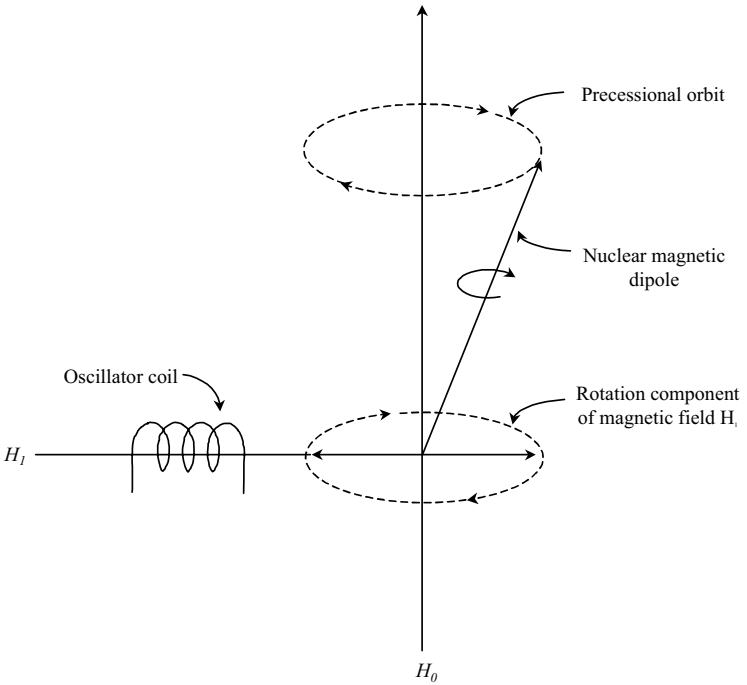

Figure 7. *Magnetic component H_1 of the applied radiofrequency electromagnetic energy rotating with the precessing proton*

The natural width of a spectral line is inversely proportional to the lifetime of the excited state. Thus, sharp resonance lines are observed for states of prolonged excitation, and broad lines are observed for short-lived excited states. Both spin-spin and spin-lattice relaxations contribute to the width of a spectral line. Most solids and viscous liquids show very long spin-lattice relaxation and very short spin-spin relaxation. In case of nonviscous organic liquids and solids in solution, resonance lines are broadened and, therefore, relaxation times are short. Two other factors influence the width of a spectral line. The presence of paramagnetic molecules, such as dissolved oxygen, results in reduction of T_1 due to the large magnetic fields associated with the paramagnetic lattice components. Because the electron magnetic moment is about 1000 times greater than nuclear magnetic moment, nuclear relaxation will be rapid, being dominated by the unpaired electron of oxygen. The

sample can be degassed before obtaining NMR spectrum. It is not known whether degassing results in any alteration of the efficacy of a homeopathic potency in water or aqueous ethanol, the usual diluent medium of a homeopathic drug. However, for purposes of comparison between a homeopahtic potency and its diluent medium no degassing is necessary. This is because of the fact that both the medium and the potentized drug are likely to contain an almost equal amount of the dissolved oxygen. Nuclei with spin numbers greater than ½, as with deuterium, have electric quadrupole moments. These nuclei have short spin-lattice relaxation times. The relaxation rates R_1 and R_2 are expressed in terms of relaxation times T_1 and T_2 as $1/T_1$ and $1/T_2$, respectively.

In the NMR experiment, as stated earlier, an excess spin population is moved from one energy level to another by electromagnetic radiation of the appropriate frequency. This is excitation. The radiationless return to equilibrium is called spin-latice relaxation because the excess energy passes from the spins to the lattice as heat. The relaxation requires magnetic fields which are fluctuating at the appropriate frequency. The dominant fields arise from the magnetic moments of protons in the same molecules as they tumble in solution. This is the dipole-dipole interaction. The rate of dipole-dipole relaxation depends on the strength and frequency of the fluctuating magnetic fields. These in turn depend on three factors. These factors are : (i) the distance between the nuclei involved, (ii) the effective correlation time, τ_c, of the vector that joins the nuclei, and (iii) the nature of the nuclei themselves. τ_c is the average time taken to rotate through one radian or roughly the reciprocal of the rate of tumbling in solution of the relevant piece of the molecule (Sanders and Hunter, 1993).

Only a single peak should appear from the interaction of radio-frequency energy and strong magnetic field on a proton. The peak area, as measured by an integrator, is proportional to the number of protons it represents. However, the nucleus is shielded to some extent by its electron cloud whose density varies with the environment. Protons in different environments are shielded by circulations of surrounding electrons to different extents. The result is usually expressed as a shifting of the resonance frequency of the proton by the electron cloud, and the position of the signal of the particular proton as the chemical shift of the proton. The chemical shifts are measured by using a proton in a suitable compound as a reference. The most commonly used compound for this purpose is tetramethylsilane (TMS). It is chemically inert, magnetically isotropic, volatile (b.p. 27^0C) and soluble in most organic solvents (Figure 8). It absorbs at a higher field than almost all organic protons. Homeopathic potencies are prepared in distilled water or aqueous ethanol. When water or deuterium oxide is the solvent, TMS can be used as an external reference. Here TMS is kept in a sealed capillary immersed in the sample. DSS, acetonitrile and dioxane are used as references in aqueous solution (Silverstein *et al.,* 1981).

The position of the NMR signal is recorded in Hertz (Hz) as a difference between the positions of the observed and reference signals. The range is normally 500 Hz, which is called the sweep width. When the chemical shifts are given in Hz (designated $\underline{\upsilon}$), the applied frequency must be specified. Chemical shifts can be

expressed in dimensionless units, independent of the applied frequency but dependent only on the nature of the proton. This can be done by dividing $\underline{\upsilon}$ by the applied frequency and multiplying by 10^6. Since the resultant term is too small, it is expressed as parts per million (ppm). The delta δ system assigns 0 to the reference signal and expresses the positions of other signals in ppm. A higher δ value indicates

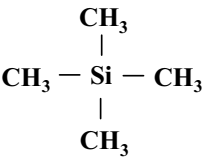

Figure 8. *Structure of tetramethylsilane*

a lower resultant magnetic field around the proton. An alternative unit tau τ assigns a value of 10 to the reference signal (TMS). Thus $\tau = 10 - \delta$. For shifts at a higher field than TMS (δ 0.00, τ 10.00) δ values are given with a negative sign when τ values simply increase numerically.

In homeopathic potencies the molecular formula of the diluent medium, water or ethanol-water, is known. Thus height ratios of peaks can be converted into the number of protons giving each peak. In all spectra, UV, IR and NMR, signals (or bands) are not strictly linear but have a finite width. The factors that influence the width are: (i) instrumental factors, (ii) factors relating to the environment in which the molecules exist and (iii) factors which depend on the molecules themselves (Dani, 1995). The group (ii) factors are important in Homeopathy because other factors (i and iii) would remain unaltered when a potentized drug, say *Nux vom* 30, is compared with its diluent medium (aqueous ethanol or water) using the same instrument under the same experimental conditions. The environmental factors like collision between molecules, exchange reactions and hydrogen bonding introduce slight variations in the transition energies which result in broadening of the signal. Broadening can also occur due to Heisenberg's uncertainty principle, specifically in NMR signals. The principle states that there is a minimum uncertainty involved in the estimation of any two simultaneous (dependent) variables. The two variables here are ΔE (energy) and Δt (life time of excited state). The lifetimes vary but a common value in NMR is 10^{-7}s. The shorter is the life time the broader is the signal. Broadening due to this effect is insignificant in UV and IR spectra where higher frequencies (10^{13} - 10^{15}) are employed. The life time is related to the rate of relaxation. Factors that influence chemical shifts are hydrogen bonding, dipole moment and bulk magnetic susceptibility of the solvent used.

3.1.1. NMR spectra of homeopathic potencies

We have already discussed about the basic principles of NMR specectroscopy. Now we would deal with NMR studies in relation to potentized homeopathic drugs. Early works of the NMR spectra of homeopathic potencies were done by Smith and Boericke (1966, 1968). We measured spin-lattice relaxation time (T_1) in ms (milliseconds) of naturally abundant ^2H in 90% ethanol, *Ethanol* 30, *Ethanol* 30 prepared without succussion and different potencies of other drugs with the help of a AMX 400 NMR spectrometer operating at 61.4 MHz at 22^0C. Measurements were, however, taken at different times. The data are presented in Table 1. We observed deuterium nuclei instead of proton for the following reasons. Deuterium is a quadrupolar nucleus having a small quadrupolar moment 1. Quadrupolar relaxation depends upon the interaction of the electric quadrupole moment with an electric field gradient. Since the qudrupolar moment is small here (^2H), the interaction is small and the relaxation is slow. Like all quadrupolar nuclei the relaxation of ^2H is sensitive to τ_c. We have already described τ_c. It is the average time taken to rotate through one radian or roughly the reciprocal of the rate of tumbling in solution of the relevant pieces of the molecule. The molecules concerned here are H_2O and CH_3CH_2OH. T_1 values of the OH of water, OH, CH_2 and CH_3 of ethanol were measured from the stacked spectra with a computer. Spectra of a few potentized drugs are given in Figure 9. The ethanol hydroxyl has the highest chemical shift value, followed by the water hydroxyl, methylene and methyl groups (Table 4).

The 4 chemical groups of different drugs and their diluent medium show distinct variation with respect to their chemical shifts and T_1 values. In some cases variations are very small and almost insignificant while in others they are large. Variations are observed not only in different drugs but also in different potencies of the same drug. This would be evident in case of potencies of *Cina* and *Nux vomica* (Table 4). Both chemical shifts and T_1 values show marked variation with respect to water and ethanol hydroxyl groups of different drugs (Table 4). T_1 values are larger in CH_2 and CH_3 than in the OH of water or ethanol. Because hydroxyl groups possess binding sites they are more immobilized by binding and thus have the shortest relaxation time. Both spin-lattice and spin-spin relaxation depend on the rates of molecular motion, because relaxation results from the interaction of fluctuating magnetic fields set up by nuclei in the spin system and in the lattice (Connors 1987). T_1 values are related to the molar volumes of the substances. Substances in which the magnetic nuclei are far apart or have small moments show longer T_1 values (Bovey, 1969). In non-ideal solutions such as aqueous ethanol, molecules of different types either attract or repel one another more than molecules of the same type. Hence the mixing either increases or decreases the average distance between molecules which in turn leads to the molar volume being different from the sum of the component volumes (Vemulapalli, 1997). Haseba *et al.,* (1993) observed that sonicated ethanol was more compact and homogeneous than unsonicated one. Thus sonication or succussion would alter the molar volume of a potentized dtug resulting in a change in the T_1 values. Different T_1 values observed in the same deuterium nucleus of different drugs and their diluent medium (90%

ethanol) (Table 4) may be attributed to this factor of altered distance between the molecules.

Potencies prepared by sonication for 30s or 5 min at every step have been mentioned. Iodine 29 was prepared without succussion but by UV radiation at every step. Some potencies were degassed for 1h to remove dissolved oxygen. All other potencies were prepared by manual succussion. All the drugs including mother tinctures were in 90% ethanol.

Alteration in distance between molecules of the drug medium may also occur due to molecular association. Because of the formation of O — H ---- O bonds, all compounds of the ROH type, e.g. water, alcohols and phenols are associated. The association is of a general type, involving the linking together of a relatively large, but indefinite, number of single molecules by H-bonds. The fact that oxygen atom in

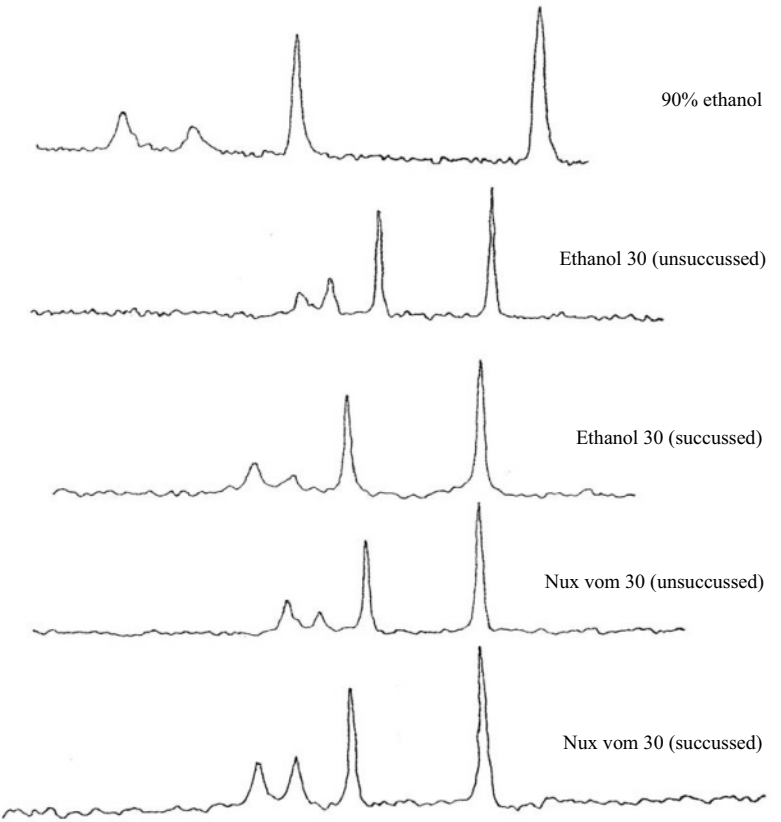

Figure 9. *Part of the stacked ^{2}H NMR spectra of 90% ethanol, Ethanol 30 (unsuccussed), Ethanol 30 (succussed), Nux vom 30 (unsuccussed) and Nux vom 30 (succussed) obtained with a AMR 400 NMR spectrometer operating at 61.4 MHz at 22°C*

Table 4. Spin lattice relaxation time (T_1) of ^2H of 90% ethanol and potentized homeopathic drugs in 90% ethanol. Figures in parentheses represent chemical shifts in ppm. Measurements were taken by a AMX-400 NMR spectrometer operating at 61.41 MHz at 22^0C. Starting with the control other drugs are given in alphabetical order.

Drugs	**Water**	**Ethanol**		
	OH	OH	CH_2	CH_3
Ethanol 90%	110.79	106.90	846.48	822.57
	(4.95)	(5.65)	(3.93)	(1.50)
Ethanol 90%	97.26	97.66	864.58	751.95
(degassed)	(5.9)	(6.64)	(4.87)	(2.44)
Ethanol 30	122.82	74.87	935.69	819.74
(Unsuccussed)	(4.96)	(5.66)	(3.95)	(1.53)
Ethanol 30	87.53	100.20	969.44	876.65
	(4.99)	(5.74)	(4.03)	1.59)
Ethanol 30	115.50	109.03	857.89	802.69
(sonicated 5 min)	(5.02)	(5.73)	(3.99)	(1.56)
Agaricus θ	221.94		894.35	739.27
Agaricus θ	117.94	104.29	847.33	854.75
(sonicated 5 min)	(4.96)	(5.67)	(3.95)	(1.52)
Agaricus 200	101.09	95.58	916.39	735.51
	(5.29)	(5.99)	(4.25)	(1.82)
Agaricus 1000	115.51		860.68	718.68
	(5.52)		(4.21)	(1.78)
Cantharis θ	125.66		954.24	855.32
	(5.35)		(3.99)	(1.56)
Cantharis 200	150.58		942.04	856.79
	(5.54)		(4.04)	(1.63)
Cina θ	104.30		883.45	776.07
	(5.28)		(3.85)	(1.92)
Cina 32	65.61	124.41	1019.00	864.29
	(5.93)	(6.67)	(4.98)	(2.55)
Cina 200	102.50	108.23	867.44	792.67
	(4.70)	(5.42)	(3.67)	(1.24)
Cina 1000	86.77	106.66	971.69	857.57
	(5.01)	(5.70)	(3.98)	(1.55)
Cocculus indicus 30	107.71	104.69	923.42	798.69
	(5.03)	(5.75)	(4.01)	(1.58)
Iodine 29	113.86	93.75	895.50	778.23
(UV radiation 5 min)	(5.16)	(5.88)	(4.12)	(1.68)
Kali nitricum 30	97.88	101.72	826.80	722.52
(degassed)	(5.88)	(6.58)	(4.83)	(2.39)
Kali nitricum 30	87.25	89.76	849.63	711.10
(sonicated 30s)	(6.04)	(6.74)	(4.98)	(2.53)
Mercurius cor 30	97.31	91.88	842.14	744.12
(degassed)	(5.91)	(6.62)	(4.85)	(2.39)
Mercurius sol 200	139.22	106.28	991.38	807.57
	(4.99)	(5.74)	(4.01)	(1.59)
Naja tripudians 200	99.02	91.94	849.99	790.44
	(5.16)	(5.87)	(4.12)	(1.70)
Nux vomica θ	112.23	93.05	894.71	777.66
	(5.16)	(5.9)	(4.12)	(1.68)

(Cont.)

Table 4 (Cont.)

Drugs	Water	Ethanol		
	OH	OH	CH_2	CH_3
Nux vom 30	68.54	109.63	977.18	885.88
(Unsuccussed)	(5.03)	(5.74)	(4.01)	(1.59)
Nux vom 30	119.58	109.83	840.40	758.78
	(4.57)	(5.26)	(8.50)	(1.07)
Nux vom 30	123.38	111.82	872.91	839.73
(sonicated 5 min)	(4.97)	(5.69)	(3.96)	(1.52)
Nux vom 200	102.67	93.33	827.41	790.59
	(4.58)	(5.31)	(3.56)	(1.13)
Nux vom 1000	128.24		879.64	839.66
	(5.52)		(3.95)	(1.53)
Phosphorus 32	98.54	83.34	854.68	745.12
(sonicated 30s)	(6.74)	(6.06)	(4.98)	(2.55)
Phosphorus 32	88.48	87.09	807.67	734.57
(sonicated 30s, degassed)	(5.78)	(6.50)	(4.74)	(2.29)
Santonin 200	94.77	95.15	994.95	752.21
	(5.81)	(6.54)	(4.80)	(2.36)
Strychnine θ	101.66	99.12	845.06	819.41
(0.5mg/ml)	(5.10)	(5.80)	(4.04)	(1.62)
Thuja 30	69.95	81.46	844.16	778.20
	(5.89)	(6.64)	(4.89)	(2.43)

water or ethanol has two lone pairs of electrons makes it possible for each such atom to form two H-bonds, as well as two covalent bonds (Figure 10). The difference in T_1 values in the same column of Table 4 may be due to intermolecular association. We have already mentioned that T_1 values are dependent on the rates of molecular motion. Haseba *et al.* (1993) reported that the thermal motion of water molecules was greater in sonicated ethanol than in unsonicated one. They measured the T_1 values of the naturally abundant ^2H of water molecules ($^2HO^2H$/ - O^2H) in pure water and aqueous ethanol solutions. Obviously, the thermal motion of water molecules in potentized drugs, prepared by succussion or sonication, may change resulting in altered T_1 values. Paramagnetic molecules such as dissolved oxygen greatly reduce T_1 values owing to the large magnetic fields associated with the paramagnetic lattice component (Dyer, 1994). Table 4 shows that this might be the reason for the reduced T_1 values of the three ^2H nuclei in degassed 90% ethanol as compared to 90% ethanol (Table 4). *Phosphorus* 32 (degassed) and *Phosphorus* 32 show almost similar type of difference with respect to T_1 values in 3 out of 4 nuclei. However, the situation is just reverse in case of *kali nitricum* 30 (degassed) and *kali nit* 30. Dissolved oxygen can be regarded as a common factor when a comparison is made between 90% ethanol and other potentized drugs. Here the medium and the drugs contain an almost equal amount of dissolved oxygen. Demangeat *et al.* (1992) reported an increased 4 MHz proton relaxation time (T_1) in high saline dilutions of a silica/lactose mixture as compared to the control (0.9% NaCl).

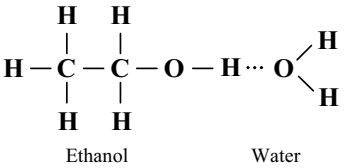

Figure 10. *Covalent bonds (—) and hydrogen bond (….) in ethanol and water molecule*

Because NMR data were obtained at different times there is a possibility of drift in chemical shifts. This can be ascertained by comparing differences in chemical shifts in one row with those in another row. Barring these situations there are also differences in chemical shifts in a column (Table. 4) which cannot be interpreted in terms of the drift due to instrumental condition at different times. The variation in chemical shift values between potentized drugs and their diluent medium is due to varied shielding effects on the deuterium muclei. In linear molecules such as ethanol an important contribution to the total shielding a proton experiences is the result of paramagnetic effect. Paramagnetic electronic circulations about atoms such as carbon and oxygen may contribute to the shielding of adjacent or neighbouring protons. The degree of electronic shielding is clearly dependent on the electron density around the proton; the higher the electron density around the proton, the higher the shielding and the higher the field (higher τ value) at which the proton absorbs (Dyer, 1994). The hydroxyls of water and ethanol participate both in self-association as well as intermolecular association by H-bonding. Protons in H-bonding particularly in self-association exhibit a very marked deshielding (Bovey, 1969). Thus variation in chemical shifts between potentized drugs and their diluent medium with respect to the deuterium nuclei, as in Table 4, may be attributed to varied types of self and inter-molecular association in water and ethanol molecules. Table 4 shows that some mother tinctures such as *Agaricus* θ*, Cantharis* θ and *Cina* θ have three peaks instead of four. In all these cases the hydroxyl peaks of both ethanol and water have merged. The exact shape of the hydroxyl proton signal depends on the time that the proton spends on a given ethanol molecule. In a given period of time a single hydroxyl proton may be attached to a number of different ethanol molecules. The rate of chemical exchange (proton transfer) in pure ethanol is relatively slow, but this rate is very markedly inceased by acidic and basic substances. In the above mother tinctures presence of various phytochemicals might have resulted in a fast chemical exchange. The marging of two hydroxyl peaks has also been observed in such potentized drugs as *Agaricus* 1000, *Cantharis* 200 and *Nux vom* 1000. Here also very rapid chemical exchange between two hydroxylic species resulted in a single resonance line (Table 4).

Abel *et al*. (2001) obtained 1H NMR spectra and T_1 values of potencies of *Sulphur*, D_4 to D_{30} and of *Betula* 30C at 300 and 500 MHz and found no difference in spectra and T_1 values between the potencies and the controls. Milgrom *et al*. (2001) studied T_2 spin-spin relaxation times of potentized and unpotentized *Nitric*

acid and found no difference between the potencies and the control with respect to T_2. This was a repetition of an earlier positive result reported by Corte *et al*.

3.2. Infra Red spectroscopy

The basic principles of infra-red (IR) spectroscopy would be described first. After that we would discuss about its application to Homeopathy. A molecule is made up of atoms which do not remain in fixed positions. Let us assume atoms as balls. So, a molecule is an assemblage of balls of varying masses and springs of varying lengths, corresponding to the chemical bonds of the molecule. A molecule as a whole rotates, the bonds undergo vibrations and even the electrons move. There are two kinds of fundamental vibrations for molecules : (i) stretching, in which the distance between two atoms increases or decreases, but the atoms remain in the same bond axis and (ii) bending (or deformation) in which the position of the atom changes relative to the original bond axis (Figure 11). Each of these kinds of motions is quantized, i.e. the molecule can exist only in distinct states which correspond to discrete energy contents. For example, a rotating molecule cannot rotate with any random velocity and rotational energy, but can have only certain allowed velocities and energies. The

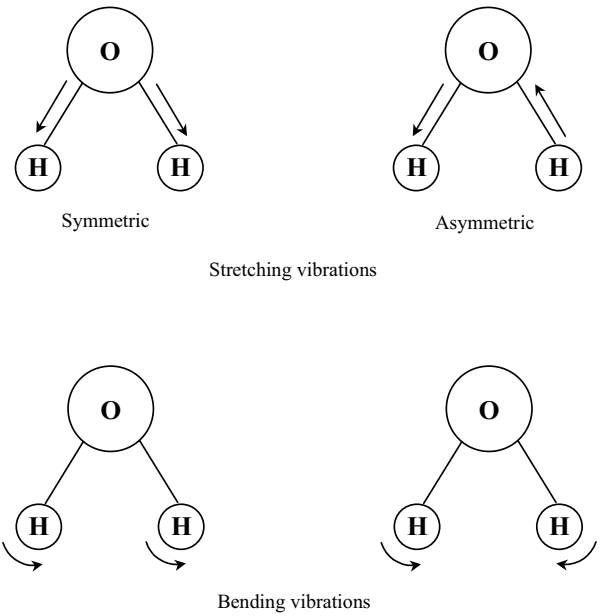

Figure 11. *Stretching and bending vibrations in water molecules indicated by arrows*

same is true for vibrational and electronic energies. Thus various stretching and bending vibrations occur at certain quantized frequencies. When infrared light of the same frequency is incident on the molecule, energy is absorbed and the amplitude of that vibration is increased. The absorbed energy is released as heat when the molecule reverts from the excited state to the ground state. Each type of electromagnetic radiation, i.e., radio waves, ultraviolet, infrared, visible etc. has both the properties of a wave as well as a particle. Electromagnetic radiation can be described as a wave occurring simultaneously in electrical and magnetic fields. It can also be described as if consisting of particles called quanta or photons. In addition to its wavelength (λ) the radiation can also be characterized by its frequency (υ), which is defined as the number of complete cycles per second (cps), also called Hertz (Hz). Wavelength and frequency are inversely proportional.

$$\upsilon = \frac{C}{\lambda}$$, where c and λ are speed of light and wave length in cm, respectively

The energy of a quantum of electromagnetic radiation has direct relation with its frequency. The higher is the frequency of radiation the greater will be its energy. $E=h\upsilon$, where h is Plank's constant.

Organic molecules absorb radiation in discrete 'packets' of $\Delta E = h\upsilon$. Absorption occurs only when radiation supplying exactly the right 'packet' or quantum of energy impinges on the compound. Each state is characterized by one or more quantum numbers and the energy difference between two such states, ΔE, is related to a light frequency υ by Plank's constant h. A molecule can only absorb a particular frequency of radiation, if there exists within the molecule an energy transition of magnitude $\Delta E=h\upsilon$.

3.2.1. IR spectra of homeopathic potencies

As mentioned earlier infrared radiation causes vibrational excitation of the molecular framework of a compound (ΔE is 1 - 10 Kcal mol $^{-1}$). The vibrational frequencies of O — H and related bonds are sensitive to interaction of hydrogen with a third atom to form a hydrogen bond. For this IR spectroscopy is an excellent means of detecting and studying H-bonds (Connors 1987). Using an infrared spectrophotometer (Hitachi model 260-10) absorption spectra of 90% ethanol and some potentized drugs were obtained in the wavelength range of 2.5 μm (4000 wave number Cm^{-1}) to 4 μm (2500 Cm^{-1}). The thickness of the sodium chloride cell was 0.5 mm at 20^0C. A few IR spectra are presented in Figure 12. Wave length (μm) and wave number Cm^{-1} of infrared absorption bands of the chemical groups of 90% ethanol and some potentized drugs are presented in Table 5. Absorption intensities of those groups are also included in the same Table. The figure shows a broad band of OH stretching vibration and a relatively narrow band of CH stretching vibration. The potentized drugs and their diluent medium (90% ethanol) differ from each other

with respect to the wave length/wavenumber Cm^{-1} of the OH and CH bands (Table 5).

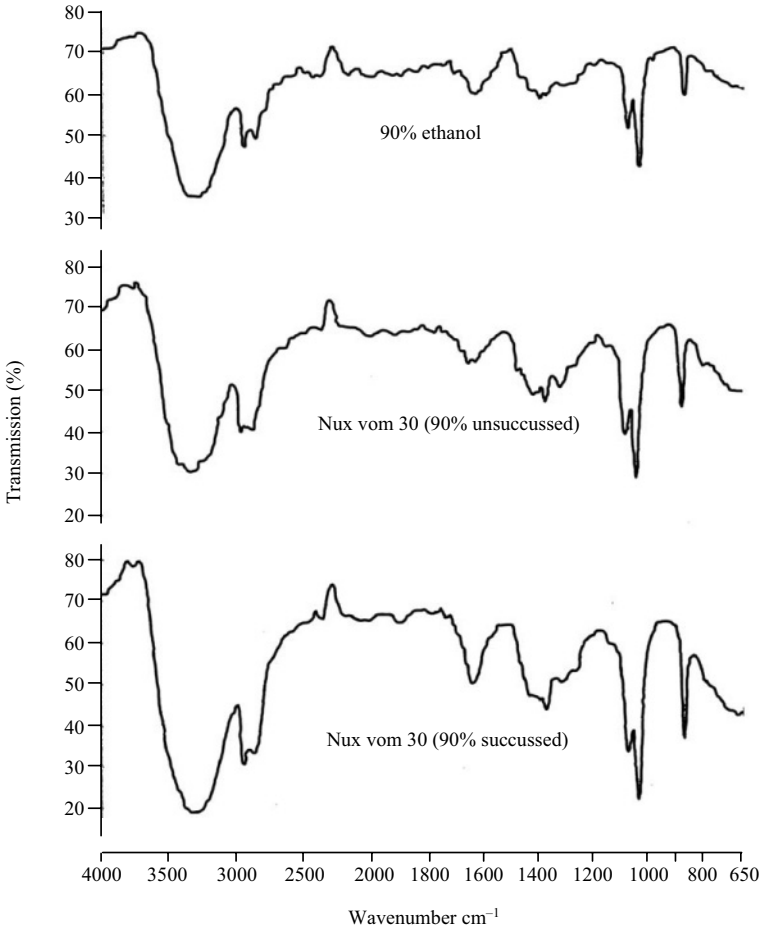

Figure 12. *Infra red (IR) spectra of 90% ethanol, Nux vom 30 (unsuccussed) and Nux vom 30 (succussed) in 90% ethanol obtained with an IR spectrometer (Hitachi, model 200-10). (Modified and reproduced, with permission, from Sukul et al. : Nux vomica 30 prepared with and without succussion shows antialcoholic effect on toads and distinctive molecular association. Br Hom J 2001; 90 : 79-85. Copyright © 2001 by Nature Publishing Group)*

Table 5. IR absorption spectra of 90% ethanol and Nux vom 30 succussed and unsucsussed obtained with the help of an IR spectrometer (Hitachi, Model 260-10). Figures in parentheses indicate intensities of absorption (%).

	Wavelength (μ)/ wave no cm^{-1} of absorption bands			
SAMPLE	O-H stretching	O-H Bending	C-O stretching	C-H bending
Ethanol (90%)	3350 cm^{-1} 2.98μ (64.5%)	1400 cm^{-1} 7.14μ (40.5%)	1060 cm^{-1} 9.43μ (58%)	880 cm^{-1} 11.36μ (41%)
Nux vom 30 (90%) (uncussussed)	3325 cm^{-1} 3.01μ (69.5%)	1370 cm^{-1} 7.30μ (53%)	1040 cm^{-1} 9.62μ (71%)	870 cm^{-1} 11.49μ (54%)
Nux vom 30 (90%) (succussed)	3350 cm^{-1} 2.98μ (81.5%)	1380 cm^{-1} 7.24μ (56%)	1040 cm^{-1} 9.62μ (78%)	880 cm^{-1} 11.36μ (63%)

Hydrogen bonding generally decreases stretching frequencies and increases bending frequencies (Dyer, 1994). H-bonding involves a lengthening of the original O — H bond. The bond is consequently weakened, so the stretching frequency is lowered (Vemulapalli, 1997). Shifts in group frequencies can arise as a result of interactions between different molecules. The -OH stretching frequency of alcohols is very much dependent on the degree of H-bonding, which lengthens and weakens the — OH bond, and hence lowers the vibrational frequency. Shifts in group frequency position caused by resonance or intermolecular effects are in themselves highly characteristic and very useful for diagnostic purposes (Banwell and McCash, 2000). The frequency shift of OH band of potentized drugs with respect to their diluent medium reflects a variation in H-bonding between the drugs and their medium. This is indicative of a dynamic polymeric association of ethanol and water molecules in the potentized drugs through H-bonding. The CH stretching vibration represents both methyl and methylene groups of the ethanol component of the drugs or their medium. The position of CH stretching vibration of the drugs shows variation from that of the same group of 90% ethanol. This indicates a change in the H-bonding in the drugs with respect to the medium.

Fourier transform (FT) spectroscopy

A major disadvantage of obtaining a spectrum using the conventional method is its inherent slowness. In the conventional method of recording a spectrum, the frequency is swept smoothly, across the whole span of the spectrum. The spectrum may contain one or two peaks and the spectrometer, therefore, records only the background noise most of the time. The FT spectroscopy provides simultaneous and almost instantaneous recording of the whole spectrum in the magnetic resonance, microwave and infrared regions. It is known that the background noise imposes a limitation on the sensitivity of any spectroscopic technique. Computer averaging of data reduces the probability of noise to some extent. The combination of computer

averaging with Fourier transform improves the quality of spectra to a great extent (Banwell and McCash, 2000).

We have obtained FTIR spectra of some homeopathic potencies. 1R spectroscopy is one of the most promising methods for the study of the distribution of hydrogen-bonding strength of the water molecules in the aqueous alcohol mixture because of the short time-scale of measurements. Since the O — H stretching vibrational bands (ν_1, ν_3) of water overlap the alcoholic O — H band, the IR spectra in the stretching region are of no use for studying hydrogen bonds of the water molecules in aqueous alcohol mixtures. In the region of the bending vibrational band (ν_2) of water, on the other hand, alcohols have no absorption bands so that the IR spectra in the bending region reveals the H- bonding strength of water molecules. The frequency of the ν_2 band is reciprocally proportional to the hydrogen-bonding strength of water molecules (Mizuno *et al.,* 1997). The authors observed blue shifts of ν_2 bands, i.e., strethening of H-bonds, for the water molecules for which ^{1}H NMR studies also revealed the formation of stronger H- bonds.

FTIR spectra of 90% ethanol, *Ethanol* 30 and some potentized homeopathic drugs obtained by a FTIR Spectrometer (Jasco-420) at 20° C are given in Fig. 13. The figure shows a distinct variation in the number of ν_2 bands, their width and wave number cm^{-1} and absorption intensities (%) in different potentized drugs. This shows a variation in H-bonding in different potentized homeopathic drugs. By convention, vibrations are labeled in decreasing frequency within their symmetry type. The symmetric vibrations of H_2O are labeled υ_1 for the highest fully symmetric frequency (3651.7 cm^{-1}) and υ_2 for the next highest (1595.0 cm^{-1}) (Banwell and McCash, 2000).

3.3. Electronic spectroscopy

Molecules can absorb, or emit, radiation not only as a result of changes in their rotational and vibrational energies but also as a result of changes in their electronic arrangement and, therefore, their electronic energy. Electrons can move and rearrange themselves much faster than the nuclei to alter internuclear distance. An electron in a Bohr orbit of an atom completes a revolution around the nucleus in about 10^{-15} sec while a typical molecule vibrates with a period of about 10^{-13} sec. According to the Frank-Condon principle, an electronic transition in a molecule takes place so rapidly compared to the vibrational motion of the muclei that the internuclear distance can be regarded as fixed during the transition. The absorption of light energy by organic compounds in the ultraviolet (wave length 100 - 400 nm) and visible region (400 - 800 nm) involves promotion of electrons in δ, π and n-orbitals from the ground state to higher energy states. These higher energy states are described by molecular orbitals that are vacant in the ground or unexcited state and are commonly called anti-bonding orbitals. The energy needed for this transfer lies in the range of 40 - 300 kcal mol^{-1}.

Electrons in the vast majority of molecules fall into one of the three classes : $\underline{\delta}$ electrons, $\underline{\pi}$ electrons and non-bonding or $\underline{n}$ - electrons. A single bond

between atoms such as C — C, C — H, O — H etc., contains only $\underline{\delta}$ electrons while a multiple bond, C = C, C ≡ C, C=N etc., contains $\underline{\pi}$ electrons in addition. Many molecules contain electrons that are not directly involved in bonding; these are called $\underline{n}$ electrons and are mainly located in atomic orbitals of oxygen, sulphur, nitrogen, and the halogens. In general $\underline{\delta}$ electrons are most firmly bound to the nuclei and hence require a great deal of energy to undergo transitions to antibonding orbitals ($\delta \rightarrow \delta^*$) while $\underline{\pi}$ and $\underline{n}$ electrons require less energy, the $\underline{n}$ electrons usually requiring lesser energy than π (Figure 14). As the $\underline{n}$ - electrons do not form bonds, there are no antibonding orbitals associated with them. Thus $\delta \rightarrow \delta^*$ transitions fall into the vaccum ultraviolet, $\pi \rightarrow \pi^*$ and $n \rightarrow \delta^*$ appear near the borderline of the near and far UV, and $n \rightarrow \pi^*$ come well into the near - UV and visible regions. The life-time of an excited electronic state is about 10^{-8} sec.

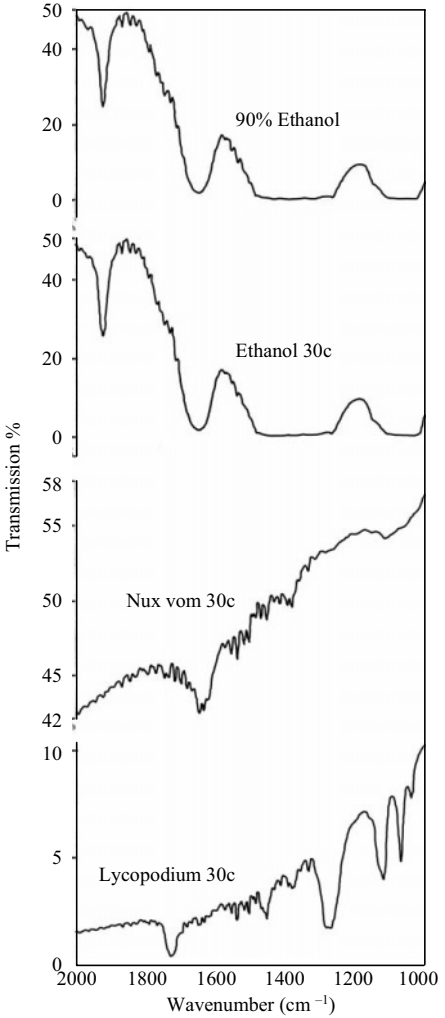

Figure 13. *FTIR spectra of 90% ethanol, Ethanol 30, Nux vom 30c and Lycopodium 30c obtained by a Jasco FTIR spectrometer (model 420) at 20ºC in the wave number region of 2000-1000 cm^{-1}. All the potencies were in 90% ethanol. There is marked variation in v_2 bands (around 1596 cm $^{-1}$) in the spectra.*

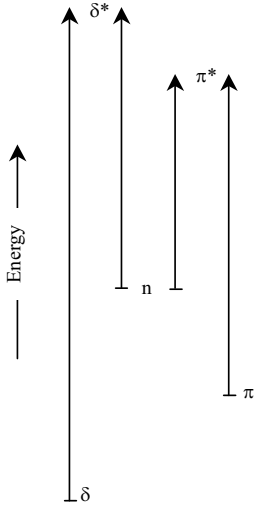

Figure 14. *Diagram showing electronic energy levels under excitation*

The plots produced by most UV-VIS spectrophotometer show absorbance A (or optical density, OD) versus wavelength (λ). When a compound does not absorb any radiation at a particular wavelength there would be 100% transmission. Absorption of radiation at a particular wavelength leads to a decrease in the percent transmission to appear in the spectrum as a dip, called a peak, or absorption band. Absorbance (A) is a measure of the absorption of radiation by a sample.

$$A = \log \frac{Io}{I}$$

where 1o is the intensity of incident light and I, the intensity of transmitted light. The calculation of the intensity of an absorption band involves the use of Lambert's and Beer's laws. Lambert's law states that the intensity of transmitted light passing through a homogeneous medium decreases geometrically as the thickness of the layer increases arithmetically. Beer's law states that each molecule of a solute absorbs the same fraction of light incident upon it, regardless of concentration, in a non-absorbing medium. Beer's law does not hold over the entire concentration range, but in very dilute solutions. These laws can be formulated by the relationship

$$\varepsilon = \frac{A}{cl}$$

where ε is the molar extinction coefficient, c the molar concentration and l the path length in cm. Experimental results are usually reported

in terms of the molar extinction coefficient ε or its logarithm log ε. The magnitude of the molar extinction coefficient for a particular absorption is directly proportional to the probability of the particular electronic transition; the more probable a given transition the larger the extinction coefficient.

There are three main factors which contribute to spectral intensities. These are: (1) **transition probability** or the likelihood of a system in one state changing to another state, (2) the **population or** the number of atoms or molecules initially in the state from which the transition occurs and (3) the **concentration** or path length of the sample. The first factor involves a knowledge of the precise quantum mechanical wave functions of the two states between which the transition occurs. Transition between all the electronic, vibrational and rotational states are not equally allowed. Some are forbidden. The rules which govern such transitions are known as selection rules. The interaction of electromagnetic radiation with matter leads to absorption only if a dipole moment is created as a result of such interaction. The strength or intensity of absorption is related to the dipole strength of transition. The second factor can be explained in the following way. If there is a total N molecules distributed between two energy levels, a lower and an upper, then most of the molecules are expected to occupy the lower state. The third factor involves the path length of the sample. If a sample is absorbing energy from a beam of radiation, the more sample the beam traverses the more energy will be absorbed from it (Banwell and McCash, 2000; Rohatgi Mukherjee, 1992). The relationship between concentration (c), path length (l), and the incident and transmitted intensities of radiation (Io and I, respectively), which is based on Beer-Lambert law, has already been mentioned.

Charge-transfer (CT) interaction

When two molecules, which form loose complexes, are mixed together, a new band appears. Here the electron of one molecule absorbs a quantum of radiation and is excited, not to a higher energy level of the same molecule but rather to one of the vacant high energy levels of the neighbouring molecules. The transition ocurs from the highest filled molecular orbital of the donor to the lowest empty molecular orbital of the acceptor. Promotion of an electron to higher unoccupied orbitals of the acceptor can occur to give additional CT absorption bands. There are two types of donors, $\underline{\pi}$ - donors and $\underline{n}$ - donors. In the former, the electron available for donation is located in the π - molecular orbital of the molecule, e.g. aromatic hydrocarbons, alkenes, alkynes etc. They form $\underline{\pi}$ - complexes. In the latter, a non-bonding electron is transferred, e.g. alcohols, amines, ethers etc. The energy needed to transfer the electron is related primarily to the ionization potential; the lower the quantum of energies, the longer is the wavelength of radiation absorbed (Rohatgi-Mukherjee, 1992; Singh and Dikshit, 1995).

Solvation and electronic spectra

The ground and excited-state energies of a solute are modified by the organization of a solvent about the solute molecules. Solvation is a complicated process that depends on both nonspecific interactions arising from electrostatic and polarization forces as well as specific forces such as hydrogen bonding. The effects of a solvent are both static and dynamic. Many molecules exhibit solvatochromism, i.e., the position of the molecule's electronic UV/VIS absorbance band shifts with the polarity of the solvent. The direction and magnitude of a solvatochromic spectral shift depends on the solvent/solute interactions. Generally, a red shift with increasing solvent polarity occurs when the excited state is more dipolar than the ground state. $\mu g < \mu e$, where μg and μe are the ground and excited-state permanent dipole moments, respectively. The electronic transition energy decreases because the more dipolar excited state is stabilized by more polar solvents. A blue shift with incerasing solvent polarity occurs when the excited state is less dipolar than the ground state ($\mu e < \mu g$). The electronic transition energy increases because the less dipolar excited state is less stabilized than the ground state for more polar solvents.

Solvation has also dynamic properties as in the case when a solvent reacts to an instantaneous photophysical change in the solute's charge distribution. The process is responsible for the ultrafast time dependent shift of the fluroscence spectral peak (Stoke's shift). If the ground and excited states of a solute have sufficiently different permanent dipole moments ($\mu g \neq \mu e$), the polar solvent reorganizes about the solute upon photo-excitation. Initially, the solvent shell is organized in a way to minimize the free energy of the ground state with dipole moment μg. Immediately upon excitation the solute has the dipole moment μe. However, the solvent shell is still in a configuration best suited for the ground state. The solvent shell rapidly reorganizes in reaction to the changed dipole moment of the excited state solute, decreasing the energy between the ground and excited states. This solute reorganization occurs on a time scale characteristic of the solvent (Zimdars and Eisenthal, 2001).

3.3.1. Electronic spectra of homeopathic potencies

Homeopathic potencies are usually produced in 90% ethanol or pure water. We used three different UV-VIS spectrophotometers (Beckman DU 640/Jasco-V-530/Shimadzu UV-1601) and obtained electronic spectra of mother tinctures and potencies of many homeopathic drugs. The spectra of mother tinctures and potencies are given in Figures 15-18. Earlier we reported the spectra of 90% ethanol and such drugs as *Cina* θ, *Cina* 30, *Nux vom* θ and *Nux vom* 30 (Sukul *et al.,* 1999, 2001 ab). The spectra were obtained in the wavelength range of 190 nm to 700 nm or 190-290 nm at room temperature against appropriate solvent blanks. The samples were kept at the specified temperature for at least 10 min to allow for thermal equilibration. The spectra were run in matched quartz cuvettes and were corrected for instrumental base line errors. In general, the figures show characteristic spectral absorption pattern of different mother tinctures and their potencies. While mother tinctures show absorption in both the ultraviolet and visible ranges of radiation, the potencies

absorb only in the UV region. Potencies in water usually show a blue shift as compared to the potencies in 90% ethanol (Figure 18). Stable rigid structures of water molecules similar to ice VI can be grown in water at room temperature and normal pressure by ultra small amounts of acid, base or salt. These structures have different UV transmission characteristics from pure water (Lo, 1996).

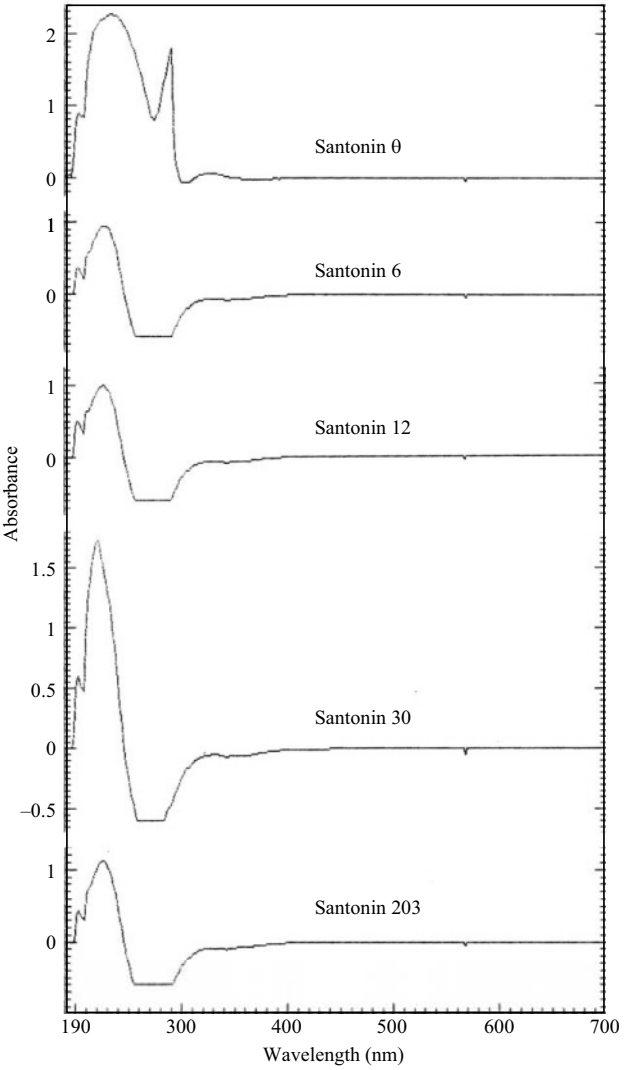

Figure 15. *Electronic spectra of Santonin θ, 6, 12, 30 and 206 in 90% ethanol obtained against blank 90% ethanol with a UV-VIS spectrometer (Shimadzu UV-1061)*

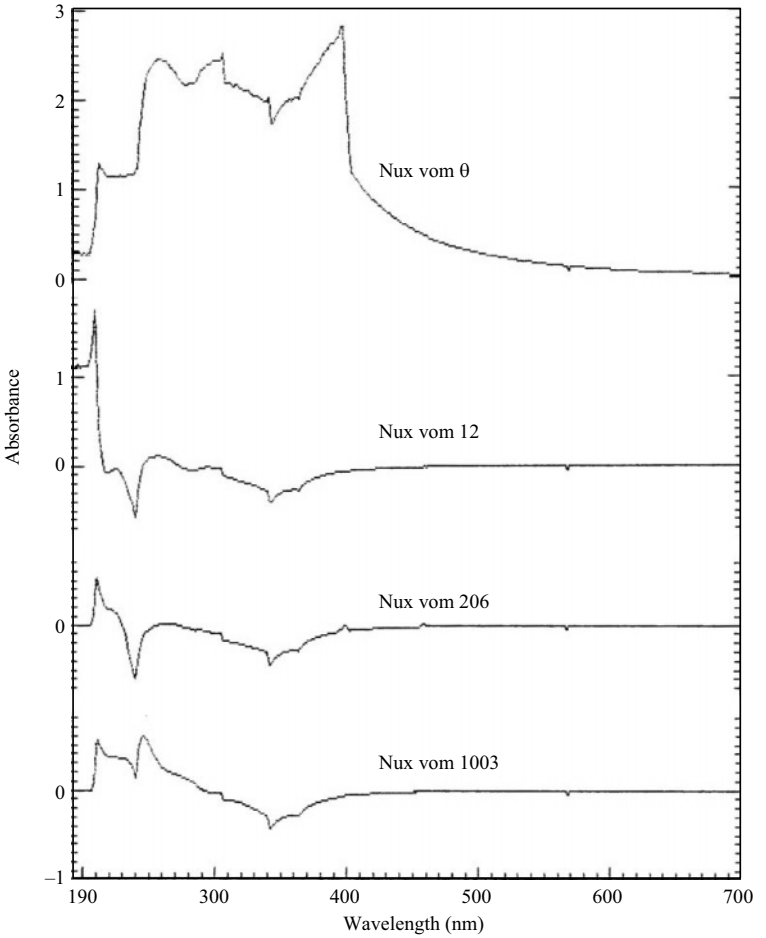

Figure 16. *Electronic spectra of Nux vomica* θ*, 12, 206 and 1003 in 90% ethanol obtained against blank 90% ethanol with a UV-VIS spectrometer (Shimadzu UV-1601)*

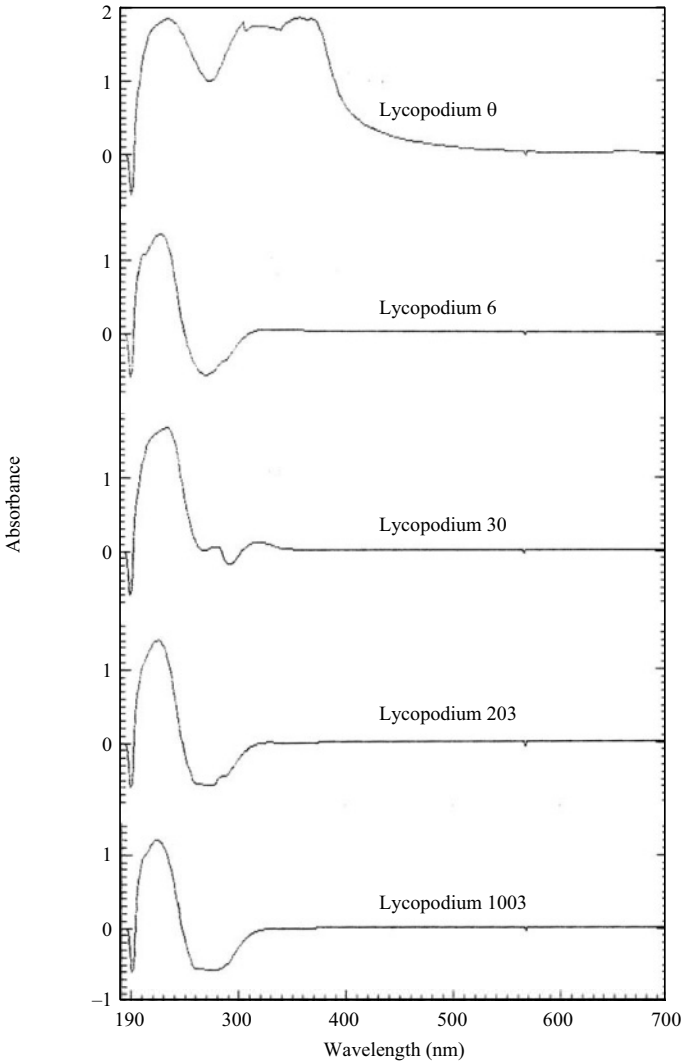

Figure 17. *Electronic spectra of Lycopodium* θ*, 6, 30, 203 and 1003 in 90% ethanol obtained against blank 90% ethanol with a UV-VIS spectrometer (Shimadzu UV-1601)*

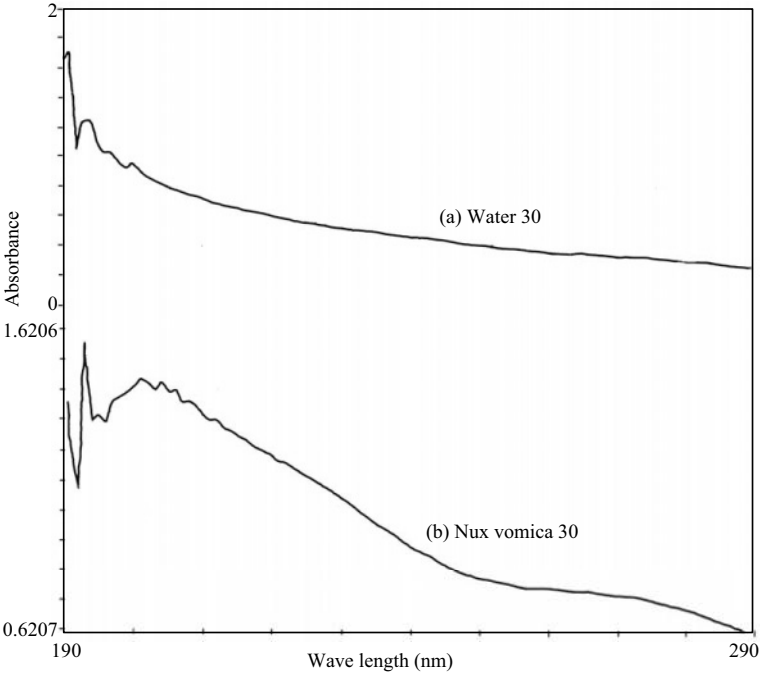

Figure 18. *Electronic spectra of water 30 (a) and Nux vomica 30 (b) in water against blank distilled water obtained with a UV-VIS spectrometer (Beckman DU 640). Both water 30 and Nux vom 30 were freshly prepared in distilled water*

Energy of UV radiation absorbed by a molecule was correlated with the transition of an electron in the molecule from a lower to a higher molecular orbital (Dani, 1995). Solvation by a polar solvent stabilizes π, π* and n orbitals ; the stabilization of non-bonding orbitals is particularly pronounced with hydrogen-bonding solvents (such as water or ethanol) and π* orbitals are more stabilized by solvation than are π orbitals, presumably because π* orbitals are more polar (Kemp, 1991). Both water and ethanol have an auxochromic group OH. This group has non-bonding (n) electrons. Transitions involving these n electrons are responsible for the increased intensity of absorption peaks as in potentized drugs. The absorption characteristics in the UV region depend on the electronic transitions ($n \rightarrow \delta^*$) that occur and the effect of the atomic environment on the transitions (Silverstein *et al.,* 1981). Self-association and intermolecular association in water and ethanol produce strongly bound complexes resulting in increased intensity of absorption in the UV region. Mechanical agitation such as succussion or sonication produces random collision of molecules, cluster of molecules or solvated micelles of ethanol resulting in the formation of strongly bound complexes (Connors 1987). Sonication of

aqueous ethanol makes it more compact and homogeneous with increased density (Haseba *et al.,* 1993). The increased density is also responsible for an increase in the intensity of spectral absorption. While mother tinctures show marked variation in spectral pattern, potentized drugs do not show much variation with this respect.

Alcohols self-associate via H-bonding resulting in the formation of many different structures such as free units, cyclic dimers, trimers, and tetramers, linear dimers and higher multimers (Schwager *et al.,* 1996). Water molecules also self-associate to form complex arrangements of trimers, tetramers, pentamers and hexamer systems (Kusalik and Svishchev, 1994). The H-bond network in liquid water is roughly characterized by tetrahedral arrangements of bonds where on the average about 50% of the molecules engage in four H-bonds (Ladanyi and Skaf, 1993). For alcohols, the fraction of molecules participating in n H-bonds is more sharply distributed and centered around n = 2. The network consists of chains that have considerable degree of branching and free ends (Ladanyi and Skaf, 1993 and references therein).

3.4. Spectrofluorometry

Luminescence is divided into two categories, fluorescence and phosphorescence. Fluorescence is the emission of light by excited molecules as they return to the ground state (GS). In the excited singlet states, the electron in the excited orbital is paired (of opposite spin) to the second electron in the GS orbital. Consequently, the return to the GS is spin-allowed and occurs rapidly by emission of a photon, a unit of radiation. A typical fluorescent lifetime is 10 ns. The life-time of a fluorophore is the average time between its excitation and its return to the GS. Let us explain the term singlet. The electrons in the GS molecule are paired and have opposite spin orientations, i.e., they have antiparalled spins, $\uparrow\downarrow$. When one of the electrons undergoes transition to a higher electronic level, its spin orientation is unchanged. This is Frank-Condone principle. Thus the two electrons in the excited state (ES) are in antiparallel spin orientations and this is called a singlet state. Molecular spins are additive, and in the singlet excited state only one net spin energy level results. The singlet state molecule can lose energy to form the triplet state. In the latter, spins of the two orientations are parallel and this leads to three slightly different spin energy levels. The triplet state is stabler than the singlet state.

Phosphorescence is emission of light from triplet excited states, in which the electron in the excited orbital has the same spin orientation as the ground state electron. Transitions to the GS are forbidden and the emission rates are slow, so that phophoresence life times are typically milliseconds to seconds.

In the fluorescence emission, the most probable transition to the ground state depends on the interneuclear equilibrium geometries of the molecules in the two states. If the internuclear geometries are not very different, as in many polyatomic molecules, the fluorescence spectrum appears as a mirror image of the corresponding absorption spectrum with a region of overlap (Rohatgi-Mukherjee, 1992). Due to this working of the Frank-Condon principle and thermal relaxation of vibrational modes, the fluorescence spectrum is always observed on the red side of

the absorption spectrum. This red shift of the fluorescence implies that the emitted quanta are of lower energy than the absorbed quanta, the Stoke's shift. Mirror image relationship in fluorescence spectra is not observed if the excited state has very different geometry from that of the ground state as is usually the case for small unconjugated molecules.

There are two types of solute-solvent interactions which affect absorption and emission spectra. These are universal interaction and specific interaction. The universal interaction is due to the collective influence of the solvent as a dielectric medium and depends on the dielectric constant D and the refractive index n of the solvent. Thus large environmental perturbations may be caused by van der Waals dipolar or ionic fields in solution, liquids and in solids. The van der Waals interactions include (i) London dispersion force, (ii) induced dipole interactions, and (iii) dipole-dipole interactions. These are attractive interactions. The repulsive interactions are primarily derived from exchange forces (non bonded repulsion) as the elctrons of one molecule approach the filled orbitals of the neighbour. If the solute molecule has a dipole moment, it is expected to differ in various electronic energy states because of the differences in charge distribution. In polar solvents dipole-dipole inrteractions are important.

Many solute-solvent interactions are more complex and specific than the universal interaction described above. If there are low lying unfilled orbitals in the solvent, it is likely to have a strong affinity for electrons. If the solvent accepts electrons from the solute molecules, a charge-transfer-to-solvent complex is formed. The donor capacity of the solute in the ground and excited states are expected to be different. Another specific type of solute-solvent interaction is the formation of hydrogen bonded complexes and exciplexes. The latter occurs during the transient complex formation in the excited state. In $n \rightarrow \pi^*$ transition nonbonding lone pair on the heteroatom is hydrogen bonded in the ground state. This results in a greater decrease in the ground state energy for more polar and hydrogen bonding solvents. The excited state is not much depressed as the promotion of the n-electrons into the π^* orbitals reduces the hydrogen bonding forces in the excited state. The result is a blue shift. A charge transfer state has a much greater permanent dipole moment than the ground state and, therefore, change to a polar solvent results in a considerably larger red shift. The effect of solvent on the absorption and emission spectra may give valuable information regarding such physical properties as dipole moment changes and polarizability of the molecule in the two combining states (Rohatgi-Mukherjee, 1992).

3.4.1. Application of fluorescence spectroscopy to homeopathy

Although all molecules are capable of absorption, only a few of them are capable of fluorescence. The phenomenon is generally observed in those organic molecules which have a rigid framework and not many loosely coupled substituents through which vibrionic energy can flow out. Fluorescence occurs mostly in molecules with conjugated system of double bonds such as C = C, N = O, - N = N, - C = O, - C = S. These parts of the molecules are called fluorophores. Some substituents enhance

fluorescence and are called fluorochromes. These are electron donors such as -OH, NH_2 etc. The diluent media of homeopathic potencies are water and aqueous ethanol which are not fluorescent. But they may produce solvent effect on a fluorescent organic molecule thereby altering the peaks and intensity of absorption and emission spectra. They may also form complexes with fluorescent molecules thereby altering the fluorescence intensity. Most applications of fluorometry to binding have made use of fluorescence intensity measurements. Suppose S is the fluorescence species, and L, the ligand (here a homeopathic potency). On binding of these species, a decrease in fluorescence occurs. This phenomenon is called quenching, and it takes place when the emission band of fluorescing species overlaps the absorption band of the quencher. At low concentrations the fluorescence intensity is directly proportional to fluorescent solute concentration according to $F = 2.3\ I_o\phi\varepsilon bc$, where F is fluorescence intensity, I_o is the intensity of the excitation source, ε is molar absorptivity at the excitation wavelength, b is path length, c is molar concentration, and ϕ is fluorescence quantum field. Because of this direct proportionality, the quantitative treatment of fluorescence binding data can be carried out in the same manner as that for absorption spectroscopy (Connors, 1987).

Homeopathic potencies are administered through the oral route where they come in contact with saliva which comprises 99% water. Saliva contains a number of proteins including amylase (Mandel 1989, Aguirre *et al.,* 1993). In proteins, the dominant fluorophore is the indole group of tryptophan. Indole absorbs near 280 nm and emits near 340 nm. The emission spectrum of indole is highly sensitive to solvent polarity. The emission of indole may be blue-shifted if the group is buried within a native protein, and its emission may shift to longer wavelengths (red shift) when the protein is unfolded.

α-Amylase contains tryptophan and tyrosine which are fluorescent. With Exλ at 280nm, tyrosine has fluorescence peaks at 336 nm (intensity 9696) and 628nm (intensity 9714). With the same Exλ tryptophan has the peak at 368 λ (intensity 1437). The amino acids were dissolved in sodium acetate buffer (pH 4.7) with distilled water (5ml). Using a Jasco spectrofluorometer (model FP-777) emission spectra were obtained with α-amylase in 90% Ethanol 30, *Merc cor* 30 in 90% ethanol, distilled water 30 and *Merc cor* 30 is distilled water. The pure aquous potencies were prepared fresh. The results are presented in Table 6.

Table 6. Fluorescence peaks and intensities in parentheses with excitation λ at 280nm of α-amylase containing tyrosine and tryptophan dissolved in 90% ethanol 30, distilled water, Merc cor 30 in 90% ethanol and Merc cor 30 in distilled water. Sodium acetate buffer (pH 4.7) was also used.

Substances	Peak 1 (intensity)	Peak 2	Peak 3 (intensity)
1. α-amylase + 90% ethanol 30 + buffer	282.5 (7688)		563 (2251)
2. α-amylase + Merccor 30 in 90% ethanol + buffer	284 (6030)		564.5 (2849)

(Cont.)

Table 6 (Cont.)

Substances	Peak 1 (intensity)	Peak 2	Peak 3 (intensity)
3. α - amylase + distilled water 30 + buffer	283.5 (2521)		562.5 (838)
4. α - amylase + Merc cor 30 in distilled water + buffer	283.5 (7536)	343 (1154)	562.5 (2060)

The peaks of emission spectra show marked variation in the Stokes' shifts. This is because of the variation in unfolding of the protein exposing the fluorescing amino acid residue in different ways. This variation is due to different solution structure in potentized drugs as compared to their diluent media.

3.5. Thermoluminescence

Thermally stimulated luminescence or thermoluminescence is applied to study the structure of solids, mainly ordered crystals. The sample material is activated at low temperature by radiant energy like UV, X-rays, γ-rays etc which creates electrons-holes pairs which are separately trapped at different energy levels. When the irradiated sample is warmed up, a characteristic glow is emitted in the form of different peaks according to the depth of the initial traps. Using this technique Rey (2003) demonstrated that *Lithium chloride* 15c and *Sodium chloride* 15c produced thermoluminescence characteristic of the original solution of the respective salts. The effect has been attributed to a specific change in the hydrogen bond network of the ultra high dilution of each salt.

3.6. Molecular association and water structure

We have already mentioned that effective homeopathic potencies are prepared in water or a mixture of water and ethanol. So, in order to understand the physical basis of a potency we should look for the structure of water and also of ethanol/water mixture. The physical properties of liquid water are not yet completely understood (Bruscolini and Casetti, 2001). However, attempt would be made to focus on that physical aspect of water which carries the information of a drug at an ultra high dilution.

Water is composed of one oxygen and two hydrogen atoms. Oxygen has six electrons, two of which are used in bonds with hydrogen. A lone pair of electrons exists on oxygen which is, therefore, electro-negative. So, molecular association would occur with oxygen attracting positively charged atoms such as hydrogen, and hydrogen attracting negatively charged atoms such as oxygen. Unlike water, ethanol can only hydrogen-bond at one end. Water and ethanol can serve as both electron donors and electron acceptors.

In the results of *in vitro* tests mentioned earlier in Chapter II, we have noted that pure water can serve as an effective medium for a homeoapthic potency. However, unlike the potency in aqueous ethanol, the efficacy of a potency prepared

in pure water deteriorates over time. So, the main role played by ethanol, in aqueous ethanol medim, is to retain the specific property of the aqueous potency. The specific property of an aqueous potency lies in the specific structure of water acquired during the process of potentization. Ethanol molecules are amphiphilic having a polar head and a non-polar tail (Figure 23). Since the ethanol molecule has a large non-polar part it can preserve or promote water structure specific to a potentized drug (Sukul *et al.,* 2002).

We have already mentioned that water molecules self-associate through hydrogen bonding, and various forms of polymers exist in bulk water. Same is the situation with ethanol. In hydrated ethanol inter-molecular association between water and ethanol molecules occurs through H-bonding. A hydrogen bond exists when (1) there is evidence of a bond and (2) this bond involves a hydrogen atom already bonded to another atom. Thus the bond formation process can be written

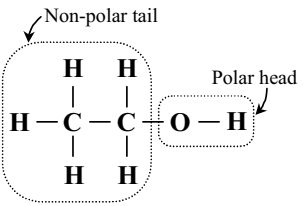

Figure 19. *Polar head and non-polar tail of an ethanol molecule*

$$A — H + B \qquad A — H \ldots B$$

with the nature of the hydrogen bond denoted by dots, being of paramount interest. Calculations of the contributions of the several interaction energies to hydrogen-bonded complexes suggest that the electrostatic component is usually dominant, with a small but significant contribution from charge transfer. There is an indication that very strong hydrogen bonding is primarily electrostatic and that very weak hydrogen bonding has a relatively larger charge-transfer component (Connors, 1987). In a homeopathic potency prepared with aqueous ethanol there is both intra and inter-molecular association between water and ethanol molecules through H-bonding. Since the electrostatic component is the dominant force in H-bonding, succussion or mechanical agitation would contribute to an increase in that electrostatic force and make the H-bonding stronger. This is one of the possible roles succussion or sonication can play during the process of dynamization of a drug.

3.6.1. Structure and dynamics of liquid water

As mentioned earlier each hydrogen atom of a water molecule shares an electron pair with oxygen atom. The outer electron orbitals of the oxygen atom form a rough tetrahedron, with a hydrogen atom at each of two corners and unshared electron pairs at the other two corners (Figure 20). The oxygen nucleus attracts electrons more strongly that the hydrogen nucleus. This unequal electron sharing results in two electric dipoles in water molecules, one along each of H — O bonds. The oxygen atom bears a partial negative charge (2 δ^-), and each hydrogen atom a partial positive charge (δ^+). As a result there is an electrostatic attraction between the oxygen atom of one water molecule and the hydrogen of another, called the hydrogen bond. The nearly tetrahedral arrangement of the orbitals about the oxygen atom allows each water molecule to form hydrogen bonds with four neighbouring water molecules. Water molecules surrounded by four hydrogen bonds tend to clump together, for both statistical and energetic reasons. Hydrogen-bonded chains (i.e. O-H….O-H….O) are cooperative (Dannenberg, 2002). In liquid water at room temperature and atmospheric pressure, water molecules are disorganized and in continuous motion. Characterizing and visualizing water structures, their dynamical properties and their distortion resulting from the presence of solutes have been possible through computer simulation. Experimental characterization of the structure of H-bonded liquids involves a combination of X-ray, neutron, and electron diffraction techniques (Ladanyi and Skaf, 1993).

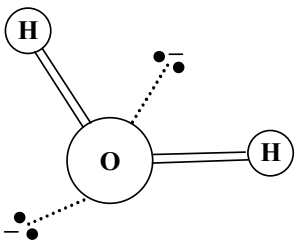

Figure 20. *Unshared electron pairs on the oxygen atom of a water molecule*

The structure of water is related to the structures of hexagonal boat-form and chair-form ice that exist at atmospheric pressure. The structure may be folded, in three dimensions, to form an icosahedral network, based on the regular arrangement of 14-molecule units. Twenty of the 14-molecule units, comprising in total 280 molecules of water, may form a 3nm diameter icosahedral structures which may be strained with increased size (Doye and Wales, 2001). Icosahedron means a solid figure having twenty faces. The stability of the network is finely balanced, being able to fluctuate between an expanded low density structure and a more dense

collapsed one without breaking any hydrogen bonds and consequent on small changes in the hydrogen bond strength relative to the bonded interactions. The expanded structure with central dodecahedra is formed when stronger hydrogen bonds are present (Henry, 2002).

Dodecahedron means a solid figure having 12 faces. If the hydrogen bonds are such that non bonding interactions are more important, then the cluster forms the partially collapsed structure due to the formation of cubic cavity puckered dodecahedra. The calculated oxygen radial distribution function from the icosahedral cluster model is in agreement with the x-ray diffraction data thereby providing direct evidence for the model (Narten *et al.,* 1967). There are many examples of the formation of dodecahedral clathrates in aqueous solutions (Sobott *et al.,* 1999). The minimum number of possible arrangements of hydrogen bonds in the fully occupied low density icosahedral network (expanded structure) is 2^{130} x 7^{12} as determined during the molecular building. This is in agreement with the minimum entropic factor expected of structural variations per molecule (Eisenberg and Kauzman, 1969).

In the liquid state 50% of water molecules engage in four H-bonds, 30% in three bonds and smaller fractions participate in one, two, and five bonds (Ladanyi and Skaf, 1993). H-bonds are weaker than covalent bonds. The energy required to break an H-bond in liquid water is about 20 KJ/mol, compared with 348 KJ/mol for the covalent C — C bond. At room temperature, the thermal energy of an aqueous solution (the Kinetic energy of motion of the individual atoms and molecules) is of the same order of magnitude as that required to break H-bonds (Nelson and Cox, 2000). Defining H-bond life-time (τ_{HB}) is difficult, because H-bonds may appear to be broken by fast librational motion of the molecules though in fact they may just be distorted from their **equilibrium geometry**. At room temperature τ_{HB} has been estimated to be 0.8 ps (Ladanyi and Skaf, 1993 and references therein). Hydrogen bonds break and reform continuously. The strength of bonding must depend on the orientation and position of other bonded and non-bonded atoms. There is a trade-off between the covalent and hydrogen bonding strengths; the stronger is the H….O bond, the weaker the O-H covalent bond, and the shorter the distance. The weakening of the O-H covalent bond gives rise to a good indicator of hydrogen bond energy; the fractional increase in its length on hydrogen bonding (Grabowski, 2001). Every hydrogen bond formed increases the hydrogen bond status of two water molecules. The network essentially completes at ambient temperatures; almost all molecules are linked by at least one pathway. Broken bonds probably reform to give the same hydrogen bond (Tikhonov and Volkov, 2002). Dissociation is a rare event occurring only twice a day i.e. only once for every 10^{16} times the hydrogen bond breaks. Thus clusters can persist for much longer times(Higo *et al.,* 2001). Hydrogen bonding carries information about solutes and surfaces over significant distances in liquid water. The effect is synergistic, directive and extensive. The effect is reinforced by additional polarization effects and the resonant intermolecular transfer of O-H vibrational energy, mediated by dipole-dipole interactions and hydrogen bonds (Woutersen and Bakker, 1999). Reorientation of one molecule induces corresponding motions in the neighbours. The relative proportions of different

polymers of water are at dynamic equilibrium of specific geometrical configuration. It is assumed that this **dynamic geometric configuration** of water clusters in a collective way confers specificity on a potentized homeopathic drug. For ethanol in solution the hydrogen bond reassociation lifetime τ_r does not depend on the ethanol concentration. This implies that in solution the sequential hydrogen bond dissociation and reassociation occurs between the same O — H groups (Bonn *et al.,* 1996 and references therein).

Many of the unqiue properties of water are related to the existence of hydroxide (OH^-) and hydronium (H_3O^+) ions in pure water. The size of aqueous clusters, OH^- $(H_2O)n$, H_3O^+ $(H_2O)n$, of these fundamental ions associated with water can be varied to effect the degree of solvation (Wood *et al.,* 1994). Water clusters are divided into two classes : bulk clusters, which are in contact with a site ooccupied by water, and shell ones, which are in contact with a site occupied by a monomer. The m water molecules belonging to a given cluster are allowed to form a maximum of 3m/2 hydrogen bonds (Bruscolini *et al.,* 2001). In water, cyclic structures are the most stable for trimer, tetramer and pentamer. For the hexamer, a non-cyclic cage-like structure is found to be most stable. The water octamer and larger clusters are known to prefer three-dimensional structures (Gregory and Clary, 1996 and references therein). Several experiments have demonstrated the $(H_2O)_2$ H^+ cluster to be 'magic', in the sense that it is significantly more stable than the neighbouring clusters under different experimental conditions. Natural water clusters of the size of 20 molecules and above are already bulk-like with 4-fold coordinated molecules, and the clathrate or fullerene-like 3-fold coordinated structures are much higher in energy (Laasonen and Klein, 1994).

Transport phenomena in water

Two classes of transport phenomena are recognised in liquid water : ordinary mass diffusion and the abnormal proton mobility. Proton conduction is a crucial process in many areas of Chemistry and Biology. Compared to all other electrolytic species typical proton mobilities appear to be anomalously high, indicating that normal ionic diffusion plays only a secondary role. Instead, the charge migration is driven by successive jumps of protons from one oxygen to the next (Tuckerman *et al.,* 1995 and references therein). Proton mobility occurs on the time scale of water reorientation. Dielectric relaxation and self-diffusion are slower and show a stronger temperature dependence. They correspond to water molecules hopping across tetrahedral site-vacancy distance l = 3.3 Å. Water dynamics show numerous cooperative, random-like, and chaotic type behaviours which are difficult to rationalize. However, embedded in these complex dynamics, one could identify quasi-simple molecular motions which are functionally important. Water reorientation and tetrahedral displacement seem to be such motions, as they give rise to transport properties of water (Agmon, 1996).

Using spectroscopic techniques, it has been demonstrated that the defect charge in water is localized in the form of long-lived simple water ions : the approximately symmetrical and planar H_3O^+ (hydronium) cation and the linear OH^- (hydroxyl)

anion. Hence, the rate-limiting step for proton transport in the liquid might not be the actual proton transfer through a hydrogen bond between water ion and a neighbouring molecule, but the formation and breaking of hydrogen bonds in the solvation shells of the ions. This is the basis of the **structural diffusion model**, which has been developed in detail for the H_3O^+ ion. In accordance with the original Grotthus picture (jumping of proton from one oxygen to the next), it is this solvation structure that migrates, not the particles themselves (Tuckerman *et al.,* 1995 and references therein).

Action of a homeopathic potency

It appears that the pattern of structural diffusion (formation and breaking of H-bonds) is specific to a particular homeopathic potentized drug. This pattern varies with different potentized drugs. Biological membranes are bathed in continuous water molecules. The moment a potentized drug is introduced into this continuous water film, the structural diffusion pattern is reset in accordance with the inherent structure of the particular homeopathic potency. Since the water film adheres to protein and oligosaccharide molecules of plasmamembranes, the structural pattern is stabilized until a new diffusion structure is added. This resetting of structural parttern triggers a cascade of biochemical reactions inside the cells exposed.

Water structure in presence of solutes

We have already noted that homeopathic potencies are produced from mother tinctures of a drug. The mother tincture may contain a single compound, such as, sodium chloride or a mixture of many different compounds such as *Nux vomica* θ. During the process of potentization, the mother tincture is diluted with a solvent medium, water or aqueous ethanol, successively. During this process, the original drug molecules are progressively depleted and at a certain point, say after the 12th potency, all the drug molecules are lost. The potentized drug then consists of only the molecules of the diluent medium, water or a mixture of water and ethanol. It is assumed that the medium is specifically structured through specific hydrogen bonding. The specific structure is retained under various conditions even when the potency is diluted with tap water. A potency mixed with tap water and then administered on a patient or on experimental animals has been found to be effective. Let us now examine what happens when a solute is added to the diluent medium, say water.

Water tends to hydrate many solutes. The hydration number is the number of water molecules bound to solute sufficiently strongly so as to become part of it. The hydration number varies with different solute molecules : Na^+, 3.9 ± 0.5; Ca^{2+}, 12 ± 2; Al^{3+}, 22 ± 2; glycerol, 2 ± 0.5; sucrose 5 ± 0.5; Fe^{3+} 18 ± 2. Water molecules form a hydration shell around the solute molecules. In case of molecules such as Al^{3+}, the large number of water molecules is unlikely to be accommodated in the first hydration shell. X-ray diffraction has shown a highly ordered second hydration shell around Al^{3+} (Zavitsas, 2001).

Both water and ethanol are polar solvents. Ions interact strongly with polar solvents, causing substantial modifications in the local structure and changes in the dynamics of the surrounding solvent molecules. Simulations indicate that the H-bond network of water is perturbed in the vicinity of singly charged cations. Specifically, the number of H-bonds per solvent molecule in the vicinity of Na^+ ions is significantly smaller than in the bulk solvent (Ladanyi and Skaf, 1993). According to Frank and Wen (1957) and Bockris and Saluja (1972) models cited by Dutta (1997), there are two layers of water molecules surrounding the ions. The solvational coordinated molecules (SCW) of water are frozen to the ion and move along with the ion during its motion through the solution. The non-solvational coordinated molecules (NSCW) of water, though present in the vicinity of the ion, are not rigidly bound and hence are left behind.

During successive dilution, ions and their SCW molecules are progressively depleted and NSCW molecules are increased in number. It is the hydrogen-bonded structure of NSCW molecules that retain some sort of structural specificity of solute molecules in a potentized drug and are responsible for the biological or therapeutic effect. Computer simulations show that water residence times are much larger in the first solvation shells of small alkali ions (Li^+, Na^+) than in the first solvation shells of larger ions. The fact that water is more strongly attached to small ions also is evident in the behaviour of the ion VACF φi (t) and the velocity correlation φ_S (t) of the surrounding solvent shell. VACF means velocity autocorrelation function. For small alkali ions the two functions are nearly identical, indicating that this moves with the ion on the time scale relevant for the velocity correlations. In the case of larger ions and of water itself, the two functions are substantially different, indicating that the coupling of the first shell to the surrounding solvent plays a significant role in its relaxation. For polyatomic ions such as ammonium the effects of coupling to the surrounding solvent are evident in orientational relaxation. Reorientation of ammonium ion in water is hindered, i.e. it exhibits high torque behaviour according to Steele criteria (Ladanyi and Skaf, 1993 and references therein).

Apolar solutes are only slightly soluble in water, because their interactions with water molecules are much less attractive than are water-water interactions. Experiments show that water becomes more structured and less mobile in the vicinity of these molecules. For example, the entropy of solvation of these species is large and negative, and NMR and dielectric measurements show the decrease in water mobility in their vicinity. H-bonds in the vicinity of apolar solutes are stronger than those in the bulk and longer lived. The H-bonds are arranged predominantly at a tangent to the surface of apolar solutes forming clathrate-like structures that rearrange on the time-scale of a few picoseconds at room temperature. Ions with high charge density and apolar species represent the two extremes of aqueous solvation. The differences between the bulk and first solvation shell are less pronounced for other solute types (Ladanyi and Skaf, 1993 and references therein). Solute with the smaller site diameters is a much better hydrogen-bond acceptor than the larger diameter solute. There exists evidence for specific solvation due to solute-solvent H-bond formation (Skaf and Ladanyi, 1996 and references therein).

In case of non-ionic surfactants in water, the behaviour of the water structure outlines three main concentration regions, which closely coincide with the three phases intersected by the experimental isotherms. In the micellar solution phase, no significant changes in the water structure are indicated, while, in the lamellar phase, rapid destruction of the tetrahedral hydrogen bond network occurs due to the confinement of the water between the hydrophilic surfaces of the lamellae. The dehydration of the surfactant head groups was found to start near the border between the lamellar and the reverse micellar solution phases. At higher concentrations, water demonstrates its trend to form clusters of tetrahedrally bonded molecules even at the very low content in the system. The results with surfactant solutions have been obtained by Raman spectroscopy (Marinov *et al.,* 2001).

Trituration and nano particles

As mentioned earlier (Chapter I, 1.4), dry solids and substances which are not soluble in water, are potentized initially by trituration to liberate their dormant medicinal properties. Actually by repeated grinding with lactose powder medicinal substances are broken down into nano (10^{-9}m) particles. Nano particles assume special importance because of their bio-activity. In the second edition of *Chronic Diseases,* Hahnemann recommended that all medicines, the juices of plants, as well as the earths, metals, salts, dry woods or barks, should be prepared by trituration up to the third attenuation and then by dilution with aqueous ethanol followed by succussion for higher potencies. When potencies are produced from the 3rd attenuation, the nano particles would induce specific water structures. It has recently been discovered that specific nano particles appear in organs of patients suffering from a disease. Spherical nano particles have been isolated from the cerbrospinal fluid of patients with schizophrania. These particles may be involved in the development of schizophrania or may result from the disease process in brains of schizophranic patients (Wetterberg *et al.*, 2002). Micro and nano particles have been found in livers and kidneys affected by cryptogenic granulomas. These are inorganic particles of varying chemical composition, but consistent in size. The particles in the liver are larger in size than those in the kidney. A new word ‘nano-pathology’ has been coined to correlate particles of specific sizes with a disease (Gatti and Rivasi, 2002). During proving, a substance may induce generation of nano particles in the provers resulting in development of symptoms of a certain disease. A potency prepared from that substance may ameliorate or cure a disease showing similar symptoms. Since diseased tissues are used to prepare nosodes, it may be that the nano particles in the tissues, when potentized, produce the opposite effect of curing a disease. Isolation of nano-particls from diseased tissues would help in the production of purer and more effective nosodes.

It is evident from the above that different solutes produce different water structures. In case of potencies produced from a single element such as *Ferrum met*, *Phophorus, Argentum metallicum, Bromium* etc. it is likely that the potency would contain one species of water structure having specific H-bond network with specific H-bonding strength. The water structure would depend primarily on the diameter of

the atom and also its charge. Potencies prepared from a mixture of compounds would consist of a mixture of different species of water structures. In case of nosodes prepared from proteins, lipids, decaying tissues etc., there would be several species of water structures of different H-bond networks and conformation. It appears that a potentized drug, derived from a single element, single compound or a mixture of many compounds, assume an integrated H-bonded network of water structures having a **geometric configuration** specific to the drug. The specific structure is maintained even if the potentized drug is diluted with distilled water or tap water.

Aqueous ethanol as a medium

Homeopathic potencies are available in the market in the medium of aqueous ethanol where ethanol constitutes 80-91% of the liquid drug. Ethanol having a large non-polar part usually stabilizes water structures and preserves them for a pretty long time. As mentioned earlier complete dissociation with respect to H-bonds in watery medium occurs only twice a day. So, the H-bonding pattern specific to a homeopathic potency would degenerate in a few days. We have already mentioned in Chapter II (2.4.1) that *Merc cor 30* prepared in pure water and stored for about a year, failed to alter the activity of ∝-amylase *in vitro*. In the alcohol water mixture, the components tend to preserve to some extent the pattern of H-bonds they had prior to mixing (Skaf and Ladanyi, 1995). Alcohols with one hydroxyl group are characterized by H-bonding patterns which may be largely regarded as linear chains. This is very much different from the tridimensional network typical of water. Both structural and dynamical properties of liquid ethanol are strongly influenced by intermolecular H-bonds. Its structure is dominated by winding chains made up of a higher number of monomers at lower temperatures (Saiz *et al.,* 1997). Data from low angle X-ray scattering and Rayleigh light scattering indicate the formation of associated complexes between hydrated and non-hydrated alcohol molecules at increased alcohol concentration above 0.1 mole fraction. Alcohols behave in water similarly to other well known amphiphiles, and at relatively high concentrations can form some kind of pools or extended structures (Kuprin *et al.,* 1995 and references therein).

Summary

Homeopathic potencies above the 12th cross the Avogadro number, and as such do not contain any drug molecules. So, all higher potencies contain only the molecules of the medium, i.e. water and ethanol. However, NMR studies show that potentized drugs differ from each other and also from the medium 90% ethanol with respect to the spin-lattice relaxation time (T_1) and chemical shift of the deuterium nuclei. Infra red (IR) spectra of potentized drugs show variation in the vibrational frequencies of O-H, C-O and C-H bands. Fourier transform infra red (FTIR) spectra of potentized drugs show marked variation in O-H bending vibration (υ_2 band). Electronic and fluorescence spectra of potentized drugs show variation with respect to spectral

pattern, peaks and absorbance or intensities. All these results are indicative of the variation in hydrogen bonding and H-bonding strength among the potencies.

In a homeopathic potency in aqueous ethanol there is specific intra and inter-molecular association between water and ethanol molecules through H-bonding. Since the electrostatic component is the dominant force in H-bonding, succussion or any mechanical agitation would strengthen the H-bonding. The relative proportions of different polymers of water are at dynamic equilibrium of specific geometrical configuration. This dynamic geometric configuration of water clusters in a collective way confers specificity on a potentized homeopathic drug. The sequential H-bond dissociation and reassociation occur between the same O-H groups. It appears that the pattern of structural diffusion (formation and breaking of H-bonds) is specific to a particular homeopathic potentized drug. This pattern varies with different potentized drugs. Water tends to hydrate solute particles. The solvational coordinated molecules of water (SCW) are frozen to the particle and move along with the particle during its motion through the solution. The nonsolvational coordinated molecules of water (NSCW) are not rigidly bound and are left behind. During successive dilution, solute particles and their SCW molecules are progressively depleted and NSCW molecules are increased in number. It is the hydrogen bonded NSCW molecules that retain some sort of structural specificity of solute molecules in a potentized drug and are responsible for the biological effect.

Water-insoluble solids are potentized initially by trituration. This process breaks down drugs into nano particles which assume special importance because of their biological activity. Nano particles naturally occur in diseased tissues from which nosodes are prepared. The basis of liberation of dormant medicinal properties of drugs by trituration is the production of nano particles. Ethanol having a large non-polar part usually stabilizes specific water structures obtained during potentization of a drug.

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CHAPTER IV

MECHANISM OF ACTION OF POTENTIZED DRUGS

Before describing the details of the mechanism of action of homeopathic potencies in the living system we would discuss first the scientific basis of some aspects peculiar to Homeopathy. These are lateralities, time modalities, similia principle, non-linear systems, miasms, polycrests and holism.

4.1. Lateralities and Homeopathy

Homeopathy has long recognized the importance of the laterality of symptoms for the selection of the right remedy for a disease. The conventional medicine does not bother much whether the eruption or pain are on the right side of the body or on the left. There are many homeopathic medicines which are more effective on the right side than on the left and vice-versa. This aspect comes under the localized modalities of symptoms. As for example, *Kali carb, Lycopodium, Apis mel, Belladonna, Causticum, Merc sol, Naja* etc. are in general remedies for the right side of the body. *Lachesis, Spigelia, Sulphur, Argentum nit, Graphites, Psorinum, Rhus tox,* etc. are usually the remedies for the left side of the body. Sometimes symptoms move from the left side to the right (*Lachesis*) or from the right to the left (*Lycopodium*). Patients wonder why the doctor is asking for the laterality of symptoms.

Let us examine whether the laterality of symptoms has really any biological basis. It is true that the body of a man and of most of the animals are bilaterally symmetrical. There is some difference with respect to the internal organs. While the liver is located on the right side, the stomach and spleen lie on the left. The human brain, though bilaterally symmetrical anatomically, shows considerable asymmetry with respect to its function. About 95% of persons are right-handed, and in these people the left cerebral hemisphere plays a predominant role in speech, and the associational paths are situated in the left hemisphere. Nearly 60% of lefthanders show speech representation on the left, 20% on the right and 20% bilaterally (Bannister 1992). *Bothrops, Zincum met* etc. are given for the treatment of aphasia, a disorder in speech and writing. Aphasia may arise due to cerebral stroke or brain injury, particularly of the left hemisphere.

Treatment of cerebral stroke patients with selective serotonin reuptake inhibitor antidepressant showed difference in improvement with respect to laterality. The post-stroke major depressive disorder improved much more in right stroke subjects in comparison with the left stroke ones (Spalletta *et al.,* 2003). In case of unilateral brain injury due to stroke or parkinsonism, the intact hemisphere plays an important role in recovery and compensation for the lost motor function (Schallert *et al.,* 2003). A well-selected homeopathic potency may facilitate recovery by acting on the intact hemisphere. It has been demonstrated that the brain can asymmetrically

modulate neurochemical, neuroendocrine and immune response to lipopolysaccharides. The asymmetry in the functioning of the sympathetic nervous system may be one of the mechanisms by which the brain hemispheres asymmetrically modulate immune reactivity. The catecholaminergic modulation of immune reactivity depends on lateralization (Dong *et al.,* 2003 and references therein). The left and right neocortex of the brain has been shown to exert asymmetrical effects on the immune system. In an experimental study on wister rats the influence of unilateral central nervous system ischemia was observed on spleen cell number and function. The cerebral ischemia induces mobilization of certain immune cells from the periphery to the brain where they may contribute to the local inflammatory response. Cerebral ischemia is followed by a systemic activation of T and B lymphocytes (Gendron *et al.,* 2002). A patient having an ischemic lesion in the left thalamus showed spike and dome discharges in the electroencephalogram recorded from both the hemispheres. The spike and dome discharges are characteristic of petit mal epilepsy (Inghilleri *et al.,* 2002). Memory formation following the one-trial discriminated bead task in the chick falls into three stages, short-term, intermediate and long-term. Recall of the memory in the chick shows cyclical changes that differ in period between left and right hemispheres. Memory in the left hemisphere is largely responsible for performance at test and that processes involved in its consolidation generate the phases of memory (Gibbs *et al.,* 2003). During development of the frog *Xenopus,* the transmembrane proteoglycan syndecan-2 cell nonautonomously regulates left-right development in migrating mesoderm by an unknown mechanism leading to left-right asymmetric gene expression and left-right orientation of the heart and gut. Protein Kinase C gamma mediates phosphorylation of the cytoplasmic domain of syndecan-2 in right, but not left, animal cap ectodermal cells (Kramer *et al.,* 2002). Cameron and Minoshima (2002) demonstrated that there are specific brain regions and hemispheric laterality of function associated with visceral function. It is possible that some potentized drugs differentially activate the brain regions of the two hemispheres and thus produce specific action on the right or left side of the body. The dorsal horn of the spinal cord, through which impulses move in ascending and descending tracts, seems to play an important role in this matter. This aspect has been further discussed under 4.4.

4.2. Time modalities and Homeopathy

Unlike conventional medicine Homeopathy has recognized the importance of time modalities of symptoms of a disease for treatment with appropriate remedies. Kent (1877) laid great emphasis on this aspect of disease symptoms for the selection of right remedies. Kent's *Repertory of Homeopathic Materia Medica* bears numerous examples of aggravation and amelioration of symptoms in relation to time. As for example, *Lycopodium* shows aggravation of symptoms at 4-8 PM, *Arsenicum album* at mid-day/ mid-night, *Rhus tox* at 7-8 PM, *Kali carb* in early morning and so on. The question is whether there is any scientific basis for time modalities of symptoms. Almost all organisms including man show an internal clock or biological

rhythms. The circadian rhythm is disturbed in many diseased conditions. Delayed sleep phase syndrome involving late bedtimes and arising times is one of them. It can be corrected by chronotheraphy which is designed to have a training effect on the circadian system. Treatment with melatonin also has a positive effect (Quera Salva *et al.,* 2001). Melatonin is synthesized by pinean parenchymal cells and secreted by them into blood and the cerebrospinal fluid. There is diurnal variation in the secretion of melatonin. The influence of melatonin on circadian rhythms and the presence of melatonin receptors in the suprachiasmatic nucleus suggest a direct action of the hormone on the circadian pace maker (Sharma *et al.,* 1999 and references therein). Circadian rhythm exists in the severity of bronchial asthma. The efficacy of a bronchodilator drug varies according to this rhythm (Lahdensuo and Alanko, 1976). There is circadian rhythm in the plasma concentration of thyroid stimulating hormone (TSH). TSH is secreted from the anterior pituitary gland of the brain (Lattanzi *et al.,* 1979). Sotalol, a β-adrenoceptor blocking agent, can modify the circadian rhythm of ventricular premature beats mainly during the morning. The drug is effective in the control of idiopathic ventricular arrhythmias (Maia *et al.,* 1994). It is, therefore, interesting to note that chronobiology has been effectively incorporated into the drug selection strategy of high dilution therapy. Some potentized drugs may act on the mid-brain influencing secretion of melatonin and the circadian rhythm. The drugs thus put to order the disturbed circadian rhythm in a patient.

4.3. Similia principle and Homeopahty

The mechanism of action of homeopathic potencies involves two basic aspects : (1) similia principle and (2) action of drugs at ultra high dilutions. The similia principle is accepted in certain areas of conventional medicine and scientific investigation (Schwartz *et al*., 2000). Experimental evidences in support of the similia principle have been described in Chapter II (2.4). There are mathematical models which set the similia aspect as a general expression of action reaction principle within the concept of the dynamic systems theory. The similia principle can be better explained from the perspective of the complexity science (Bellavite *et al*., 1999; Bellavite, 2003). *Nux vomica* increased the variability in the electroencephalogram (EEG) of healthy rats due to irritation in the central nervous system (CNS). The drug, which produces irritation in the CNS, also ameliorates sleep disorders in patients thereby providing evidence in support of the similia principle (Torres, 2003).

The similia principle shows that drugs have two types of biological effects. In high doses they produce some symptoms and in ultra low doses they abolish or ameliorate those symptoms. Evidences from toxicological studies of drugs and from clinical observation with their potencies establish the dual action of the drugs. Examples can be given from such drugs as strychnine, morphine, arsenic trioxide, lead, mercury etc. (Sukul, 1997). Some homeopathic drugs produce therapeutic effects both in the form of mother tinctures as well as their potencies, and the effects are almost similar. The examples are *Nux vomica, Chelidonium, Passiflora, Rananculus bulb* etc. But homeopathic mother tinctures are applied at essentially

low doses, 5-10 drops with water. So, the dual action may not be found in these cases. The difference in effects between high and low doses of a drug may be due to difference in the mode of action of the two doses. Govoni *et al*. (1994) reported that prolonged treatment with a very low dose (3%) of alcohol in drinking water produced beneficial effect on rats in terms of better performance in a two-way avoidance learning test. This behaviour is disrupted by high doses of alcohol. The authors suggest that a discontinuity exists between the effects of low and high doses of alcohol. Homeopathic drugs also show this discontuinity in effect between high and ultra low doses. There may be a **critical dose** for each drug where the action of low doses ceases and that of the high doses begins. It seems the critical dose for toxic drugs is relatively low as compared to that for non-toxic drugs. In conventional pharmacology, this critical dose represents the lower limit of dose-response curve of a drug. In homeopathy, the dose response is just the reverse; the higher is the dilution the stronger is the effect.

The effect of a drug below its critical dose appears to depend mostly on the susceptibility of the individual. The susceptibility can be induced by a drug or by a disease. The more the individual is susceptible to a particular drug, the more intense is the action of the ultra low dose of that drug. The similia principle represents the biological effects of a drug on the two sides of its critical dose, the upper and the lower. The ultra low dose effects are not as generalized as the high dose ones because the former would be evident only in special subjects. These subjects are susceptible to the low dose of the drug. On the upper side of the critical dose lies Allopathy and on the lower side Homeopathy. We would now discuss in more details about the non-linear systems.

4.4. Non-linear systems and Homeopathy

Random motion of fluid in living organisms shows complex behaviour. Good mathematical models exist for systems showing irregular oscillations in forms of deterministic non-linear (or piece-wise linear) differential or difference equations. Theoretical as well as experimental studies of these systems show that the time history is sensitive to initial conditions and that the future behaviour is unpredictable. Nereby trajectories diverge form each other exponentially in time and this behaviour is called **Chaos**. The bifurcation parameter in chaos is of interest in a dynamic system (Steeb and Louw, 1986). Some theoretical models have been proposed to describe the non-linear properties of deoxyribose nucleic acid (DNA), a macromolecule in cells that carry coded information of heredity (Daniel and Latha, 2000). Chaotic phenomena in cellular neural networks have been studied. Non-linear systems have been applied to model the study of pathological conditions resulting from instabilities in physiological control systems (Thangavel *et al*., 2000 and references therein). Based on the clinical and basic research data concerning the theories on the neural basis of consciousness, it is concluded that the brain works by largely nonlinear parallel processing and much intramodal shifts of attention may be effected by intracortical, or multiple corticothalamic mechanisms (Smythies, 1997).

In conventional medicine treatment is disease-specific or organ-specific. Homeopathy treats the patient as a whole by mobilizing self-organization towards restoration of health from the diseased state. For this, some scientists believe that responses of patients to homeopathic remedies follow the patterns of the non-linear complex systems (Shepperd, 1994; Bellavite and Signorini, 2000). A minute dose of a homeopathic potency can bring about prolonged and widespread changes in the physical and mental state of a patient. This kind of response fits in with the non-linear concept because the magnitude of the output is disproportionately compared with the magnitude of the input (Bell, 2003 and references therein). An appreciation of the three main properties of the complex systems such as non-linearity, self-organization, and dynamicity would help in understanding the homeopathic phenomena and in opening new avenues of research in homeopathy (Bellavite, 2003).

Considerable experimentation is necessary to provide supportive evidences for the relationship between non-linear systems and homeopathy. It is necessary to identify the physical basis of a homeopathic stimulus and the biological correlates of response to that stimulus. These correlates should be common to all organisms. Holistic and vitalistic theories of homeopathy are very often associated with the complexity science. The vitalistic theory has no place in modern biology. But some biological phenomena can be interpreted in terms of the holistic concept. Animals with a nervous system very often respond to a stimulus in a holistic manner. Protistans, whose body consists of single cells, behave as the whole organism and are called acellular rather than unicellular. Plants without a nervous system also respond to an external stimulus, infection or injury. If a part of the root is infected, other parts of the root and the aerial parts of the plant show defense response in the form of expression of pathogenesis-related (PR) proteins (Bowles, 1992; Ogallo and McClure, 1996 and rerences therein).

In animals, the immune system and the nervous system respond to the external stimuli in a holistic manner. Fever or pyrexia occurs in response to infection with microorganisms which usually do not survive at a temperature higher than the normal body temperature. Thermoregulatory mechanisms operate with a view to maintaining body temperature at a more or less constant level in spite of fluctuation in environmental temperature. The mechanisms involved consist of receptors in the hypothalamus and spinal cord, which monitor temperature of blood, and those in the skin which monitor environmental temperature. The hypothalamus appears to be the site of integration of sensory information for initiation of suitable responses. As fever occurs, the body temperature is reset to a new point above the normal temperature. Endotoxin from invading microorganisms serves as exogenous pyrogen causing fever. This toxin acts on some types of leucocytes which synthesize and secrete interleukin 1 or IL1, a polypeptide known as endogenous pyrogen. Fever, caused by a wide range of infectious diseases, is a natural holistic response which tends to restore health by suppressing pathogens at a higher temperature. Appropriate homeopathic remedies appear to promote synthesis of IL1. The fever produced by IL1 is due to the release of prostaglandins, notably PGE2, from the hypothalamus. Antipyretic drugs such as paracetamol block the synthesis of PGE2

thereby reducing fever. However, the pathogens of fever are not destroyed by these drugs (Sukul, 1997 and references therein). We would further discuss holism under 4.6.

4.5. Miasms and their biological basis

Sometimes patients suffering from chronic diseases do not respond to properly selected remedies. The failure of response in these cases is attributed to the specific inherent condition of the patients called miasms. This condition can be corrected by applying appropriate antimiasmatic drugs. Miasms have been discussed in details in the earlier book by Sukul (1997). According to homeopathic principles chronic diseases arise from miasms such as psora, sycosis and syphilis. The chief of the three miasms is psora whose primary expression is in itching eruption on the skin. Hahnemann did not offer any suggestions about what the miasms might be in themselves. Here we propose a hypothesis relating to the biological basis of the chief miasm psora. We propose that 'itch and tickle' sensation represents psora. Relatively mild stimulation, if produced by something that moves across the skin, produces itch and tickle. Itch spots can be identified on the skin by careful mapping. Like pain spots, itch spots lie in regions in which there are many naked endings of unmyelinated nerve fibres. Itch persists along with burning pain in nerve block experiments when only C fibres are conducting. Itch, like pain, is abolished by section of the spinothalamic tracts. Itching occurs only in the skin, eyes, and certain mucous membranes and not in deep tissues or viscera. Stimulation of the skin over an area of visceral inflammation produces some relief of the pain due to the visceral disease. The old-fashioned mustard plaster works on this principle (Ganong, 1999). Thus we see that manipulation of itch and tickle sensation can produce some effect on visceral organs. This may be the reason that chronic diseases show improvement only after application of a strong antipsoric drug such as *Sulphur*. This drug in material doses can stimulate 'itch and tickle' nerve fibres producing itching of skin. Results of clinical studies with homeopathic remedies may be anomalous due to miasmatic blockages in some patients under the study group.

Primary afferent neurons for 'itch and tickle' sensation in skins have their cell bodies in the dorsal root ganglia of the spinal cord or equivalent ganglia in cranial nerves. They make multiple synaptic connections to motor neurons and connections that relay impulses to the cerebral cortex. Somatic and visceral afferents converge on the same spinothalamic neurons. Thus the same pathway may be stimulated by the activity in the visceral afferents or somatic afferents. The dorsal horn of the spinal cord represents a gate in which the impulses in the sensory nerve fibres are translated into impulses in ascending tracts, and passage through this gate depends on the nature and pattern of impulses reaching the outer laminas of the dorsal horn. The gate is also affected by impulses in descending tracts from the brain (Ganong, 1999). Thus suppression of eruptions by ointments may modify the usual sensory input into the ascending and descending tracts and affect the autonomic outflow. This may lead to visceral disorders. An appropriate antipsoric drug may bring about homeostasis by restoring normal signals.

4.6. Polycrests, reappearance of past diseases and holism

Polycrests are homeopathic remedies which produced during proving a wide variety of symptoms common to many diseases. They have many-sided action, and are allowed to exhaust their action before repetition. Examples are *Arsenicum album, Calcarea carb, Causticum, Graphites, Natrum mur, Psorinum, Silicea, Sulphur* etc. Polycrests may act through multifunctional neural networks which can produce many outputs. It is the common experience of homeopathic physicians that during treatment of a chronic disease with a high potency of a polycrest, past diseases, apparently unrelated with the current one, reappear briefly in the same sequence as they affected the patient in the past. The phenomenon can be explained by the nature of changing pattern of neural circuits. The same neurons form part of several different functional networks, some of which may exist operationally for a short time if the behaviour they control is not continuous (Meyrand *et al*., 1991). Since same neurons can be part of several different functional networks, any change produced on the synapses connecting these neurons by a disease is likely to affect different other networks, thereby producing a variety of symptoms apparently unconnected with the disease. As for example, the pain in the region of liver may be more aggravated during stepping and may be accompanied by constipation, frequent eructation, dry mouth with thirst etc. provided the neurons processing the impulses from the pain receptors of the liver region form part of other functional networks. An appropriate polycrest for the liver pain would briefly activate in chronological succession neurons of other networks which are connected with the sensory neurons supplying the liver (Sukul, 1997).

The time that has elapsed since a recent event must be represented in neural activity. The essential analog information describing the time interval since past events may be quantitatively contained in and represented by the cellular Ca^{2+} level or other slow cellular variables. In a cricket neuron Ca^{2+} concentration is used as a computational variable relevant to sequence processing (Hopfield, 1996 and references therein). During recapitulation of past diseases in response to a homeopathic potency, it is usually the pain component that reappears. Pain is produced by the output of a widely distributed neural network in the brain rather than directly by sensory input from injury, inflammation or other pathology. According to the **'neuromatrix'** theory pain is multidimensional experience produced by characteristic **neurosignature patterns** of nerve impulses generated by a widely distributed neural network (Melzack, 1999). A large number of functional imaging studies have shown that multiple central regions are involved during the experience of pain. These regions process information in circuits which are thought to process the affective, sensory, cognitive, motor, inhibitory, and autonomic responses stimulated by a noxious event. The concept of a 'neuromatrix' for pain processing is, thus, well-supported (Derbyshire, 2000). Altering the activity of one network neuron by a homeopathic potency would alter the activity of other network neurons resulting in the experience of past pains.

We have already mentioned under introduction that homeopathy involves holism. During selection of a homeopathic remedy, all the physical and mental

symptoms of a patient are taken into account as a whole besides his or her specific disease symptoms. The 'neuromatrix' concept fits in with the holistic approach of homeopathy. The body is felt as a unity with different qualities at different times, and the brain mechanism underlying this feeling also comprises a unified system that acts as a whole and produces a neurosignature pattern of a whole body. Every moment millions of nerve impulses from all the sensory systems of the body arrive at the brain. The genetically built-in neuromatrix produces a continuous message that represents the whole body of the individual in which details are differentiated within the whole as inputs come into it. The neuromatrix is a template of the whole which gives the characteristic neural pattern for the whole body as well as subsets of neurosignature patterns that relate to events at different parts of the body (Melzack, 1993). During the onset of a disease the neuromatrix is affected resulting in manifestation of symptoms and a generalized feeling of indisposition. The totality of symptoms, obtained from the patient, represents his or her neurosignature pattern affected by the illness. The constitutional remedy, selected on the basis of neurosignature pattern through the totality of symptoms, would modulate the affected neuromatrix to its normal condition giving the patient the feeling of well-being. In a healthy individual the same drug acts on the normal neuromatrix thereby generating a set of symptoms similar to those of the illness.

4.7. Action of drugs at ultra high dilutions

In order to understand the mechanism of action of a homeopathic potency we must have an idea of the following aspects: (1) physico-chemical property of the concerned drug, (2) the primary biomolecular target in the organism with which the drug molecules interact first, (3) the initiation of biochemical events in the concerned cell or cells acted upon by the drug and (4) manifestation of the therapeutic effect of the drug in the organism. We have already got some idea of the physico-chemical nature of a potentized drug, which is actually structured water. So, water structures are expected to carry information about drug molecules.

4.7.1. Water as informational molecules

We know that in living organisms there are two kinds of informational macromolecules. These are protein and nucleic acid. Sequences of 20 different amino acids make innumerable variety of proteins. There is also variation in the three-dimensional structure of proteins due to difference in folding from disulphide bridges. In case of nucleic acids, a limitless variety of sequences can be made from the 4 subunits like Adenine (A), Thymine (T) or Uracil (U), Guanine (G) and Cytosin (C). Polysaccharides with only a single kind of subject or with two different alternating units are not informational molecules in the same sense as are proteins and nucleic acids. However, short sugar polymers made up of six or more different kinds of sugar connected in branched chains have the structural and stereochemical variety to carry information recognizable by other macromolecules. The most abundant polysaccharides in nature, starch and cellulose, consists of repeating units

of D-glucose. Other polysaccharides are composed of a variety of sugar molecules derived from glucose.

The two diluent media of homeopathic potencies are water and aqueous ethanol. Since each homeopathic potency has unique properties characteristic of the mother tincture or the drug from which it is prepared, there is reason to believe that both water and aqueous ethanol serve as informational molecules. However, water and ethanol are simple molecules without any subunits. How do they serve as informational molecules? The answer lies in the fact that both water and ethanol can self associate through hydrogen bonding and form dimers, trimers and multimers. In aqueous ethanol water molecules hydrogen bond with ethanol molecules and form different structures. We have already mentioned that ethanol molecules having large non-polar part tend to preserve water structures. The three-dimensional structure of the molecular aggregates of water carries the information of drug molecules from which it has originated through the process of dynamization. Thus water can form an innumerable variety of structural configurations through H-bonding strengthened by succussion or sonication and preserved by ethanol. Different biological effects of different potentized drugs indicate that water, like proteins and nucleic acids, is capable of serving as informational molecules. In liquid water at room temperature hydrogen bonds break and reform continuously. We have mentioned in the previous Chapter III that H-bond dissociation and reassociation occur between the same O — H groups. Different polymers of water remain at dynamic equilibrium of specific geometric configuration representing a particular drug.

4.7.2. Two components of a homeopathic potency

It is evident from clinical results that a drug would be effective on a patient if symptoms agree. Usually in acute cases, lower potencies work better and quicker. In case of chronic diseases, higher potencies are selected and they produce a lasting effect on the patients. It is, therefore, evident from clinical results that a potentized homeopathic drug should have two physical components, one constant and another variable. The constant component would not undergo any change during the process of potentization of a drug through successive dilution and succussion. This component carries the identity of a particular drug. Any mechanical agitation or dilution would not alter the specificity of this component. We now call this as **identity component**. The variable component, on the other hand, bears the mark of the potency of a particular drug. This component undergoes a change due to dilution and mechanical agitation. This would be referred to as the **dilution component**. The most interesting point is that both the components are capable of producing biological effects. In other words, both the components are recognizable by biomolecules. The question is whether the two components are dissociable. It may be one component with a variable site or an association of two different units. Since H-bonding results from electrostatic force, succussion or any mechanical agitation can strengthen hydrogen-bonding in different species of water polymers in a homeopathic potency. So, strengthening of H-bonding may have a relation with the dilution component.

It is known from the clinical experience that a properly selected drug would produce curative effect on the patient whatever is its potency. This indicates that the identity component plays a major role in producing therapeutic effect on a patient. If tridimentional water structure carries the identity component, what would be the features that carry the mark of the dilution component? Is it simply the strength of H-bonding in the water structure? We have observed in one of our earlier experiments that *Nux vomica* 30 prepared by sonication showed stronger antialcoholic effect on rats than *Nux vomica* 1000 prepared by succussion (Paul *et al.,* 1992). Since sonication produces a much stronger mechanical agitation than manual succussion, H-bonding in water structures would be more strengthened by sonication rather than by succussion. We have already mentioned that H-bonding occurs mainly due to electrostatic force. The dilution component may, therefore, be the result of mechanical agitation contributing to the strengthening of H-bonds in water structures.

4.7.3. Primary biomolecular target for a homeopathic potency

The question is how a homeopathic potency or, in other words, a stereospecific water structure, is recognised in the body of the patient or the test organism. It is evident from the clinical and experimental results described in the foregoing Chapter II that homeopathic medicines are effective in man, mammals, amphibians, fishes, plants and bacteria.

The results of *ex vivo* and *in vitro* tests show that potentized drugs are effective on isolated organs, and cells. So, the primary targets, with which the drugs first interact and initiate action, must be present in all living organisms, isolated tissues and cells. In fact, the drug molecules or structured water molecules first come in contact with the surface of cells. The cells may be in the oral mucosa of man and animals, in the leaves of plants, in the tissues of isolated organs and in prokaryotes etc. We must, therefore, have a clear idea of the structure of cell surface.

Potentized homeopathic drugs are capable of producing effects on both prokaryotic and eukaryotic cells. Prokaryotic cells are usually smaller in size (1 - 10 μm) than eukaryotic ones (5 - 100 μm). Membrane-bound organelles like mitochondria, endoplasmic reticulum, Golgi complexes etc. are present in eukaryotic cells but absent in prokaryotic ones. While eukaryotic cells have nucleus containing DNA with histone and non-histone proteins in chromosoms, prokaryotic cells have no nucleus and their DNA with non-histone proteins lies in nucleoid without any membranous envelope. However, both types of cells are covered by plasma membrane with some common features.

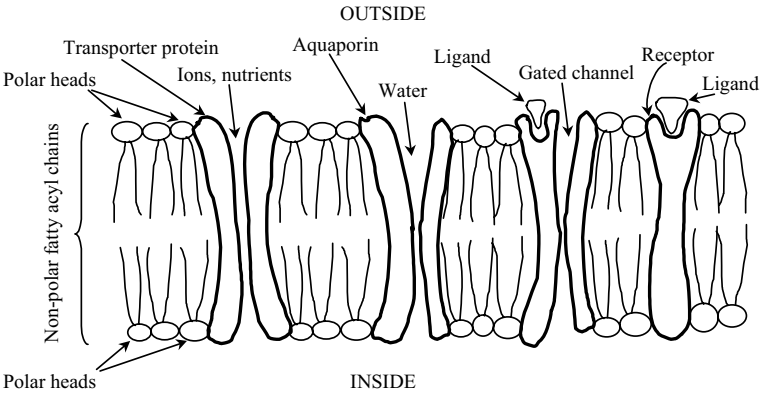

Figure 21. *Plasma membrane of a cell. Extracellular messengers interact with receptors resulting in production of second messengers inside the cell which in turn lead to appropriate adaptive changes in the cytoplasm and the nucleus*

4.7.4. The cell and the plasma membrane

We now describe major structural features of eukaryotic cells with special emphasis on the plasma membrane. The plasma membrane consists of a bilayer of amphipathic lipids (for instance, phospholipids, glycolipids, sterols) with globular proteins embedded in it (Figure 21). The two lipid bilayers are 5 - 6 nm thick and joined laterally, and tail to tail, by van der Walls interactions between their hydrophobic hydrocarbon chains. In plant cells there is an outer covering of cell wall outside the plasma membrane; protoplasts of neighbouring cells are connected by strands of cytoplasm known as plasmodesmata. The cell wall, made up of cellulose and other carbohydrate polymers, is porous allowing water and small molecules to pass through them. The lipid molecules of the bilayer being amphipathic have within their structure hydrophobic (non-polar) and hydrophilic (polar) moieties. The two faces of the bimolecular layer contain hydrophilic heads of the lipid molecules. Thus the hydrophilic ends of the molecules are exposed to the aqueous environment that bathes the exterior of the cells and to the aqueous cytoplasm in the interior. The hydrophobic ends meet in the water-poor interior of the membrane. The major lipids in the plasma membrane are phospholipids such as phosphatidylcholine and phosphatidylethanolamine. The phospholipid molecules each have 2 fatty acid chains attached to a phosphate head. In prokaryotes phospholipids are generally the only membrane lipids, but in eukaryotes, cell membrane also contains cholesterol (in animals) or other steroids (in plants). The membrane is not a static structure. But the lipid and protein molecules move in the plane of the membrane, a process known as lateral diffusion.

There are many different proteins embedded in the membrane, some on the outer surface, some on the inner one, and some transmembrane. In general, the

uncharged hydrophobic portions of the protein molecules are located in the interior of the membrane, and the charged hydrophilic portions are located on the surfaces. Some of the proteins contain lipids (lipoproteins) and some carbohydrates (glycoproteins). Proteins embedded in the plasma membrane serve as transporters, signal receptors, and ion channels. Transporters span the membrane and carry substances into and out of the cell. Some use energy to transport ions and compounds against their concentration gradients. Small molecules such as ions, sugars and amino acids can pass through protein channels of the membrane. Large molecules or macromolecules such as proteins or particles are carried into the cell by the invagination and pinching-off of pieces of the plasma membrane in a process called endocytosis. Endocytosis controls entry into the cell and has a crucial role in development, the immune response, neurotransmission, intracellular communication, signal transduction, and cellular and organismal homeostasis (Conner and Schmid, 2003). During endocytosis macromolecules or particles together with their hydration shell are carried inside the cell. Exocytosis is exactly the opposite process carrying intracellular materials outside the cell and taking with them the water of hydration. Thus there would be similarity in the structure of extracellular and intracellular water. The extracellular fluid contains water, nutrient molecules, hormones, neurotransmitters and antigens. Signal receptors have specific binding sites for extracellular signalling molecules, the ligands. When a ligand binds to its specific receptor, the receptor protein transduces the signal carried by that ligand into an intracellular message. When a ligand binds to a receptor associated with an ion-channel, the latter opens allowing entry of specific ions. Signal transduction by receptors can activate or inhibit enzymes on the inner surface of the membrane. A single ligand molecule bound to a single receptor may result in entry of thousands of ions through the opened channel or the synthesis of thousands of molecules of an intracelular messenger molecule by an activated enzyme (Nelson and Cox, 2000). Antigens bind to specific receptors and trigger the production of antibodies. Neurotransmitters bound to specific receptors initiate a cascade of cellular events resulting in a specific function. Plant cells contain hihgly slective cation-channel for H^+, K^+ and Ca^{2+} and also selective anion-channels for Cl^- and dicarboxylates such as malate (Heldt, 1997). Plant membranes in contrast to those of animals seem to possess no specific channel for Na^+ ions (de Duve, 1991). The lipid bilayer is esentially impermeable to water-soluble molecules and ions. It is an inert structure that can neither mediate nor control multiple exchanges of matter and information between the cells and their environment and between different intracellular compartments. These functions are carried out by integral membrane proteins (Peoples *et al.,* 1996). So we have to look for targets for homeopathic potencies in the integral membrane proteins.

4.7.5. Membrane proteins

Let us examine the possible candidates among membrane proteins for initial interaction with homeopathic potencies. The transporters move specific organic solutes and inorganic ions across the membrane. There are passive transporters and

active transporters. They help passage of sugars, amino acids, inorganic ions and water for biosynthesis and energy production in cells. The passive transporters, like enzymes bind their substrates with stereochemical specificity through many weak, noncovalent interactions. The negative free-energy change associated with these weak interactions counterbalances the positive free-energy change that accompanies loss of water of hydration from the substrate. This lowers the activation energy needed for transport of polar compounds through the lipid bilayer. A polar solute is stripped of its hydration shell during passage through the bilayer and rehydrated in the cytosol (Nelson and Cox, 2000). In passive transport, the solute is transported down its electrochemical gradient. An electrochemical gradient involves difference in solute concentration plus electrical gradient across the plasma membrane. Active transport occurs against a gradient and involves the breakdown of ATP (adenosine triphosphate), or the concomitant flow of some other chemical species down its electrochemical gradient. The enzyme ATPase breaks down ATP thereby releasing energy to drive a solute uphill. In the secondary active transport passage of an ion through the membrane downhill provides necessary energy to drive cotransport of a solute uphill against its electrochemical gradient. There are 4 types of transport ATPases of which P-type ATPases are present on plasma membranes of both eukaryotic and prokaryotic cells. These ATPases have two types of protein subunits, α and β. The α-subunit is essential and has its amino acid residue aspartate phosphorylated during transport. ATPases are responsible for the transport of Na^+, K^+, Ca^{2+} and H^+. Bacteria use P-type ATPases to pump out toxic heavy metal ions such as Cd^{2+} and Cu^{2+}. Plasma membrane of tumours contain an ATP dependent transporter that drives out different drugs from within the tumour cells. These multidrug transporters are responsible for the resistance of tumour cells against chemotherapy. The ABC1 protein is a member of the family of multidrug transporters. These proteins actively transport a variety of ions, amino acids, vitamins, steroid hormones, and bile salts across plasma membranes.

4.7.6. Biomolecules in continuous water medium

Whenever a potentized homeopathic drug is administered on an organism, be it human oral cavity, plant leaf or bacteria, it first comes in contact with water covering the surface of biological membranes. The extracellular fluid (ECF) constitutes 20% of the body weight. The interstitial fluid, which constitutes 15% of the ECF, forms the actual environment of most of the cells of the body. Normal cell function depends upon the constancy of this fluid, and a large number of regulatory mechanisms operating on the principle of negative feedback, maintain it (Ganong, 1999). Water is treated as a continuous liquid in and around cells. Since homeopathic potencies are structured water molecules they would first interact with the watery medium enveloping inter and intra-cellular surfaces and protein and other molecules. Different non-covalent forces may locally alter the structure of water in a number of ways. The operative forces are Lifshitz-van der Waals (LW) forces, Lewis acid-base (AB) forces and electrostatic (EL) forces. Of these, the AB forces are generally the most prevalent (90% or more) in aqueous media. This is due to the

strong cohesive and adhesive hydrogen-bonding interactions typically occurring in and by water. Among the strong AB interactions occurring in water are hydrophobic attraction and hydrophilic repulsion (hydration) pressure. Thus hydrophobic moieties would be attractive between each other and hydrophilic ones repulsive between each other. The three types of interaction energies (LW, AB, EL) decay as a function of interparticle or intermolecular distance. The molecules of hydration on polar (hydrophilic) surfaces necessarily organize themselves in a thicker and less dense layer, usually with the H-atoms bound to the hydrophilic (generally electron-donating) surface, and with the O-atoms protruding away from the surface, into the liquid, so that here the plane of each water molecule of hydration is perpendicular to the hydrophilic surface (Figure 22). Hydrophilic surfaces have two layers of water of hydration. Hydrophobic surfaces do not have more than one flattened layer of water molecules, attached via LW forces. On hydrophilic surfaces the water of hydration is attached via AB + LW forces, where AB forces may dominate (Van Oss *et al.,* 2001). A homeopathic potency or, in other words, structured water should interact first with one of the integral membrane proteins which would be ubiquitous being present in bacteria, plants, animals and man. Such a ubiquitous protein is aquaporin. Let us examine how far aquaporins serve as the primary target for a homeopathic potency.

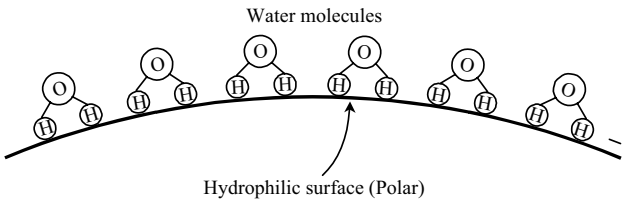

Figure 22. *Water molecules on a hydrophilic surface (–) forming thicker and less dense layer with O-atoms protruding away from the surface*

4.7.7. Aquaporins

Aquaporins are transmembrane glycoproteins which allow water or small specific solutes to pass unhindered, but block the passage of ions to prevent the dissipation of transmembrane potential (Borgnia *et al.,* 1999; Engel *et al.,* 2000). Although water can pass cellular membranes by diffusion, extensive evidence has been provided during the last decade that in tissues where water has to permeate the cell membranes at a high rate this process is facilitated by aquaporins (Deen *et al.,* 2000). Aquaporins belong to major intrinsic protein (MIP) channels and occur in bacteria, plants and animals. They exhibit a pronounced sequence homology and share functional as well as structural similarities (Engel *et al.,* 200l; Hohmann *et al.,* 2000).

Distribution in animals

More than 10 aquaporins (AQP) have been cloned from various mammalian tissues. AQP_0 is expressed in eye-lens, AQP_1 in proximal tubules of kidney and in intrahepatic cholangiocytes, AQP_2 and AQP_3 in Kidney collecting ducts; AQP_3, AQP_4, AQP_7 and AQP_8 in the gastro-intestinal (GI) tract, AQP_5 in salivary and lacrimal glands, corneal epithelium and lungs, AQP_6 in renal epithelia, AQP_8 in liver, pancreas and colon; and AQP_9 in liver (Ma and Verkman, 1999). Fluid transport is a major function of the GI tract with more than 9 litres of fluid being absorbed or secreted across epithelia in human salivary gland, stomach, the hepato-biliary tract, pancreas, small intestine and colon. At least seven aquaporins are expressed in various tissues in the GI tract : AQP_1 in intrahepatic cholangiocytes, AQP_4 in gastric parietal cells, AQP_3 and AQP_4 in colonic surface epithelium, AQP_5 in salivary gland, AQP_7 in small intestine, AQP_8 in liver, pancreas and colon and AQP_9 in liver (Ma and Verkman, 1999).

AQP_9 occurs abundantly in peripheral leukocytes of man. These are permeable to water and urea but not to glycerol. However, the role of AQP_9 in the immunological function of leukocytes is intriguing (Ishibashi *et al.,* 1998). AQP_4 is abundantly expressed in the brain and is localized in glial cells bordering the subarachnoidal space, ventricles and blood vessels (Nielsen *et al.,* 1997). These water channels could serve as osmosensor mechanism responsible for detecting changes in cell volume (Venero *et al.,* 1999; Jung *et al.,* 1994). AQP_4 plays an important role in brain water and K^+ homeostasis from the second week of development in the rat brain (Wen *et al.,* 1999). AQP_4 expression occurs in chick embryonic brain, in parallel with maturation and functioning of the blood-brain barrier and suggests that there is a close relationship between water transport regulation and brain development (Nico *et al.,* 2001).

Water Pump

Neurons, unlike glial cells, appear to lack water channels such as aquaporins. The human brain represents about 2% of body weight but consumes nearly 20% of its oxygen intake. Thus it produces and must remove metabolic water at about 12 times the rate of the rest of the body. N-acetyl-L-aspartate (NAA), the most abundant free amino acid in the vertebrate brain, is involved in the movement of osmolyte-obligated water out of neuronal cytosol. NAA is synthesized in neurons and hydrolysed in glial cells oligodendrocytes. NAA appears to turn over every 24 - 48 h by virtue of its continuous efflux in a regulated intercompartmental cycling via extracellular fluids between neurons and a second compartment in oligodendrocytes (Baslow *et al.,* 1999). It serves as a molecular water pump actively pumping water against its gradient. The system involves cotransport of water across a membrane against its gradient as a hydrophilic solute is transported down its own intercompartmental gradient. Molecular water pumps are thought to be universal in nature and a constituent mechanism in all cells. They may be related to both normal

and pathological interactions between cells, and between cells and their extracellular environment (Baslow, 2002).

Aquaporins in plants

As mentioned earlier, aquaporins are members of MIP family. In the plant Kingdom, a single plant expresses a considerably large number of MIP homologues. These homologues can be subdivided into four groups with highly conserved amino acid sequences and intron positions in each group. These groups are tonoplast intrinsic protein (TIP), plasma membrane intrinsic protein (PIP), NOD 26-like MIP (NIP) and small and basic intrinsic protein (SIP). These members of the MIP family may have diversified from an ancestral gene before the evolutionary divergence of higher plants (Baiges *et al.,* 2002). While PIPs from spinach leaves account for 15% of plasma membrane proteins, TIPs (membrane surrounding the vacuole of a plant cell is called a tonoplast) account for upto 10% tonoplast proteins (Johansson *et al.,* 1996, Karlsson *et al.,* 2000). NIP is expressed in root nodules of soybean and also in non-leguminous plants (Baiges *et al.,* 2002). In plants, aquaporins regulate the water flow through membranes during growth, development and stress responses (Harvengt *et al.,* 2000). PIP_1 aquaporins in tobacco enhances cellular water permeability, increases root hydraulic conductivity, relative osmotic pumps and support the survival of dry periods (Siefritz *et al.,* 2002). It has been suggested that the photosynthetic CO_2 uptake across the plasma membrane of the leaf mesophyll cells is facilitated by mercury sensitive aquaporins (Terashima and Ono, 2002).

Aquaporins in fungi and bacteria

Candida albicans, a fungal pathogen of humans, contains a single functional aquaporin gene, AQY1. Deletion of AQY1 had little effect on all surface hydrophobicity and no effect on the virulence of *Candida* in mice. The deletion strain shows a decreased sensitivity to osmotic shock (Carbrey *et al.,* 2001). The genome of the yeast *Saccharomyces cerevisiae* contains two highly similar aquaporin genes, AQY1 and AQY2. A survey of 52 yeast strains revealed that all industrial and wild yeasts carry the allele encoding a functional AQY1, while none of them appear to have a functional AQY2 (Laize *et al.,* 2000). Microbial aquaporins are mostly glycerol facilitators (GlpF), which transport glycerol and possibly other solutes in addition to, or even in preference to, water. They have been reported from such gram-positive bacteria as *Bacillus subtilis, Clostridium acetobutylicum, Staphylococcus aureus, Lactococcus lactis, Streptococcus pneumoniae* and gram-negative bacteria as *Escherichia coli, Haemophilus influenzae, Mycoplasma pneumoniae, Pseudomonas aeruginosa, Salmonella typhinum, Shigella flexneri, Vibrio cholerae* etc. (Hohmann *et al.,* 2000). The gram-negative pathogenic bacterium *Brucella abortus* contains an aquaporin which is an efficient water-channel (Rodriguez *et al.,* 2000).

Structure of aquaporin

The aquaporin is a small protein (~ 30 kDa) containing six transmembrane domains, in which the first three domains are homologous to the 2nd three. Both N- and C-termini of the protein are oriented to cytosol. The two sections are oriented at 180^0 to each other allowing a highly preserved asparagine-proline-alanine (NPA) motif, present in two intra-domain loops to fold inwards and form a channel (Figure 23). These segments line the path of water permeation (Jung *et al.,* 1994 Cheng *et al.,* 1997, Walz *et al.,* 1997). AQP1 is a homotetramer containing four independent aqueous channels (Smith and Agre, 1991; Jung *et al.,* 1994). The fundamental importance of aquaporins is suggested by their conservation from bacteria through plants to mammals (King *et al.,* 2000).

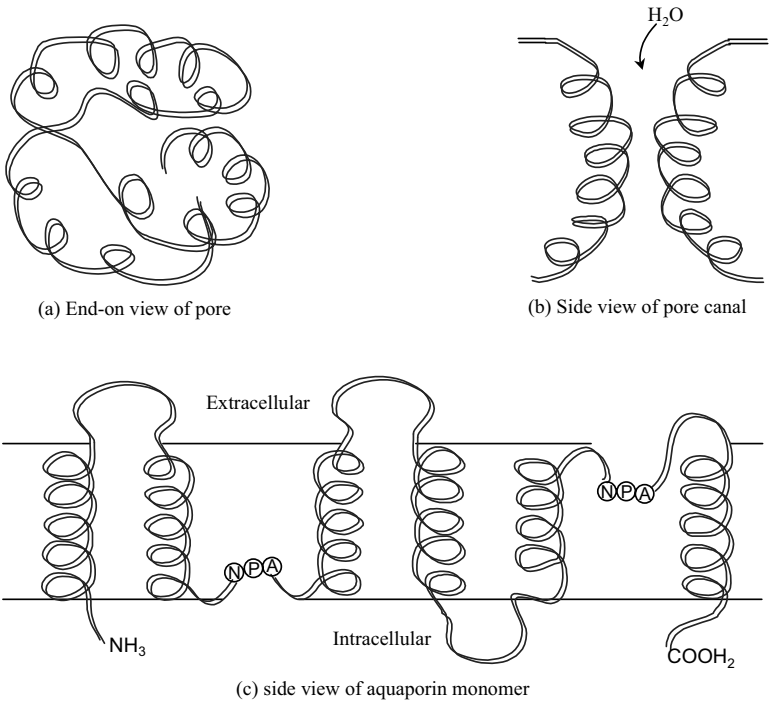

Figure 23. *Aquaporin, water-channel protein. (a) End-on view of the pore from extracellular surface, (b) side-view of the pore-canal, (c) six membrane spanning helices of the aquaporin monomer. The asparagine-proline-alanine (NPA) motifs in the second and fifth loops fold in to produce the water channel.*

Function of aquaporin : passage of water

Murata *et al.,* (2000) described an atomic model of AQP1 at 3.8Å resolution from electron crystallographic data. The pathway of water molecules follows the 'hour glass model' having a constriction in the middle which is hydrophobic, except for two central Asn residues within the conserved NPA motifs. When a water molecule passes through this constriction, the interaction of the oxygen atom with both Asn residues reorients the two hydrogen atoms and it can only form H-bonds with adjacent water molecules. The transfer of protons via H-bonding is thus prevented in this region. It is thought that in addition to spatial restrictions, the pore generates a high dielectric barrier that repels ions (Murata *et al.,* 2000). Tajkhorshid *et al.* (2002) determined the structure of the *E. coli* aquaglyceroporin GlpF with bound water, in native (2.7 Å) and in W 48 F/F 200T mutant (2.1 Å) forms and carried out molecular dynamics (MD) simulation relating to orientation of water molecules inside the channel. Two conserved asparagines force a central water molecule to serve strictly as a hydrogen bond donor to its neighbouring water molecules. Assisted by the electrostatic potential generated by two half-membrane spanning loops, this dictates opposite orientation of water molecules in the two halves of the channel thereby preventing the formation of a 'proton wire' and permitting rapid passage of water (Tajkhorshid *et al.,* 2002). Proton mobility in bulk water is explained by the Grotthuss mechanism involving proton tunneling from one water molecule to the next and rearrangement of H-bonds. Proton mobility in proteins occurs through a 'proton wire', in which a single file of properly H-bonded water molecules and polar groups of protein provides an optimal pathway for efficient proton transfer, the latter requiring a reorientation of water molecules (Tajkhorshid *et al.,* 2002 and references therein). The hydrogen bonding interactions of water molecules with the polar side chains of Asn-76 and Asn-192 of AQP1 on the strictly conserved Asn-Pro-Ala sequence motifs are essential for maintaining the connectivity of water flow in the narrow constriction region. The size of the narrow constriction fluctuates significantly during the MD simulation which frequently breaks the flow of water, and thus breaks the single file water network necessary for proton translocation (Kong and Ma, 2001). AQP1 permeates water molecules across the membrane at a rate of 3×10^9 s^{-1} per channel with an activation energy as low as the one associated with the self-diffusion rate in bulk water (de Groot and Grubmüller, 2001 and references therein).

Regulation of function of aquaporin and disease

Since most aquaporins, including aquaporin 2, are considered to be constitutively open channels, regulation of shuttling of AQP_2 to the apical membrane is considered important. This water channel is expressed in the renal collecting duct and is redistributed to the apical membrane in response to an intracellular signalling cascade, initiated by binding of the antidiuretic hormone vasopressin to its receptor (Deen *et al.,* 2000). Disregulation of aquaporins, especially AQP_2, is critically involved in many water balance disorders. Lack of functional AQP_2 is found in

primary forms of diabetes insipidus. Reduced expression and targetting is seen in several diseases associated with urinary concentrating defects such as acquired nephrogenic diabetes insipidus, post-obstructive polyurea, as well as acute and chronic renal failure. In contrast, in conditions with water retention such as severe congestive heart failure, pregnancy and SIADH both AQP_2 expression levels and apical plasma membrane targetting are increased suggesting a role for AQP_2 in the developent of water retention (Kwon *et al.,* 2001). Many AQPs are regulated by pH variation, phosphorylation and binding of auxiliary proteins (Engel *et al.,* 2000). AQP_1 may traffic from intracellular sites to the plasma membrane in cultured cholangiocytes stimulated with secretin (Marinelli *et al.,* 1999). In response to vasopressin, AQP_2 migrates from intracellular membrane vesicles to the apical membrane, thereby raising the water permeability (Nielsen *et al.,* 1995). Water channel function of all AQPs but AQP_4 can be inhibited by mercurial compounds. These compounds are believed to bind specifically to cysteine residues and block the pore of AQPs (Kuwahara *et al.,* 1997). Brassinolide, a plant hormone, has been found to be involved in the modification of water transport properties of cell membranes in a plant *Arabidopsis thaliana* (Morillon *et al.,* 2001).

Renin-angiotensin system plays an important role in the expression of AQPs and consequently the regulation of water transport in the peritoneum (Imai *et al.,* 2001). Incubation of rat parotid tissue with 10 μM epinephrine resulted in a transient and marked trafficking of AQP_5 from intracellular membranes to the apical plasma membrane that was maximal at 1 min. This effect of epinephrine was mimicked by phenylephrine (α_1 agonist), but not by clonidine (α_2 agonist) or salbutamol (β-agonist). It was inhibited by phentolamine (α-adrenoceptor antagonist), but not by propranolol (β-adrenoceptor antagonist). The results indicate that epinephrine, acting at α_{1-} adrenoceptors, induces the trafficking of AQP_5 to the apical plasma membrane by triggering the release of Ca^{2+} from intracellular stores through inositol 1, 4, 5 triphosphate and ryanodine receptors. Acetylcholine acting on M_3 muscarinic receptors in rat parotid tissue was shown to induce the translocation of AQP_5 from intracellular membranes to the apical plasma membrane by increasing the cytosolic concentration of Ca^{2+} (Ishikawa *et. al.,* 1999 and references therein). Badaut *et al.,* (2000) observed common cellular localization of cholinergic muscarinic receptors (mAChRs) and aquaporin-4 water channels in the cortex, the corpus callosum and in ependymal cells of the rat brain. The authors suggested a functional relationship between mAChRs and AQP-4. Cholinergic muscarinic response may affect water and multiple ion channels. AQP4 in cerebral microvessels is involved in the regulation of water transport between blood and brain in rats (Kobayashi *et al.,* 2001). Protein Kinase and dopamine decrease water permeability via phosphorylation at ser-180 and that the effect is likely mediated by gating of the channel (Zelenina *et al.,* 2002). Krane *et al.* (2001) observed that AQP_5 expression in mouse lung is not restricted to type I cells, but is also detected in alveolar type II cells, and in tracheal and bronchial epithelium. AQP_5 knockout mice (AQP5 (– / –)) show a significantly increased concentration-dependent bronchoconstriction to intravenously administered ACh, as shown by an increase in total lung resistance and a decrease in dynamic lung compliance. Ma *et al.* (1999) demonstrated that

pilocarpine-stimulated saliva production was reduced by more than 60% in AQP_5 knockout mice. Pilocarpine is a cholinergic agonist.

The intracellular routing of AQP_5 molecules is abnormal in the salivary glands of patients with Sjögren's syndrome (SS). SS is a common inflammatory disease characterized by lymphocytic infiltration of salivary and lacrimal glands leading to glandular hypofunction and dry mouth and eyes (Steinfeld *et al.,* 2001). In diseases with increased renal water uptake, total and apical membrane expression of AQP_2 is increased (Deen *et al.,* 2000). Induction of diabetes mellitus resulted in a significant increase in AQP_2, p-AQP_2, and AQP_3 (Nejsum *et al.,* 2001). Alterations in water metabolism are present in such diseased state as diabetes insipidus, syndrome of inappropriate antidiuretic hormone secretion, cardiac failure, cirrhosis, and pregnancy (Schrier *et al.,* 2001). Members of the aquaporin family are implicated in numerous physiological processes as well as pathophysiology of a wide range of clinical disorders (Borgnia *et al.,* 1999, King *et al.,* 2000). AQP_1 water channel is heterogeneously expressed in tumour cells and their vasculature, and that the level of expression is determined not only by the specific cellular origin of the tumour, but also by the location of the tumour in the host animal (Endo *et al.,* 1999).

Manipulation of aquaporin expression may have a therapeutic role in several disease processes (Connolly *et al.,* 1998). AQP-mediated water transport constitutes a most attractive drug target for several widespread pathophysiological conditions which may affect a variety of tissues. Agents, which modify aquaporin function, will have a significant therapeutic potential (Beitz and Schultz, 1999).

4.7.8. Interaction between a potency and biomolecules

It is known that if a homeopathic potency is diluted with water 1 : 1000 or more it still remains effective on patients. This shows that a potentized drug is capable of influencing bulk water in such a way that the latter behaves as a potency. If this is so, then a homeopathic potency dropped on cell surface could modify the organization of water covering the cell surface (chapter III 3.6.1. *Action of a homeopathic potency*). This would affect all the surface proteins of cells thereby altering such activities as receptor-ligand interaction, enzyme substrate interaction, passage of ions, glucose, water, glycerol, O_2, CO_2, macromolecules etc. We have already described that homeopathic potencies can produce both structural and functional modification of a protein (chapters II, III). Since a homeopathic potency is specifically structured water, it is likely to influence structural water molecules intimately associated with proteins and sugars resulting in functional modification of the latter. Yamada (2001) studied intracellular water of frog skeletal muscles under various physiological conditions by use of ^{1}H-nuclear magnetic resonance technique. The intracellular water of muscle fibres is structured and aligned along the myofilaments. The state of intracellular water changes with physiological conditions of the muscle fibres. The structured water suppresses the diffusion of solutes in an aniosotropic fashion. This shows that structured water can alter diffusion of solutes in cells, and play an important role in cell physiology. When the cell is in a morbid state, the structured water in it assumes a different state. An

appropriate homeopathic potency actually holds the complementary water structure which induces change in water structure of the diseased cell thereby restoring the normal state of the cellular water. The change in water structure is brought about through a change in the number of hydrogen bonds and in the strength of the hydrogen bonds.

Receptor or transporter proteins protrude outside plasma membranes. Some, for instance, aquaporins form transmembrane loops. In the protruding loops there may be polar or hydrophilic amino acids and apolar or hydrophobic amino acids. Depending on the nature of these properties of amino acids, the layer of hydration would be different. Some of them would serve as attractant while others as repellant for structured water clusters. Obviously, because of differential binding of structured water molecules to the various amino acid molecules, the overall function of the transporter or receptor molecules would be changed. This would finally lead to the production of biological effects of potentized drugs on cells. Shorter polymers of sugars (oligosaccharides) attached to proteins or lipids at the cell surface serve as specific cellular signals (Nelson and Cox, 2000). Higher plants use metabolites like glucose, sucrose and nitrate not only as nutrients but also as signals as part of their life strategies. Sugars serve as signal molecules in plant seed development (Wobus and Weber, 1999). Some sugar molecules can fit into a network structure of icosahedral water cluster with hydrogen bonding by replacing a chair-form water hexamer in a cluster. Equatorial hydroxyl groups on chair-form sugars show stronger interactions as the water molecules are optimally positioned for more extensive and stronger hydrogen bonding (Uedaira and Uedaira, 2001). Sialic acid residues in terminal positions of oligosaccharides covalently linked to glycoproteins are normally found on all surfaces, and can play an important role in cell-cell interaction and recognition process. The siglecs (sialic acid-binding immunoglobulin-like lectins) mediate sialic acid dependent cellular interactions (Freeman *et al.,* 2001). Potentized drugs may interact with hydration shell of sugars or lectins thereby modifying their effect as signal molecules. The effect of homeopathic potencies on plants may be mediated through sugar molecules.

Thus we see that a potentized drug can interact on cell surface proteins or their sugar moieties and produce a cascade of biochemical evants internally in the cells in contact with the drug. How is the effect propagated beyond the area of contact between biomolecules and drug molecules? If the contact area has sensory neurons, the propagation of the primary effect of a potency can occur through nerve impulses. Affected sensory neurons would transmit impulses to the brain which would process the message and produce appropriate reaction through efferent nerves. Details of this kind of propagation in man and mammals have been described in an earlier book by Sukul (1997). Irrespective of neuronal propagation, the action of a potency would spread in all directions from the point of contact through the continuity of watery medium covering cell surfaces.

Homeopathic medicines can cure or ameliorate many inflammatory diseases such as boils, abscess, sepsis, rheumatoid arthritis, inflammatory bowel syndrome etc., and the therapeutic effect is mediated through the nervous system (Sukul, 1997). There exists a link between inflammation and nervous system. Lipo-

polysaccharides, components of bacterial cell wall, activate the immune system's macrophages. The circulating white blood cells, monocytes, enter tissues and become tissue macrophages. These cells on activation release cytokines including tumour-necrosis factor (TNF). This protein can affect nearly all cell types and induce expression of a large number of genes that encode essential inflammatory molecules. TNF is beneficial when it is restricted to the site of infection. As it spreads through circulation, it causes serious inflammatory diseases (Libert, 2003). The nervous system, through the 10^{th} cranial nerve vagus, can inhibit significantly and rapidly the release of macrophage TNF and reduce the systemic inflammatory responses. This mechanism is known as cholinergic anti-inflammatory pathway. The vagus nerve upon stimulation releases the neurotransmitter molecule acetylcholine which binds to the receptor on macrophages thereby suppressing TNF release. The nicotinic acetylcholine receptor $\propto 7$ subunit is essential for inhibiting cytokine synthesis (Wang *et al.*, 2003). Potentized homeopathic drugs may stimulate the sensory nerve endings of the vagus and help in the release of acetylcholine. The drugs may also act on the $\propto 7$ subunit of the receptor protein on the surface of macrophages and reduce TNF release. Libert (2003) suggests a possible connection between inflammation and molecular biology of 'alternative' medicine.

While all the membrane proteins are affected by a homeopathic potency we can lay special emphasis on aquaporins as primary targets because these proteins are ubiquitous in all forms of life and are closely related to health and disease. We have described earlier how the function of aquaporins is regulated. Homeopathic potencies may influence those regulatory processes and thus alter the permeation of water through plasma membranes. We have already presented evidence for enhanced peremeation of water through red blood cells AQP_1 by potentized homeopathic drugs (chapter II 2.4.2).

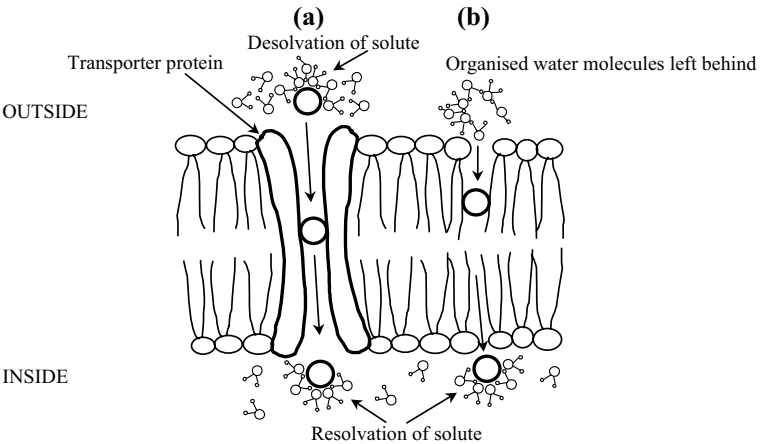

Figure 24. *Desolvation of a solute particle during passage through the channel of a transporter protein (a) and by simple diffusion through the lipid bilayer (b)*

As ions pass through ion channels of a plasma membrane they are desolvated (Figure 24), but the water molecules of the empty hydration shell left behind remain organized for 1 picosecond (Van Oss, personal communication). Since there are thousands of ion channels, there would be thousands of organised clusters around a cell in a given time. The types of organized water clusters and the number of each type existing in a given time would depend on the varieties of ion channels and the rate of movement of ions or other molecules across a plasma membrane. As mentioned earlier these organized water clusters remain in dynamic equilibrium (chapter III). The equilibrium geometry of structured water clusters would be modified and reset by a homeopathic potency introduced. Propagation of the potency-induced modification of water clusters would be accomplished by structural diffusion through water reorientation and tetrahedral displacement (chapter III).

Under diseased condition structure of integral proteins on cell surface is modified leading to differently organized water structures in extra-cellular fluid and in cytosol. An appropriate homeopathic potency would tend to reorganize the morbid structural geometry of water clusters to its native form initiating a cascade of biochemical reactions inside cells. This reaction would continue until normal health is restored in due course. It has been mentioned earlier that a single ligand molecule bound to a single receptor may result in entry of thousands of ions through the opened channel or the synthesis of thousands of molecules of an intracellular messenger molecule by an activated enzyme (Nelson and Cox, 2000). Thus a few structured water molecules bound to a receptor can initiate entry of thousands of ions and water molecules as well as synthesis of thousands of molecules inside a cell. Here organized water clusters do not serve as a specific ligand for a specific receptor but as a master key for all kinds of ligands and their receptors.

4.8. Role of carbohydrates in recognition process

We have mentioned earlier that sugars can serve as signal molecules in a biological system and can interact with water (chapter IV). We describe here the possible role carbohydrates in general can play in recognition process and can respond to a homeopathic potency. Carbohydrates with bound water molecules play an important role in recognition process. Potentized homeopathic drugs, which are essentially structured water molecules, initiate specific biochemical events in cells when they come in contact with carbohydrate molecules.

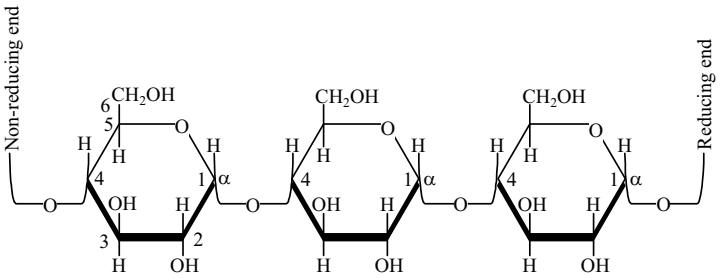

Figure 25. *A part of amylose, a linear polymer of D-glucose residues in (α1→4) linkage showing exposed OH groups for hydrogen-bonding with water*

The most important storage polysaccharides are starch in plant cells and glycogen in animal cells. Both occur intracellularly as large clusters or granules. Starch and glycogen molecules are heavily hydrated because they have many exposed hydroxyl groups available to hydrogen bond with water (Figure 25). Starch contains two types of glucose polymers, amylose and amylopectin. The former consists of long, unbranched chains of D-glucose residues connected by (α 1→4) linkages. Amylopectin also has a high molecular weight but unlike amylose is highly branched. The glycosidic linkages joining successive glucose residues in amylopectin chains are (α 1→4). The branch points occur as one per 24 to 30 residues and are (α 1→6) linkages. Like amylopectin, glycogen is a polymer of (α 1→4)-linked subunits of glucose with (α 1→6)-linked branches. However, glycogen is more extensively branched with an average of one branch per 8 to 12 residues. It is more compact than starch.

Polysaccharides, also called glycans, differ from each other in the identity of their recurring monosaccharide units, in the length of their chains, in the types of bonds linking the units, and in the degree of branching. Homopolysaccharides contain a single type of monomer. Heteropolysaccharides contain two or more different kinds of monomer. The disaccharide lactose, which yields D-galactose and D-glucose on hydrolysis, occurs naturally in milk. It is, therefore, called the sugar of milk. The anomeric carbon of the glucose residue is available for oxidation, and thus lactose is a reducing disaccharide. Sucrose is a disaccharide of glucose and fructose. It is formed by plants but not by higher animals. In contrast to maltose and lactose, sucrose contains no free anomeric carbon atom. The anomeric carbons of both the monosaccharide units, glucose and fructose, are involved in the glycosidic bond. Sucrose is, therfore, not a reducing sugar (Nelson and Cox, 2000). It is to be noted here that homeopathic potencies, prepared in aqueous ethanol, are presrved in sucrose globules (chapter I). Homeopathic potencies are also soaked in lactose powder and pellets and then given to the patients. Thus these two polysaccharides do not serve as neutral carriers but play an active role as information molecules in combination with potentized drugs.

Plants can synthesize carbohydrates through the process of photosynthesis. Animals obtain them from plant sources. Diatary carbohydrates are for the most part polymers of hexoses, of which the most important are glucose, galactose, and fractose. Most of the monosaccharides occuring in the body are the D-isomers. The principal product of carbohydrate digestion and the principal circulating sugar is glucose. The concentration of glucose in blood of mammals is about 5 mM. Once it enters the cells, glucose is normally phosphorylated to form glucose 6-phosphate. The enzyme, that catalyzes this reaction, is hexokinase. In the liver, there is in addition an enzyme called glucokinase, which has greater specificity for glucose and which, unlike hexokinase, is increased by insulin and reduced in starvation and diabetes. The glucose 6-phosphate is either polymerized into glycogen or catabolized (Ganong, 1999).

4.9. Structured water and early life

Right from the emergence of life on earth, organisms have encountered various types of chemicals in their environment. It has now been accepted that life originated in aqueous environment in early seas, lakes or ponds. Water, sugars, amino acids and ethanol are the most primordial molecules the earliest forms of life first encountered in their environment, and utilized them. The source of energy for the earliest life forms was fermentation. This is because there was no free oxygen in the atmosphere. The first organisms were acellular heterotrophs, feeding on the accumulated inorganically synthesized resources. In the reducing atmosphere respiration was anaerobic leading to the release of carbon dioxide. Fermentation of sugar by yeast leads to the production of alcohol, carbon dioxide and energy. Yeast and other microorganisms ferment glucose to ethanol and CO_2. The enzyme alcohol dehydrogenase is present in many organisms that metabolize alcohol including humans. In human liver this enzyme catalyzes the oxidation of ethanol, either ingested or produced by intestinal microorganisms, with the concomitant reduction of NAD^+ to NADH. Some marine vertebrates ferment glucose to ethanol and CO_2 in order to generate ATP.

Thus ethanol molecules stabilized some water structures and brought about a specific change in the bulk water in the vicinity. Primordial protistans might have developed a mechanism to sense the specifically structured water and orient themselves accordingly. This adaptive mechanism helped them find their way to the colony of anaerobic heterotrophs on which they lived. While beneficial chemicals provided energy for sustaining life, harmful chemicals elicited defensive responses from primitive life forms. One of the most pressing problems of aquatic life is to maintain osmotic balance in their environment. Moreover, organisms encountered other organisms and the latter invaded the former as predatoros or pathogens. Naturally, organisms developed protective mechanisms against invading organisms for their survival. In lower organisms, some macromolecules, called antigens, are synthesized and these initiate defense responses in them. So, if they could sense the impending danger earlier through specifically structured water they would be able to prepare themselves to cope with the situation. This prior sensing might have been

mediated through the major intrinsic proteins (MIPs), particularly aquaporins which are highly conserved and ubiquitous in all forms of life. Because early protistans originated and lived in aqueous environment, regulation of entry of water into their body was one of the topmost priorities for adaptation and survival. Thus aquaporins and structured water clusters have been playing an important role since the beginning of life. Together they constitute an efficient machinery for signal recognition and maintenance of homeostasis in all life forms. Homeopathy is an adaptation of this natural machinery and brings about homeostasis in the living system under abnormal condition.

Summary

Potentized homeopathic medicines have preferential action on sides of the body; some are more effective on one side than on the other. This differential effect of the medicines with respect to laterality can be traced to functional asymmetry of the human brain. The brain can asymmetrically modulate nurochemical, neuroendocrine and immune reactivity. Potentized drugs are very often selected on the basis of time modalities of symptoms of a disease. The time modalities of the drug action can be correlated with the internal clock or biological rhythms of organisms which are disturbed in diseased conditions. Melatonin, secreted in the brain, has marked influence on the circadian rhythms.

Chronic diseases arise from miasms such as psora, sycosis and syphilis. The chief of the maisms is psora which manifests itself in itching eruption of skin. It is proposed that ‘itch and tickle’ sensation, supplied by naked nerve endings in the skin, represents psora.

Some homeopathic remedies like polycrests produce a wide variety of symptoms common to many diseases. They may act through multifunctional neural networks. For this, treatment of a chronic disease with a high potency of a polycrest sometimes results in brief recapitulation of past diseases, particularly their pain components which are connected with the same multifunctional neural network. According to the ‘neuromatrix’ theory pain is a multidimensional experience produced by characteristic neurosignature patterns of nerve impulses generated by a widely distributed neural network. The neuromatrix concept fits in with the holistic approach of homeopathy.

Proteins and nuclei acids, which serve as informational macromolecules in living organisms, are made up of different units. Although water is a simple molecule, it can form different structures through hydrogen bonding. The structural configurations of water achieved by H-bonding are strengthened by succussion and preserved by ethanol. Water structures remain at dynamic equilibrium at room temperature because H-bond dissociation and reassociation occur between the same O-H groups. While specific structural configuration of water maintains the identity of a drug, the strength of hydrogen bonding, augmented by succussion, carries the information of its potency.

Potentized drugs are applied on oral cavity, leaves of plants or cell surfaces where the drugs come in contact with the proteins on plasma membranes. All the

cell surfaces are bathed in a continuous film of water which maintains a geometric configuration in normal healthy condition. The non-covalent forces that locally alter the structure of water are van der Waals, acid-base and electrostaic forces. The most dominant of these forces are acid-base forces which occur due to strong cohesive and adhesive hydrogen-bonding interactions in water. Water structure on all surfaces assumes a different configuration in a diseased state depending on the nature of the disease. As a homeopathic remedy or, in other words, a stereospecific structured water, is applied on a cell surface, the water structure on cell surfaces is modified resulting in structural changes in integral membrane proteins. This triggers a cascade of biochemical events in cytosol culminating in restoration of health.

Although all integral membrane proteins are affected due to the application of a homeopathic potency, a ubiquitous membrane protein, aquaporin, may be the primary target of a potency. This water channel protein helps in passage of water through the cell membrane and is related to health and disease. Sucrose and lactose, which are soaked with a homeopathic potency and are used as medicated globules, play an active role as information molecules in combination with a potentized drug.

Water, sugars, amino acids and ethanol are the most primordial molecules the earliest forms of life first encountered in their environment and utilized them. Ethanol molecules stabilized water structures, some of which carry information of a potential source of food. Primordial protistans might have developed a mechanism by which they can sense the specifically structured water and orient themselves accordingly. Because early protistans originated and lived in aqueous environment, regulation of entry of water into their body was one of the topmost priorities for their adaptation and survival. Thus aquaporins and structured water clusters have been playing an important role in maintaining health since the beginning of life. Homeopathy is an adaptation of this natural machinery and brings about homeostasis in living organisms under an abnormal condition.

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