

Proposition of a new system of medicine based on tolerance principle

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Summary The author proposes an explanatory model to modify Hahnemann's old tool – 'similia similibus curantur' and suggested a new experimental design to be used to detect the root cause of several chronic diseases and to treat them. All the biochemical reactions of an ideal cell are interlinked by enzymes like a network and shortfall of any one of them may create specific combination of symptoms (e.g., phenylketonuria, alkaptonuria, etc.), due to lack of one or more products that enables us to identify the responsible enzyme. Sometimes malsynthesis of an unknown enzyme(s) or receptors are responsible for a chronic disease and it becomes difficult to identify them by merely observing symptoms. Hence involvement of a normal healthy person ('prover') would be essential to detect it. If an inhibitor that comes from a drug is able to bring the same combination of symptoms in prover it may be predicted that the drug is able to bind and inhibit the responsible enzyme or its product. Minute doses of the same inhibitor(s) can cure the disease if it can act as the ligand of the same enzyme(s), by increasing the rate of transcription (by a positive feedback loop), to compensate the loss of product of the same. The ligand-inhibitor should be trapped in by an organic molecule, like ethanol by the process of potentization to increase the invasiveness of the medicine and to avoid detoxification mechanism, baffling of which increases the concentration of inhibitor inside the cell in course. The cells able to cope up with the stress by the operation of positive feedback loop or compensation cycle synthesize more enzymes and multiply rapidly, but those cells unable to tolerate such stress gradually perish. Thus Hahnemann's principle being dependent on the cause of symptoms becomes modified as 'similia similibus curantur causosymptomically'. © 2002 Elsevier Science Ltd. All rights reserved.

INTRODUCTION

Friedrich Samuel Hahnemann (1755–1843) was the father of a system of medical practice that treats a disease by administration of minute doses of remedy that would in healthy persons ('prover') produce the symptoms of the disease treated. The essence of the system of homeopathy according to its father, lies in three words – 'similia similibus curantur', which has been severely criticized by present-day scientists, because, anything similar cannot 'curantur'. The present author also thinks

that this is simply an observation, beyond the scope of any scientific theory, but certainly a valuable tool, which seems to be improvised to combat several chronic diseases that are difficult to cure. The author has also designed an experimental scheme for the demonstration of the similia principle according to the concept of modern biochemistry.

There are numerous evidences published in mainstream journals in support of Hahnemann's system, but antagonistic examples are not fewer. The majority of present-day workers believe that the Tolerance principle or 'Hormesis' (1) which can easily be demonstrated under laboratory conditions may have some relation with similia principle, as described below.

It has been found that there is an increase in tolerance of toxicant to an individual by the pre-treatment of the

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same in minute or homeopathic doses. Sensitivity of cadmium was found to decrease in a fish, *Fundulus heteroclitus*, by the pretreatment of the same poison in minute doses (2) probably due to increment of efficiency of metallothionein synthesis. Similar study was done on amphibian embryo (3), rats (4,5), and several other animals (6,7). There are several examples also, where pretreatment doses are much higher, and post-treatment doses are minute for the same toxicant that causes recovery in rats (8–10) and dogs (11) from the effect of the same. It is also evident that minute dose pretreatment of a toxicant sometimes brings protection against a different toxicant (12–15). There are also several evidences of statistically significant recovery of diseases by the post(?) treatment of minute doses of homeopathic drugs (16–20).

Vaccination against a pathogen by the minute dose pretreatment of a pathogen-related antigen seems to be the most complicated example of tolerance principle. Pretreatment of highly potentized solution of 'Bursin', a tripeptide, Lys-His-Gly-NH₂, found in the bursa of chicks was found to be reproducibly effective to produce normal quantity of antibodies against post-treatment of porcine thyroglobulin, even in embryonically bursectomized chicks (21), but unable to do the same in non-treated ones. Similarly, potentized solution of 'Thymulin', prepared from thymus extract of rats, was found effective for immunoprotection and immunomodulation (22). Immunotolerance, over-expression, and over-replication of genes in lymphocytes by the minute pretreatment of immunosuppressive and cytostatic agents have been reported by some workers (23,24). The protective effect of minute dose drugs in transcriptional level has been proved by in vitro study also (25–27). It was found that minute dose toxicant, inhibitor or even antigen may act as 'stressor', in which the system concerned appeals to all the resources of its genome to find an answer, either by amplifying a synthesis of pre-existing substance or by waking up a non-transcribed gene or by transcribing suitable segments of DNA to translate a single protein. Rearrangement of DNA sequence, though, is a very rare event.

It seems to be possible from the above works that minute dose toxic drugs can quickly penetrate into each and every cell of the system through body fluid due to two reasons. First, in high-dilution drug, molecules occupy a negligible fraction of total volume of body fluid, moving at random in the viscous fluid and colliding infrequently with each other and behave almost like gas molecules, which is not possible in low dilution of the same drug. Secondly, concentrations of high-dilution toxic drugs are far below the threshold level to trigger detoxification mechanism, involving synthesis of nonspecific biotransformation enzymes (e.g., glutathione-S-transferase) and detoxifying protein (e.g., metal-

lothionein, synthesized in liver). If an individual is pretreated with a toxicant in minute and prolonged doses, all the cells becomes tolerant against the same. If the pretreatment dose is high and post-treatment doses are prolonged and minute there is also a chance of recovery due to acquisition of tolerance in non-affected cells. Here the pretreated high-dose toxicant cannot quickly affect all the cells of a tissue due to the above reasons, but minute post-treatments easily 'vaccinate' all of them against the harmful post-effect of the pretreated toxicant. This is possible for minute pre- or post-treatment of different toxicant also, possibly if both of them are able to bind and partially inactivate the same target protein, later the loss becomes compensated by an increased rate of transcription, which enables them to combat against high post- or pretreatment stress, respectively. In diseased individuals, pretreatment is not at all required, because the target protein is originally deficient; if prolonged minute post-treatment can increase the rate of transcription of the same, the patient may be recovered. In rapidly dividing cells pretreatment not only increases transcription rate but replication also, so that the 'vaccinated cells' multiply rapidly, produce more specific proteins that are able to inactivate post-treated toxicant or pathogen or compensate the loss of target protein. Thus immunosuppressive and cytostatic drugs when pretreated in minute dose are able to increase particular kind of T-lymphocytes and pretreatment of minute pathogen-related antigen or even small peptide does so for B lymphocytes that have specific receptors for the same. Specific antibodies against a pathogen could be produced in B lymphocytes by transcribing different DNA segments and splicing them into a single mRNA.

The present author would try to prepare a self-explanatory model that can support all the above facts and thereby would try to repair Hahnemann's old tool by the establishment of new experimental design. Before doing so, he would have to solve the following problems:

1. detection of the intracellular root cause of a disease;
2. proper selection of ingredients of a medicine;
3. effect of a drug on positive and negative gene regulation;
4. utility of taking minute-dose potentized drugs as medicine;
5. permanent cure of a disease by a medicine;
6. reproducibility of effect of a medicine.

DERIVATION OF THE HYPOTHESIS

Detection of the intracellular root cause of a disease

Problem

It has been observed that several genetically controlled human diseases are mediated by proteins, the majority

of them are enzymes. Though several other kinds of proteins may be responsible for chronic and hereditary diseases, enzymes should be considered as the most important intracellular cause, because:

- (i) Synthesis of any kind of proteins requires enzymes. Deficiency of any type of metabolic product may be due to deficiency of a rate-limiting enzyme. Replication transcription and translation cannot take place without enzymes. Synthesis and processing of hormones and antibodies require enzymes. Cretinism is a thyroid hormone-deficiency disease, but the enzyme iodotyrosine deiodinase may be responsible for it.
- (ii) All the enzymes in a cell are interlinked like a network, sometimes deficiency of one enzyme may cause multiple symptoms, e.g., deficiency of phenylalanine-4-monooxygenase causes phenylketonuria (PKU). The enzyme can be detected by a specific combination of symptoms, i.e., mental retardation and excretion of phenylalanine. Since the said enzyme is metabolically linked with tyrosinase, responsible for melanin synthesis, PKU patients show less pigmentation in skin and hair. Other well-known enzyme-related diseases are albinism, acetalasia, alkaptonuria, cystic fibrosis, galactosemia, glycogen storage disease, Huntington's cholera, Tay-Sachs disease, etc. Root cause or rate-limiting enzyme of all of them is detectable by a specific combination of symptoms.
- (iii) Environmental pollutants, like heavy metals have been reported to impair the activities of several enzymes (28) and may cause several diseases (e.g., Mina Matà disease by mercury pollution), that can be identified by symptoms.

Next to the enzymes the receptors may be considered as the intracellular root cause of a disease, as they can control the activity of several enzymes by binding hormones, substrates, ions or even antigens in the case of lymphocytes. Hormones should not be considered as an intracellular root cause, because, they are carried to the concerned cell from highly specialized endocrine cells. Though the hormones are responsible for several diseases, we shall consider it to be merely a specialized product of endocrine cells. Hypo- or hypersecretion of it represents disease of the latter. For the same reason we

shall consider antibody as a specialized product of lymphocytes. Special emphasis should be given on antibodies when the disease is parasitic and chronic.

It becomes clear from the above that symptoms sometimes help to detect the responsible enzyme, but it may become rather difficult if deficiency of an unknown enzyme(s) or receptors of very minute concentration are responsible for a disease.

Proposed solution

In complicated cases as mentioned above, involvement of a 'prover' seems to be essential to detect the root cause of a disease. A prover, according to Hahnemann's principle, is a normal healthy person, who shows a combination of symptoms by the administration of a drug in low and tolerable dose. Suppose a group of patients is suffering from a symptom c only (case 1) due to malfunction of malsynthesis of an unknown enzyme E (Table 1), that is directly or indirectly responsible for formation of at least one unknown metabolic end product, lack of which is responsible for the symptom. A group of provers show the same symptom by the low-dose application of pure inhibitory drug I. So it is highly possible that the unknown enzyme E is the protein factor that can bind I and can prevent symptom c in healthy persons by mediating the formation of at least one end product. Similarly, if a patient group is suffering from two symptoms b and c (case 2) and the provers show the same symptoms by the application of a drug I', it is likely that the E' is that protein which can bind I' and becomes inhibited by it, prevents symptom b in healthy persons by mediation of formation of at least one end product, and might be metabolically linked with E (due to common symptom c). A group of patients suffering from three symptoms a, b, and c (case 3), where the provers show all the three symptoms by the application of drug I''. The unknown enzyme E'' can be defined as the binder of I'', which directly or indirectly responsible for the formation of at least one end product that prevents symptom a and might be metabolically linked with E' (due to common symptom b) and E (due to common symptom c). Now if a known enzyme that does not bind I, I' or I'', becomes inhibited by any one of them to synthesize a known product, it may be presumed that the said enzyme has close metabolic linkage with E. All the above-mentioned enzymes are synthesized in ribo-

Table 1 Detection of intracellular root cause of a disease by applying test drugs on 'prover'

Cases	Symptoms	Unknown deficient enzyme	Unknown deficient end product(s)	Test drug
1	c	E	P	I
2	b, c	E'	P', P	I'
3	a, b, c	E''	P'', P', P	I''

It is given that any of the three test drugs does not bind a known enzyme but inhibits the formation of its product.

some by mRNA, which is synthesized in the nucleus by the help of RNA polymerase and several other enzymes. So, we may draw a metabolic pathway (Fig. 1) from the available data of Table 1 that converts substrate S, which enters into the cell by receptor R, into intermediate products P₁, P₂, and P₃ by the mediation of three enzymes, E'', E', and E. Here P'', P', and P are the metabolic end products of the network and deficiency of which may cause specific symptoms a, b and c respectively. So the P'', P', and P could be defined as symptom a, b, and c preventer, respectively. Single blockage in branching water pipes can be detected by observing flow-rates of each pipe. Fig. 2 represents condition of case 1, 2, and 3

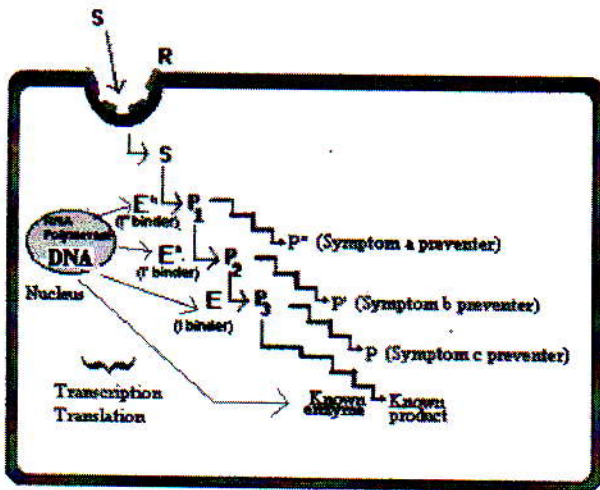


Fig. 1 Hypothetical metabolic pathway drawn from Table 1, where the three unknown enzymes have been defined on the basis of specific inhibitor binding ability. (The known product could act as a reference molecule of the hypothetical pathway).

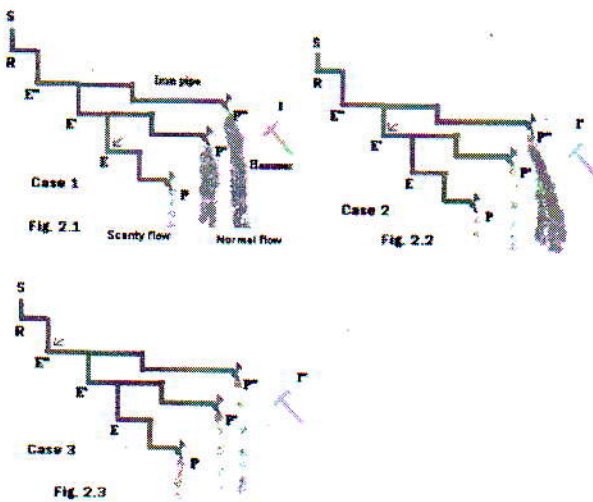


Fig. 2 Detection and removal of single blockage of branching water pipes by gentle hammering at a particular point (red arrow).

patients. Light hammering on arrowed points may clear the blockage but hard hitting on these points can make the flow scantier even in non-choked water pipes.

Proper selection of ingredients of a medicine

Problem

Suppose one becomes able to detect the responsible enzyme(s) for a disease, how could it be normalized? In allopathy there are several methods to modulate the function of a known enzyme. Take a simple example, PKU, as mentioned earlier, is a disease, involving an enzyme deficiency that is required for the conversion of phenylalanine into tyrosine. The patient is suggested to take low phenylalanine-containing food: but this is not at all a real solution.

Take a complicated example, Parkinson's disease, a neurodegenerative syndrome, caused by a defect in dopamine-synthesizing pathway, deficiency of certain receptors are also involved in it. L-dopa, a precursor of dopamine, may be supplemented as drug to cure such a disease, but for prolonged use it causes 'wearing off phenomenon', i.e., loss of efficacy of L-dopa (29), probably due to 'switch off' of the gene for dopa synthesis. Hence the enzymes responsible for it would be less synthesized and more and more L-dopa would be required as drug.

Proposed solution

There are several examples also, where inhibitory allopathic drugs like aspirin, used to check over synthesis of an enzyme, e.g., cyclo-oxygenase, (30), loses activity after repeated and prolonged use, probably due to positive feedback effect, involving over-expression of the gene, responsible for the synthesis of the responsive enzyme. Low doses of the same drug have been found to permanently cure multiple physical disorders (31,32). So why don't we use the minute dose inhibitor for the stimulation of enzyme synthesis. The minute dose inhibitor should preferably be the ligand of the responsive enzyme to ensure binding with the same in vivo.

There is continual enzyme synthesis and enzyme degradation in a tissue and the equation $dE/dt = K_s - K_d E$ (33) describes a change in enzyme content of a given tissue or cell; where E is the enzyme content of the tissue, K_s is the rate constant for enzyme synthesis, K_d is the rate constant for enzyme degradation (34). Under steady-state condition, i.e. when the enzyme concentration remains fixed, $dE/dt = 0$, the rate of synthesis is equal to the rate of degradation, $K_s = K_d E$. Cellular hydrolytic enzymes that come from lysosome naturally cause enzyme degradation. Binding of a ligand-inhibitor with a particular enzyme fraction is also a kind of degradation and that would be able to make an urge to

increase K_s , to keep the functional enzyme content of a given tissue fixed or equilibrium.

Ingredients of homeopathic drugs are found from resources and contain several inhibitor molecules that can bind specific enzymes or receptors. Plant products contain several inhibitory alkaloids. Homeopathic drugs, 'Thea' and 'Coffea' prepared from tea (leaves) and coffee (fruits) contain theophylline and caffeine respectively that are well known inhibitors of phosphodiesterase enzyme. Likewise homeopathic drug 'Physostigma' prepared from a plant, *Physostigma venenosum*, contains eserine which blocks acetyl cholinesterase. Homeopathic drug 'Agaricus' prepared from a fungus, contains muscarine, which can block acetylcholine receptors of nerve cells. Homeopathic drug of animal origin contains several small peptide that can act as antigenic determinants of several pathogens and can bind specific antigenic receptors of lymphocytes and can initiate allergic reactions if applied in a high dose; e.g., homeopathic drug 'Apis' prepared from honey bee. Homeopathic drugs of mineral origin may contain several inhibitor molecules. As for example, 'Merc Sol', a drug, contains mercury salt and 'Plumbum' contain lead salt, both can inhibit a number of enzymes, even in minute dose. Botulinum toxin, which comes from a bacteria, is an inhibitor of acetyl cholinesterase, and has been used in homeopathic drug 'Botulinum'.

Effect of a drug on positive and negative gene regulation

Problem

Regulation of activity of genes by end product of a series of enzymatic reaction is well known in prokaryotes, e.g., *Trp*-operon in *E. coli*, where tryptophan, being an end product acts as corepressor and terminates the synthesis of several enzymes by negative feedback loop. In eukaryotes the method is rather complex.

Activators and repressors of eukaryotic gene transcription act by altering the rate of formation of several transcriptional factor complexes on the promoter region. The regulator protein or *trans* factor bind to control sequences or *cis*-elements either within the promoter region, relatively near the gene, or at long distance, upstream or downstream from the gene. The long-distance control elements can either be positive control elements (enhancers) or negative control elements (silencers). The transcription of an enzyme-coding gene is regulated by several *trans* factors that interact with each other and general transcription factor to control the rate of transcriptional initiation. Many *trans* factors bind to sequence elements, close to the gene, but some bind to enhancers and silencers located long away from gene. Single enhancer or silencer can control expression of

more than one gene and they only form a contact with RNA polymerase when DNA forms a loop (35). According to many scientists, the distinction between enhancer and promoter is blurred; some elements are found in both enhancers and promoters (36). Some enhancers or silencers are activated in tissues only, but others could be active in all cells. From the above discussion we may conclude that our knowledge regarding positive and negative control of eukaryotic genes is incomplete and the effect of drugs on these control elements is simply beyond our reach.

Proposed solution

Paucity of knowledge regarding mode of operation of control elements would not be able to restrict the scope of the proposed scheme. Positive and negative feedback of all biomolecules exists in organ level, tissue level, and cellular level though their mode of action may be different. The proposed hypothesis depends on the existence of negative and positive control elements, that are present in all individuals from bacteria to man, but not on their mechanistic pathway, i.e., how the information of alteration of substrate or product of an enzyme is brought to the *cis*-element of a gene by a *trans*-factor. If a drug inhibits the majority of the enzyme molecules, it is very difficult to be compensated very soon by positive feedback loop; if an infinitely small fraction of the enzyme is inhibited, operation of compensation cycle would be infinitely slow; but if an optimally small fraction (the optimal fraction) of the same is inhibited by the drug it seems to be quicker. The partial inhibition of any enzyme of the metabolic pathway yields less end product formation to activate negative control element, so that enhancer actively initiates transcription of the gene, but silencer cannot stop the same and as a result K_s increases. As the same inhibitor bound enzyme molecule is repeatedly used in a biochemical reaction according to

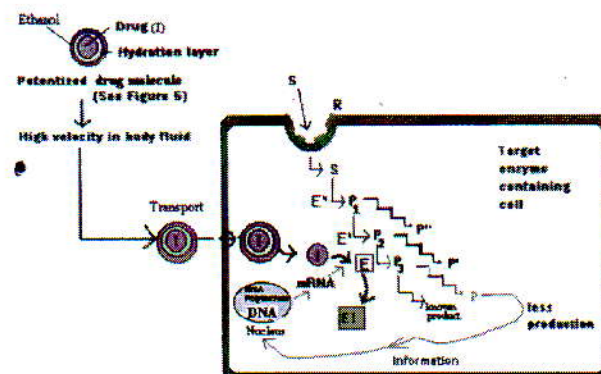


Fig. 3 Recovery of case 1 patient by potentized drug I, which initiates the continual operation of positive feedback loop (indicated by red arrow) by inhibiting negative feedback loop.

its turn over number, the frequency of inhibition to negative feedback loop per minute becomes multiplied and that causes more efficiency of positive feedback loop. Fig. 3 represents how a minute dose ligand-inhibitor drug could recover the rate of transcription of enzyme E in a case 1 patient by the continual operation of positive feed back loop or compensation cycle (for details see the next sections).

Utility of taking minute dose potentized drugs as medicine

Problem

Generalized technique of 'potentization' or centesimal dilution includes the following steps: the crude drug is dissolved in distilled water, mixed with absolute ethanol, volume made up to 100 ml, shaken well, to prepare the 'Mother tincture' (Fig. 4a). Small fraction (1 ml) of the tincture taken, again mixed with 99 ml fresh ethanol and repeated manual stroke was given at the bottom of the container to prepare 1st potency solution. A small fraction (1 ml) of the 1st potency solution taken and mixed with 99 ml fresh ethanol, repeated manual stroke given at the bottom of the container to prepare 2nd potency solution (Figs. 4b-d) and so on. Thus, the original drug is diluted by ethanol several times in a repeated and systematic way.

Now, suppose one mole pure drug, containing 6.023×10^{23} molecules, has been dissolved in water and added ethanol to make the volume to 100 ml of mother tincture. After first potentization the number of molecules would be 6.023×10^{21} , after second potentization 6.023×10^{19} and likewise after twelfth potentization 6.023×10^{-1} . Hence practically no molecule of the original remedy remains in the solution, but several workers (8,18,21,22) obtained good result by the pre- or post-treatment of such type of non-molecular dilution. How can this be explained?

Proposed solution

Ethanol bound hydrated drug molecules, when highly potentized remain concentrated at the bottom layer of the solution and show drug bound liposome like orientation of molecules.

This can be explained as follows. Cohn in 1941 derived a method for precipitation of proteins from their aqueous solution by the gradual addition of ethanol (37). The positive and negative charges of protein molecules remain separated widely in aqueous medium due to high dielectric constant of water ($\epsilon = 80$). By the gradual addition of ethanol, having low dielectric constant ($\epsilon = 24$) the attraction between opposite charges of protein increases and they aggregate and precipitate. The

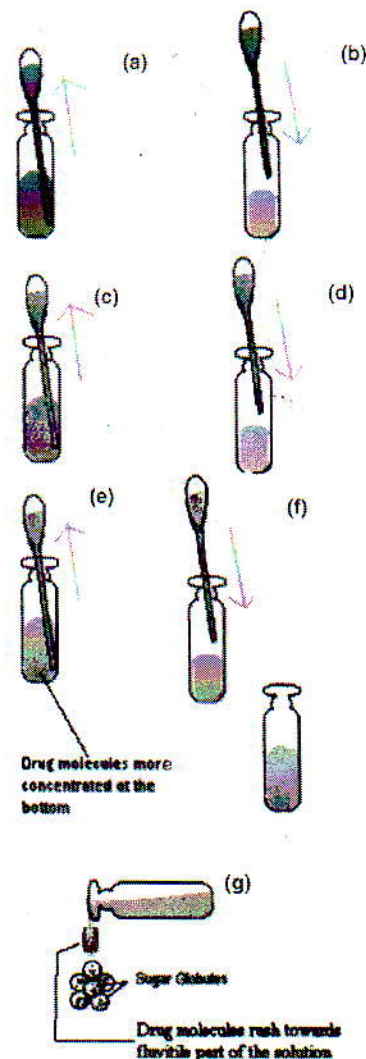


Fig. 4 Ideal method of potentization of a drug. (a) 1 ml 'Mother tincture' taken from 100 ml solution by a dropper. Now the dropper contains 1/100 drug molecules of the tincture. (b) Added to 99 ml fresh absolute ethanol, succeeded well to prepare 1st potency solution, containing 1/100 drug molecules of the tincture. (c) Again 1 ml of the first potency solution taken by a dropper. (d) Added to 99 ml fresh ethanol and shaken well to prepare the 2nd potency solution, containing 1/10000 of the original drug molecules. (e) After repetition of the process 3rd, 4th upto n th potencies are prepared containing a very negligible number of drug molecules (critical dilution). Now 1 ml of n th potency solution taken. (f) Added to 99 ml fresh ethanol and shaken well to prepare the $(n-1)$ th potency solution, but here the number of drug molecules would not differ significantly from n th potency solution because majority portion had been lifted by the dropper. (g) Preparation of $(n-1)$ th potency medicine.

concept is true for all charged molecules, not only proteins. Hydrated charged molecules become more concentrated at the bottom layer of the ethanol solution and if the bound water is removed the charged molecules

become precipitated. By the gradual addition of ethanol, hydrated drug molecules also become more and more concentrated at the bottom layer of the solution. When it reaches to a critical dilution and one tries to pick up 1 ml n th potency solution by a dropper or by any other means to prepare $(n + 1)$ th potency solution majority of drug molecules due to high velocity like gaseous molecules would rush into the dropper and remain unaltered in number in $(n + 1)$ th potency solution (Fig. 4e,f), though may be non-detectable by spectrophotometry. Potentized solution might be poured upon sugar globules for medicinal use (Fig. 4g), as described below.

Some phospholipid molecules are amphipathic in nature, containing both hydrophilic and hydrophobic moiety. As for example, phosphoglycerides contain phosphorylated alcohol as hydrophilic unit and fatty acid chains as hydrophobic unit. This special property of amphipathic molecules has been utilized for the formation of micelles, inverted micelles, and liposomes. Micelles are small (4–10 nm), stable spherical droplet like aggregation of about 50–100 amphipathic molecules, hydrophobic units of which are all hidden in its interior, away from surrounding water, while their hydrophilic polar groups are oriented on the outer surface of the droplet, closely associated with surrounding water molecules. Amphipathic molecules in non-polar medium form inverted or reversed micelles.

Liposomes are aqueous compartments enclosed by lipid bilayer. They can be formed by suspending suitable phospholipid in an aqueous medium. The mixture is then sonicated by high frequency sound to give a dispersion of closed vesicles that are quite uniform in size of about 50 nm in diameter. Ions or molecules, even several drugs (Fig. 5a), including anti tumor, anticancer and antibacterial agents (38–40) can be trapped in the aqueous compartment and by this way delivery of drugs to target tissue becomes possible. Amphipathic molecules having single long hydrophobic tail generally form micelles, e.g., higher alcohols, but that molecules having double tail generally form liposomes, e.g., phosphatidyl choline. Due to large size liposomes cannot penetrate cell membrane.

Ethanol, though have a polar head due to presence of OH group and a very short hydrocarbon tail (Fig. 5b) it does not form micelles or liposomes, but addition of it in crude drug aqueous solution (Fig. 5c) can decrease the dielectric constant and increases attraction between positive and negative charges of drug molecules in mother tincture, in which ethanol molecules show inverted micelle like orientation (Fig. 5d) around hydrated drug molecule and when it reaches to higher potency, number of ethanol molecules in relation to drug molecule increases enormously and by repeated manual stroke at the bottom of the container possibly they arrange them-

selves into drug loaded liposome like orientation (Fig. 5e) As the aggregation number of ethanol molecules is very small due to very short tail and small head it can trap a very small number of drug molecules, most possibly one, so that they cannot form visible structure under microscope. Repeated manual stroke at the bottom of the container, during potentization, may be compared to sonication, applied to prepare liposome. Repeated manual stroke creates an agitation, by which head and tail portion of ethanol molecules become reoriented covering few water molecules that form hydration sheath around drug molecule. Ion dipole interaction, hydrophilic interaction, hydrophobic interaction and van der Waals force might be the force of stabilization here, like that of liposomes. Isolation of drug molecules from ethanol becomes very difficult; because drug bound waters participate in azeotrope formation. Practically it becomes impossible with the increase of potency, but it is highly possible from agitated aqueous solution of the same drug. Ethanol molecules being supersaturated around hydrated drug molecule form capsule of enormous strength in high potency solution and their penetration power increases. If the solution is poured upon sugar globules for the preparation of medicine, outer layer of ethanol bilayer can tightly bind sugar molecules by their hydrophilic heads.

Highly potentized solution of drug becomes highly diluted also, and taking such type of solution has the following utilities as medicine:

- (i) As mentioned earlier, inhibitory drug when binds a large number of a particular enzyme molecules as a ligand in a prover he shows disease related symptoms, but when it binds a small optimal fraction of the same there is a chance of recovery, so the concentration of drug should be kept ultra low in a homeopathic medicine.
- (ii) As the same enzyme or receptor molecule is repeatedly used in a biochemical reaction, less drug molecules would be required to partially inhibit the same. The inhibitor would decrease the turn over number of the target enzyme and would proportionally increase the number of operation of the compensation of compensation cycle per unit time.
- (iii) Most inhibitors in higher concentration can bind several enzyme species, in lower concentration binds a few and in ultra low concentration can bind one, the target enzyme.
- (iv) Activity of many enzyme is under the control of hormones and upper cascade enzyme molecules, being very few in number, can activate several molecules of subordinate enzymes and the incoming signal that comes from hormone becomes amplified several fold, e.g., 25-million-fold in adrenaline cascade.

So, if an enzyme of the upper cascade is deficient and considered as target enzyme, the concentration of its ligand inhibitor should be kept ultra-ultra low.

- (v) When an inhibitor enters into the body and reaches certain concentration, detoxifying proteins and bio-transformation enzymes become actively synthesized. So that the drug molecules cannot penetrate all the diseased cells, but when the concentration of drug is kept far lower than threshold level, detoxification system becomes baffled, highly diluted drug molecules move very fast in the body fluid and can penetrate all the cells (see earlier).
- (vi) Though ethanol can dissolve a very large number of hydrated drug molecules but trap a very few of them

by potentization, which becomes almost inseparable from it, so there is no way except keeping the concentration of drug molecules ultra low.

Endomembrane system, composed of Golgi body, endoplasmic reticulum and lysosome divides the cell into several concentric and parallel compartments or chambers where different enzyme mediated reactions are going on. Some chambers anastomose with each other. The deficient enzyme (target enzyme) remains in a specific compartment to mediate a specific reaction and the drug molecules should have to reach there and bind the same. Ethanol encapsulated drug molecules being very few in number move through the body fluid

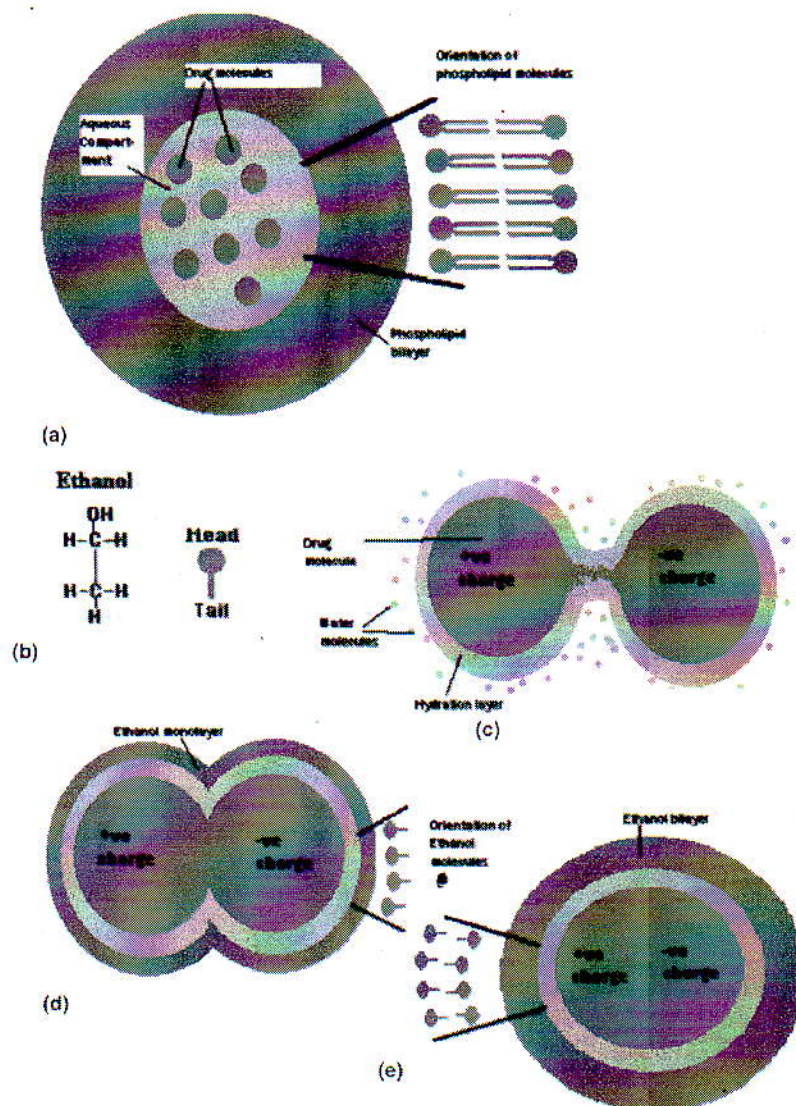


Fig. 5 Liposome-like orientation of ethanol molecules when potentized with drug. (a) Drug loaded Liposome. (b) Amphipathic nature of ethanol. (c) Aqueous solution of pure drug. (d) 'Mother tincture'. (e) 'Potentized drug'.

in a very high velocity, so that they cannot participate in any chemical reaction. Moreover, the number of ethanol molecules, packing each hydrated drug molecule, remains in supersaturated condition, which provides enormous capsular strength to high potency drugs, so that the drug molecules remain undissociated within the body fluid. The drug capsules lastly attach themselves by their hydrophilic heads with the polar heads of lipid bilayer of membrane of affected cell, and due to high partition coefficient ethanol capsule can easily penetrate through it along with drug molecules, pass through endomembrane system, until it reaches to the specific compartment, where the target enzyme remains. Penetration power of capsules increases with capsular strength, which is very high in higher potency drugs, that enables them to reach the innermost compartment of a cell. They have to cross several lipid bilayers of endomembrane system that snatches some ethanol molecules from the capsule rendering it breakable. When the specific compartment is reached the capsule breaks and the drug molecule binds the target enzyme as a ligand and attains a thermodynamically more stable form. The specific cellular compartment then could act as a sink for more ethanol trapped drug molecules that would rush towards the chamber by the attraction of thermodynamic pull.

Permanent cure of a disease by a medicine

Problem

It was established earlier that partial inhibition of an enzyme decreases the synthesis of product, the message reaches to the gene, and loss of product becomes compensated by increased rate of synthesis (K_s). After the withdrawal of drug K_s of the enzyme could fall again.

Proposed solution

The above phenomenon does not occur due to two reasons:

- (i) When a normal cell is concerned, K_s , K_d (see earlier) and K_M (Michaelis-Menten constant) of an enzyme remains adjusted in such a manner that it can utilize maximum substrate or precursor product, leaving no excess, and if there is a deficiency of the said enzyme there would be accumulation of its substrate. As for example in Fig. 3 enzyme E is deficient (case 1) and would not be able to convert product P_2 into P_3 totally, causing accumulation of P_2 . By the application of inhibitory drug I in minute dose K_d would increase and to compensate the loss K_s would also increase by the continual operation of a feedback loop. After a prolonged treatment when the drug is with-

drawn K_d would certainly fall but K_s would not, because the excess enzyme molecules would be engaged to convert P_2 into P_3 . So prolonged minute-dose drug has negligible or temporary effect on K_s of normal enzymes, but positive and long-lasting effect on K_s of deficient ones.

- (ii) The drug molecules being protected by ethanol capsule from systemic detoxification process safely reach to specific reaction compartment (see earlier). As the same enzyme molecule can be used repeatedly in a biochemical reaction, concentration of inhibitor bound enzyme hardly falls; conversely it increases with prolonged administration of the drug. The cells that could cope up with the stresses by compensation cycle survive and gradually multiply, other cells gradually perish. This phenomenon can be easily exhibited in rapidly multiplying cells i.e., lymphocytes (see earlier).

Reproducibility of effect of a medicine

Problem

Reproducible model is very difficult for Hahnemann's system and no homeopathic drug has been proved to have reproducible effect, but statistically significant recovery by the use of the same has been reported by several workers (see earlier). It is a fact that similar drugs cannot always cure similar symptoms and one can hardly believe that 'similia similibus curantur'.

Proposed solution

Reproducibility of effect of any drug in vivo is more difficult than in vitro due to influence of several external and internal factors, but the cause of non-reproducibility of homeopathic drugs seems to be little different. As shown in Figs. 1 and 2, symptom c may be caused by malsynthesis of any one of the three enzymes, E, E' or E'', and it would not be cured unless the specific target or rate-limiting enzyme is bound by a specific drug (Fig. 3), e.g., if symptom c is caused by malsynthesis of enzyme E', then only I' should be the prescribed as medicine. So a disease could be reproducibly treated by observing not only single or few symptoms, but, a combination of symptoms that focus on cause also (rate-limiting enzyme), which according to Hahnemann's principle is very difficult, specially when multiple enzymes are involved. Hence the old principle becomes modified as 'similia similibus curantur causosymptomically'.

THE PROPOSED METHOD

The new experimental design of the present proposition is as follows, which is expected to be more reproducible than any system of medicine.

- (i) Small amount of tissue or cultured cells should be taken from normal healthy individual from different tissues.
- (ii) Two-dimensional gel electrophoresis should be performed in each tissue with high resolution (Fig. 6a) and all the bands (or spots) that represent enzymes and receptors of the respective tissues should be identified on the basis of charge and molecular size. Protein content of all the identified bands should be estimated densitometrically. The bands should be blotted on a nitrocellulose sheet (Fig. 6b), like Western blotting.
- (iii) A radio-labeled pure test ingredient I-1 (see earlier Ingredients: inhibitors, alkaloids etc.) in minute dilution with a suitable buffer should be used as probe. Autoradiography should be carried out to detect the bound enzyme (Fig. 6c). After detection, the labeled probe should be eluted by adding non-labeled probe to the buffer (Fig. 6d). The experiment should be repeated several times, with new probes, I-2, I-3 etc. (Fig. 6e,f), until all the bands on the sheet are bound by at least one radio labeled ingredient. Now the bands should be defined on the basis of inhibitor binding affinity.

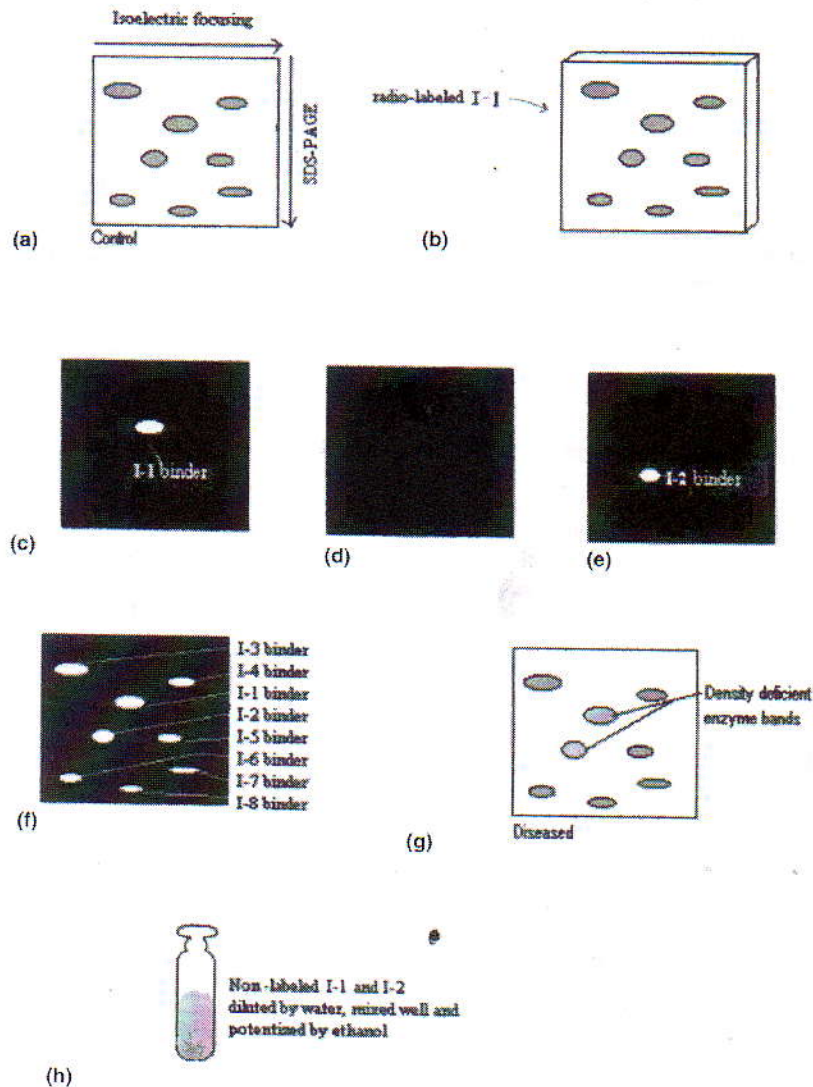


Fig. 6 Proposed method of patients specific treatment of a chronic disease. (a) After two dimensional gel electrophoresis of control tissue. (b) Target enzyme blotted on a nitrocellulose sheet, incubated with radio-labeled drug ingredients one after another. (c) Autoradiograph on a photographic plate after first successful drug I-1 (probe). (d) Autoradiograph after the removal of labeled probe. (e) Autoradiograph after second successful drug I-2. (f) Integrated autoradiograph with different successful probes. (g) After two dimensional gel electrophoresis of diseased tissue. (h) Suggested medicines of diseased person.

- (iv) Gel electrophoresis would be performed also for the affected tissue of the diseased individual (Fig. 6g) and the density of all the bands should be measured. The density deficient band(s) of diseased individual that differs significantly from the same tissue of control should be identified as target enzyme(s) or receptor.
- (v) Then the respective non-labeled inhibitors of the target enzymes should be diluted several times by water depending on their toxicity. When the drugs reach into tolerable concentrations they should be mixed well. If the mixture were applied on a 'prover' he would show all the disease-related symptoms like the diseased individual.
- (vi) The mixture should be potentized several times (see earlier) and should be used as a medicine for the diseased individual (Fig. 6h).

DISCUSSION

The main theme of the proposed experimental design emerges from Hahnemann's old principle, but practically much different from it in implementation. Hahnemann only emphasized on symptoms, but here equal emphasis has been given on the cause, i.e., rate limiting enzyme, receptors, etc. The proposed experimental design is also dependent on the involvement of a prover like Hahnemann's principle, but requires more biochemical observations. Regarding selection of test tissue from the diseased individuals much caution should be adopted; for a skin disease epithelial tissue might be initially considered as test tissue, for hormonal disease endocrine gland is the test tissue and for parasitic diseases blood should be emphasized as the test tissue, because it contains B and T lymphocytes, responsible for prevention of the parasitic infection. Hahnemann himself was against the mixture of multiple drugs for a single disease, but in the present method it has been encouraged when more than one enzyme is found responsible for a disease (e.g., gout), but prohibited in three cases only: first, if a single enzyme band is found to be the cause of the disease; second, if the same drug ingredient can bind all the target enzyme bands (see earlier) in minute dose; third, if a known target enzyme is found to be the activator or immediate metabolic precursor of few other target enzymes, represented by bands, the former should only be considered as target enzyme, others might be ignored, because treatment of the former by a single drug ingredient may cure the deficiency of dependent enzymes.

In true sense the proposed method is not much different from the conventional practice of Hahnemann's method. Here, a few low potency medicines that show similar combination of symptoms might be applied on a

patient one after another with a few days interval and any of the three results must appear for each trial and that can be explained as follows:

- (i) All the symptoms may remain unchanged: it indicates either the drug is unable to reach the specific cellular compartments, where the target enzyme mediated reactions are going on or unable to bind the target enzyme(s). Here the drug should be used in higher potencies to increase its penetration power, if still there is no result the drug should be rejected.
- (ii) One or more symptoms may be aggravated: it indicates that the drug molecules have reached the specific cellular compartment and bind the target enzyme molecules higher than optimal fraction (see earlier). If the symptoms diminish within two or three days (due to operation of compensation cycle) there is a chance of recovery. Hence, the drug should be repeated with long intervals.
- (iii) One or more symptoms may diminish: it indicates that the drug has been used in right dilution to bind with optimal fraction of the target enzyme and the drug should be continued with short intervals.

The trials should be continued until all the disease related symptoms disappear.

Three important points come out from the present work: first, activity of a homeopathic medicine not only depends on dilution but also on molecular packing. If dilution be the only criterion, a drop of medicine could cure the disease of a large number of fish in a pond; second, different individuals suffering from the same disease can be cured by the same or different potentized drug(s) and third, minute dose drugs may have positive and long lasting effect on the rate of synthesis of deficient enzymes, but temporary and negligible effect on normal enzymes. Several experimental works would be essential to define all the rate limiting enzymes responsible for each and every chronic diseases and to treat them on the basis of drug binding and that is not possible by a single worker. Pilot experiments should be conducted on sub-human creatures also. The author expects that the total process would not be costlier than human genome project, but more natural, safe and valuable.

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