Laboratory Research in Homeopathy: Con

John R. Moffett, PhD, Peethambaran Arun, PhD, and M. A. A. Namboodiri, PhD

Alternative medical approaches to human diseases such as cancer are becoming increasingly popular, but reports on their success rates have been highly variable. Homeopathy is an alternative medical practice often applied to less critical human diseases but one that has also been applied sporadically to the treatment of cancer. Animal studies on the use of homeopathy to treat experimental cancer are few and the evidence provided to date is far from conclusive. The debate presented here concerns the utility of animal studies on cancer treatment with homeopathic preparations. As part of a Point-Counterpoint feature, this review and its companion piece in this issue by Khuda-Bukhsh (*Integr Cancer Ther*. 2006;5:320-332) are composed of a thesis section, a response section in reaction to the companion thesis, and a rebuttal section to address issues raised in the companion response.

Keywords: *alternative medicine; water; glass; silica; borate; epitaxy*

Thesis

The topic to be discussed is whether laboratory research using experimental biological systems provides useful information for homeopathic complementary and alternative medicine approaches to cancer treatment. Our laboratory has been actively involved in investigating so-called "potentized" homeopathic preparations in model biological systems for several years, but we have been unable to document any reproducible biological effects in animals or cell culture. A great deal of the difficulty in doing homeopathy research lies with the fact that, unlike mature sciences, there are no scientifically demonstrated first principles to guide hypothesis generation and experimental design.[1] Previous research has not uncovered any mechanisms explaining the mode of action for homeopathic preparations, nor has it provided any credible evidence for an active agent in such preparations. Furthermore, decades of research have failed to elucidate a single biological assay that can determine the presence and potency of any supposed active agent in homeopathic remedies. The major Achilles' heel for biological homeopathy research may be the lack of reproducibility of results between different laboratories. For these and other reasons outlined below, we believe that basic laboratory research into homeopathy will not be a fruitful avenue of investigation into the treatment of human cancer.

The history of homeopathy is intertwined with the history of the early "medical systems" or doctrines of 17th- and 18th-century Europe. Before the germ theory of disease (and subsequent discovery of genetic diseases, carcinogens, etc), numerous medical doctrines competed for favor, such as that advocated by John Brown (Brunonian theory) on imbalances in "nervous energy," or "hydropathy," based on humoral theories of disease. Allopathy and homeopathy emerged as popular opposing medical systems in the early 19th century, each based on distinct views of health and disease that were not grounded on scientific research. C. F. S. Hahnemann coined the term "allopathy" in 1842 to differentiate the established practice of medicine from homeopathy, an alternate system of therapy founded by Hahnemann. Homeopathy was based on the concept that diseases can be treated with minute doses of compounds thought to produce the same symptoms in healthy people as the disease itself. Hahnemann believed that nothing could be known of the underlying nature of a disease, because disease does not arise from material causes but rather from a perturbation of the "vital spirit."[2] Based on personal experience with Peruvian tree bark containing quinine, a treatment for malaria, Hahnemann experienced malaria-like symptoms, thus leading to his formulation of the basic principle of homeopathy that "like shall be cured by like." Allopathy, in this early simplistic view, treated symptoms with drugs having actions opposing the symptoms of disease.

Hahnemann defined the "law of similars" as the central principle of homeopathy. Drugs or toxins

JRM, PA, and MAAN are with Uniformed Services, University of the Health Sciences, Bethesda, Maryland.

Correspondence: John R. Moffett, Uniformed Services, University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD. E-mail: jmoffett@usuhs.mil.

DOI: 10.1177/1534735406294795

that were known to cause symptoms similar to a particular disease were given to patients in extremely diluted form.[3] This was said to induce a restorative process in the body that would counteract the effects of the disorder being treated. Homeopathy enjoyed great success in part because the extremely diluted preparations used by practitioners invariably caused fewer negative side effects in patients compared with the often dangerous "allopathic" medications and treatments of the day (eg, blood letting). The concept of allopathy has become outmoded as the practice of medicine shifted from countering the symptoms of a disease to disrupting specific pathophysiological processes, for example, by the treatment of a bacterial infection with antibiotics, or cancer with surgery, radiation, and chemotherapy.

It's in the Water

Homeopathy is not a singular practice, and at least 2 general classes of homeopathy can be distinguished based on the degree of dilution applied to the starting material.[4] One type of traditional homeopathy involves diluting natural compounds extensively, but low, presumably biologically relevant levels of the active ingredients remain in solution. This type of homeopathy can be referred to as the "hormetic method" (Arndt-Schultz law[4]). A modified method of homeopathy has also been used extensively, where the starting solutes are diluted to a point calculated to be "beyond the reciprocal of Avogadro's number" (BRAN). These extremely diluted BRAN solutions are calculated to have no remaining molecules of the starting compounds. According to homeopathic clinicians and researchers, homeopathic preparations are made more potent, or are "potentized," by this extreme dilution process. We have focused our research on this latter type of homeopathy, where the starting solutes have been diluted away, because principles of modern biology and biochemistry conflict with hypotheses of increasing potency as medications are diluted to BRAN levels.

Homeopathic preparations are distinct from standard laboratory dilutions of bioactive agents both in the method of mixing the solution and in the degree to which they are diluted.[5] Standard laboratory dilutions of bioactive agents in aqueous solution are done in as few steps as possible in plastic containers, and brief mixing of the solution is done at each step to generate the required dilution. Typically, the calculated dose per kilogram of body mass will provide blood and tissue levels of the drug that are near the dissociation constant (Kd) for the target receptor, or the Michaelis-Menton constant (Km) for the relevant enzyme system. In contrast, homeopathic solutions are almost universally prepared in glass vials rather than in plastic containers, and this distinction is critical as will be discussed below. Homeopathic preparations are made by starting with a tincture of the active agent or mixture, often dissolved in an ethanol/water mixture, and this tincture is repeatedly diluted with water and vigorously agitated between each dilution step. The agitation process is done in a stereotypical manner, by striking the glass vial repeatedly on an elastic surface (a process called "succussion"). In BRAN-type preparations, the repetitive dilution and forceful agitation process is continued long after the starting solutes are exhausted, such that the concepts of Kd or Km of the active agent for their molecular targets are not taken into consideration.[6] It is claimed that the succussion process is critical for "potentizing" homeopathic preparations and that the further the solution is diluted and "succussed," the more effective it will be. These ideas were based on anecdotal observations, not on scientific data.

Several modern explanations of the effective agents in BRAN-type homeopathic preparations have been offered, but rigorous studies that test these hypotheses in model biological systems are few. One such hypothesis is that the effectiveness of homeopathic preparations results from specific changes in physical or chemical properties of the bulk water phase caused by the combined effects of the starting material, the dilution, and forceful agitation processes.[7] This hypothesis states that specific water clusters, or clathrates, can be formed by interaction of the starting material with bulk water via hydrogen bonding and that these specific water structures could exist even when the dilution process essentially removes most or all of the starting material. Mathematical calculations of water cluster stability in pure water at room temperature suggest that ordered water clusters would be very short lived in solution in the absence of solutes,[8] and yet homeopathic preparations are thought to retain potency for weeks, months, or years.[9] Recent nuclear magnetic resonance studies of homeopathic solutions have shown that no long-lived alterations are present in the hydrogen bonding pattern of the bulk water[10,11] and that silicates leaching from the glass vials used to make the preparations may be responsible for some differences observed in relaxation times between different solutions.[12,13] The water cluster theory of homeopathy runs into additional trouble in light of the fact that many homeopathic preparations are soaked onto sugar pills, which are then dried to remove the solvent.

There has been a debate in the homeopathy literature on the nature of any therapeutically active ingredient in BRAN-type homeopathic preparations.[14] The concept of a soluble active ingredient in homeopathic preparations is not universally accepted by practitioners and researchers in the field. Indeed, some

researchers cite the purported increase in potency of remedies the further they are diluted and agitated as proof that any active agent in homeopathic remedies is nonmaterial and involves some form of energy or information contained in BRAN preparations.[15,16] This concept was reviewed recently by Anick.[10] Homeopathic preparations are not thought to exhibit dose–response relationships, and laboratory studies of homeopathy cannot demonstrate a biological effect that can be diluted away with pure water. Short of demonstrating heretofore unknown principles of nature, it is impossible to reconcile homeopathic claims about the efficacy of BRAN-type preparations with modern biochemical and biological principles.

Some authors have indeed attempted to propose new principles of nature, by extension of certain theories in chemistry, physics, or materials science. For example, in their extensive 2005 review of structured water and its relevance to homeopathy, Roy et al[17] suggested that a materials science view of water provides great insight into possible mechanisms of action of homeopathic preparations. At the heart of this particular view of water structure as it relates to homeopathy is the concept of epitaxy. Epitaxy is a property of matter wherein 1 structurally organized material layer (usually a solid) influences the atomic or molecular order of a deposited layer (usually deposited as a vapor, liquid, or molecular beam). This property is often used in the manufacture of semiconductors. If the deposited layer is made of the same substance as the solid substrate, and the solid substrate is a single crystal, then the deposited material will add additional layers to the crystal surface, aligned in register with the crystal's molecular lattice.

Roy et al stated that epitaxy provides a mechanism for the formation of highly structured water phases that they proposed are at the foundation of how homeopathic preparations could function. They introduced the issue this way:

> This paper does not deal in any way with, and has no bearing whatsoever on, the clinical efficacy of any homeopathic remedy. However, it does definitively demolish the objection against homeopathy, when such is based on the wholly incorrect claim that since there is no difference in composition between a remedy and the pure water used, there can be no differences at all between them. We show the untenability of this claim against the central paradigm of materials science that it is structure (not composition) that (largely) controls properties, and structures can easily be changed in inorganic phases without any change of composition. The burden of proof on critics of homeopathy is to establish that the structure of the processed remedy is not different from the original solvent."[17(p 578)]

Despite such bold claims, it is not incumbent on biologists and chemists to prove that homeopathic remedies and the solvents they are made from are identical; it is incumbent on homeopaths to show that a biologically active agent is present in homeopathic preparations, that target receptors exist in the body, and that a clear mode of action can be demonstrated. As outlined below, we have found differences between plain water and homeopathic preparations, but not of the sort Roy et al proposed.

Roy and colleagues suggested that silicon dioxide (the primary ingredient in glass) and water have similar properties[17,18] and that because glass has distinct, microscopic internal phases lending it a nanoheterogeneous structure, then so it is with the structure of liquid water. However, the glass analogy makes the opposite case because glass is, as will be discussed below, a composite of silicon dioxide (sand), boric oxide, sodium oxide, and other minor components.[19] The metal oxides and minor components form inclusions in the glass, creating an extremely heterogeneous structure. Pure silicon dioxide would not have the same heterogeneous microstructure as modern composite glasses, and therefore the analogy is seriously flawed. Furthermore, Roy et al offered no mechanisms whereby nanostructured water could be stable over long periods of time or how small amounts could effect changes in an organism, what mechanism of action is involved, or how the structured water would not be reordered in a biological system by the high salt, protein, and carbohydrate environments present in all body fluids. Studies on water in biological systems have found 2 basic classes of water: bound water, which is in close association with proteins or carbohydrates, and bulk-phase water.[20] Other more exotic forms of liquid water are short lived and are not relevant to pure water at room temperature and pressure. Homeopaths are necessarily vague on how a tiny amount of ordered water could enter the body and effect changes in the functioning of the entire organism.

Homeopathy Research

Two major distinctions between human homeopathy trials and animal studies or cell culture studies of homeopathy are the placebo effect and the doctor–patient interaction effect. Experimental studies in animals or cell culture have many pitfalls, but those do not include psychological factors among the subjects of the study. Much has been said about potential placebo effects in homeopathy research and the need to control for these effects in homeopathy trials as rigorously as in pharmaceutical trials.[21,22] Perhaps somewhat less has been said about doctor–patient interaction effects on the outcomes of clinical homeopathy trials.[23] It is arguable that

a significant amount of variability reported in the literature on human homeopathy trials is attributable to the degree to which these 2 variables are controlled for or not. In contrast, the marked variability of the results within and between various animal studies or *in vitro* studies of homeopathy requires other explanations.

Laboratory and clinical investigations into the effects of homeopathic remedies offer mixed results ranging from no effect to remarkable efficacy. Our laboratory has done several preliminary pilot studies on BRAN-type and standard homeopathic preparations in experimental systems. One study was done to reproduce unpublished data from another laboratory that had claimed significant efficacy in treating experimental cancer in mice using homeopathic preparations. Under the same reported laboratory conditions, opposite results were obtained relative to the efficacy of the homeopathic treatments in the 2 labs, wherein the other lab reported 40% to 90% reduction in tumor rates under different treatments, and our lab found no effect whatsoever. Being mutually exclusive, both sets of results cannot be correct. Methodological differences could be important determinants of the differing results, but researchers from our laboratory traveled to the other laboratory on more than 1 occasion to observe the techniques and methods used, and following the same protocols we still could not show any efficacy whatsoever using the same commercial homeopathic preparation. Our observations are outlined briefly below.

In the first attempt to confirm the positive results obtained in the other laboratory, 1×10^6 melanoma cells each were injected into 20 mice through lateral tail vein as described by the other laboratory's protocol. Ten animals (control) were given oral administration of 50 µL each of phosphate-buffered saline on the same day and continued for 10 days, and the other 10 animals (treated) received the same volume of a homeopathic preparation designated as "1M Thuja" for 10 days. The experimental protocol indicated a 21-day interval before making comparisons between the experimental and control groups. At 20 days, 3 animals from the control group and 4 animals from the treated group had died. The remaining animals were sacrificed on that same day and their lungs were inspected for tumor nodules. The lungs of all the animals were almost entirely filled with black nodules, which formed thick masses that we were unable to count individually. There were no differences between the control and treated groups. These results clearly indicated that the described protocol involved injecting drastically too many melanoma cells, resulting in massive cancer infiltration of the lungs. The reason for this discrepancy remains unclear.

In the second round of experiments, we reduced the cell count injected per animal by factors of 2 and 10, and the experiments were repeated. Mice were divided into 4 groups having 7 animals in each group. Group 1 animals received 1×10^5 melanoma cells followed by 50 µL of water treatment for 10 days. Group 2 animals received 1×10^5 cells followed by 50 µL of 1M Thuja treatment for 10 days. Group 3 animals received 5×10^5 cells followed by 50 µL of water treatment for 10 days. Group 4 animals received 5×10^5 cells followed by 50 µL of 1M Thuja treatment for 10 days. After 20 days, all the animals in group 3 and 4 died, again indicating that the described procedures were problematic. Four animals from group 1 and 3 animals from group 2 were killed on the 20th day, and the number of nodules in their lungs was counted. The results obtained were that the control group had 14 ± 5 and treated group had 22 ± 3 nodules. After 26 days, 3 animals from group 1 and 4 animals from group 2 were sacrificed, and we counted the number of nodules in their lungs. Control animals had 40 ± 30 nodules, and 1M Thuja-treated groups had 45 ± 20 nodules in their lungs. Because this was a preliminary study with a small number of subjects, and because approximately half of the animals in each group died before the experimental regimes were completed, there was no statistical power or significance between groups. Despite the small group sizes, these results showed that the homeopathic preparation, which was claimed to be very effective against experimental melanoma in the other laboratory, had no effect in our hands with the same strain of mice under the same experimental conditions.

Our lab has also attempted to show that pretreatment with toxic compounds diluted to BRAN levels (30, 100× dilutions) had protective effects when the animals or cells were subsequently subjected to the same toxin administered at high doses. In 1 set of preliminary experiments, we administered BRAN-level sodium cyanide solutions to mice intraperitoneally, and then after multiple pretreatments, we administered toxic doses of cyanide to look for any level of protection from the homeopathic pretreatments. Mortality rates were no different between BRAN-cyanide pretreatment groups and control animals. In another set of experiments, we pretreated SH-SY5Y human neuroblastoma cells (ATCC, Manassas, VA) in culture with BRAN-diluted paraoxon solutions and then challenged the cells with toxic (LD_{50}) doses of paraoxon. Cell viability was measured by the MTT colorimetric assay, and we found that BRAN-level paraoxon pretreatment of neuroblastoma cells resulted in a slight but statistically significant ($P < .05$) increase in cell death in response to

paraoxon challenge, rather than a decrease in cell death. Although these were only preliminary studies, we found no beneficial effects from any BRAN-type treatment in preventing cancer progression or ameliorating toxicity. Larger scale studies do not appear warranted based on our preliminary findings.

Chronic, low-level arsenic poisoning is common in some areas of the world where groundwater is contaminated with oxides of this toxic metal. Excessive arsenic in tube well water is a well-documented problem in certain areas of India, particularly in West Bengal Province. Many aquifers in this region are naturally contaminated with arsenic levels that exceed 50 µg/L. This is also an area of the world where homeopathy is a popular form of medical practice and where homeopathy has been applied to arsenic poisoning. Experimental studies on homeopathic treatment of arsenic intoxication in mice performed by Khuda-Bukhsh and colleagues reported significant efficacy using BRAN-type preparations of arsenic trioxide.[24] The measures of efficacy involved reported decreases in enzyme activity levels of aspartate aminotransferase and alanine aminotransferase and increased glutathione levels in blood and liver. Both homeopathic preparations of "arsenicum album" (30, 100× dilutions and 200, 100× dilutions) were prepared in an alcohol/water mixture and given orally to mice subjected to single dose of 0.1% As_2O_3 solution (1 mL/100 g body weight) by intraperitoneal injection. Aspartate aminotransferase and alanine aminotransferase activity levels were reported reduced in blood and liver by approximately 50% on day 30 after onset of treatment. However, there were some curious data values, including lower enzyme activity levels on day 30 in the treated animals compared with controls that did not receive arsenic.

Success in these preliminary investigations into treating arsenic poisoning in mice led to several field studies on homeopathic treatment of groundwater-associated arsenic poisoning in humans. These studies were conducted in areas of India where groundwater arsenic poisoning is common, and they were funded in part by Boiron, one of the world's largest producers of commercial homeopathic preparations.[25,26] In these studies, a number of pathological parameters were reported improved by the administration of homeopathically prepared arsenic solutions, including blood levels of aspartate aminotransferase and alanine aminotransferase. In these homeopathic trials for the treatment of chronic groundwater arsenic exposure, Khuda-Bukhsh and coworkers reported that "potentized" (BRAN level of dilution) arsenic trioxide preparations were effective in normalizing some of the abnormal biological parameters associated with chronic, low-level arsenic poisoning. They reported dramatic, time-dependent reductions in blood enzyme levels following 10 days of treatment with sugar pills that had been soaked with the homeopathic preparation. They also reported reductions in "antinuclear antibody" titers, elevations of which have been reported to be associated with arsenic poisoning.[25] Only some of these field studies were placebo-controlled, and even those had significantly fewer patients receiving placebo than receiving "potentized arsenicum album." Additionally, water purification facilities had been installed in the local villages, tube wells were capped by the government, and the people were informed of the dangers of drinking tube-well ground water. As such, it is possible that some of the improvements in blood enzyme levels and other parameters could have been attributable to withdrawal of arsenic exposure and random variance in the recovery rates among the local population. As such, these results should be interpreted with caution.

The Glass Effect

To investigate the chemical nature of BRAN-type homeopathic preparations, we have analyzed them by sensitive elemental analytical techniques, including inductively coupled plasma optical emission spectroscopy (ICP-OES) and mass spectroscopy. We produced water-based, BRAN-type homeopathic preparations in our laboratory in borosilicate glass vials (screw-cap 24-mL borosilicate glass tubes from VWR Scientific, West Chester, PA; item 66011-358) and tested them for trace elements. We found that silicon, boron, and sodium were all present at micromolar levels. Furthermore, we found that these solutes were derived from the borosilicate glass tubes used to make the solutions. Boron was present in BRAN solutions at a level of approximately 2 mg/L. The most likely form of boron dissolving from borosilicate glass into water at near-neutral pH would be the borate anion. Borate has been reported to be biologically active but not at such low concentrations.[27] The mean concentration of silicon in the BRAN-type homeopathic solutions made in glass vials was approximately 1.5 mg/L. In water samples stored in glass vials, but not "succussed," the mean value was approximately 0.8 mg/L, indicating that the process of forceful agitation increased the level of glass-derived solutes. The most soluble silicate at neutral pH is orthosilicic acid ($Si[OH]_4$), but silicon dioxide and other silicates are also present in glass-exposed solutions.[19] Sodium was present at about 2 mg/L in all glass-exposed solutions. Trace metals were also found in BRAN-type preparations, with aluminum, scandium, titanium, and other metals being present in nanomolar concentrations. Similar observations

have recently been reported for homeopathic preparations made and stored in glass vials.[28] In those studies, sodium was found to be the most concentrated solute in homeopathic preparations made in glass, and silicon (in the form of silicates) was the second most concentrated solute. Succussion was found to increase the concentrations of glass constituents in solution.

A review of the literature indicates that silicon oxides and other glass constituents dissolve from borosilicate glasses exposed to water, both by diffusion of solutes out of the glass matrix and by breakdown of the glass network itself.[29] Glasses are amorphous solids that can be composed of widely varying starting materials, each of which affects the solubility of the resulting glass. All types of glass are composed predominantly (55% to 80%) of silicon dioxide (sand or silica), with the addition of lesser amounts of sodium oxide (and/or potassium oxide), calcium oxide or carbonate, and, in the case of borosilicate glasses, boric oxide. The various metal oxides exist as inclusions in the silicate matrix of glass and, with the exception of boric oxide, tend to increase the solubility of glass in water relative to pure silicon dioxide. It has been found that solutes derived from the interaction of glass with water increase the rate at which glasses dissolve in water, such that deionized water is less corrosive to glass than is a solution containing soluble glass constituents.[29] The process of forceful agitation (succussion) increases the dissolution of the borosilicate glass matrix into water by disrupting the hydrated silica gel that forms at the glass–water interface.

We have carefully analyzed BRAN-type homeopathic preparations made in our laboratory for biological activity. We found that the activity of enzymes, such as horseradish peroxidase and acetylcholine esterase, was stabilized in BRAN-type homeopathic preparations produced in glass vials relative to enzymes stored in deionized water. We found that silicates leaching from the glass vials into solution stabilized enzyme activity in these dilute solutions for days at room temperature, whereas enzymes in deionized water lost activity within hours. In contrast, when we attempted to elicit biological responses ranging from superoxide dismutase expression to superoxide production, we were unable to produce any responses in neuroblastoma or macrophage cell lines in culture, even with relatively high concentrations of dissolved silicates (up to 100 µM). Very high concentrations of silicates are typically required to elicit biological responses (500 µM to 1 mM in vitro),[30,31] and, therefore, there is no evidence that the low levels of silicates present in our BRAN solutions (~20-30 µM) would be sufficient to have any biological effects if given to people or animals in small volumes.

If low concentrations of biologically active compounds become potentized by the forceful succussion process used to make homeopathic preparations, then every homeopathic remedy ever made in glass is basically a "potentized" form of silicates, sodium, borate, and the trace metals that dissolve from the glass vials used to make them. Furthermore, when homeopathic preparations are diluted well beyond the reciprocal of Avogadro's number, then substantial quantities of glass constituents are the only solutes remaining in solution as the succussion and dilution process is continued. How could any of the original so-called "signal" not be overwhelmed by new "signals" coming from the compounds that are dissolving from the glass? This issue has been brought up previously, and indeed some homeopathic researchers have turned the criticism on its head and claimed (without evidence) that the contaminants from glass vials are necessary for the proper functioning of homeopathic remedies.[28] Nonetheless, the failure of most homeopathic researchers to account for the fact that glass constituents dissolve into water presents an insurmountable obstacle to their contention that extreme dilution and succussion make homeopathic preparations more potent by virtue of the fact that the original solutes have been diluted away, and only a specific "signal" remains in the structure of the water solvent. Indeed, if hypotheses such as those of Roy et al[17] concerning specific, long-lasting, biologically active water structures are correct, then succussion and dilution should generate BRAN-type homeopathic remedies even when made with ultrapure water and produced in plastic containers.

Reproducibility and Controls

The onus to demonstrate clear effectiveness of homeopathic preparations for the treatment of any human condition lies squarely with homeopathic researchers for the simple reason that laboratory biologists who are engaged in basic biological research have been given no clear reason to pursue such studies. The inability of basic research laboratories to reproduce positive homeopathic results reported by other laboratories is a significant problem for homeopathy proponents, because the hallmark of science is reproducibility. Lacking fundamental principles regarding mechanisms of action, homeopathy researchers are left without guidance in their attempts to design hypothesis-driven experiments and analyze data. Furthermore, because there are no bioassays or analytical methods that can demonstrate the potency of homeopathic preparations, it is impossible to know if any particular preparation was made

correctly and what its potency is, if any. Until homeopaths unequivocally demonstrate a mechanism of action and develop bioassays to determine potency, they have failed to provide even the most basic tools for further scientific research.

If homeopathic research data are to be published in quality peer-reviewed journals, the practitioners must design well-controlled experiments that include both positive and negative controls. That is, the effectiveness of homeopathic remedies cannot simply be compared with vehicle or water but also must be compared with standard pharmaceutical agents with well-documented effects. For example, if anti-inflammatory effects of BRAN-type preparations of Arnica Montana are to be studied, not only should their effectiveness be compared with that of so-called "vehicle" but also they should be tested against known anti-inflammatory pharmaceuticals such as acetaminophen and acetyl salicylic acid at pharmaceutical doses. If the latter are clearly effective in reducing inflammation, but the homeopathic remedy shows marginal or no effect in comparison, then the researchers have failed to demonstrate significant effectiveness of the treatment.

We would like to end this portion of the discussion with 2 quotes, including 1 from the Introduction to this section of the issue: "We know from placebo and behavioral medicine research, for example, that manipulation of the social and cultural context, practitioner–patient–family communication strategies, the physical environment, and feedback of information markedly changes outcomes, often to a much greater extent than specific drug and even surgical treatment effects." If true, then basic laboratory research into the effects of homeopathy in animal or cell culture systems may remain fruitless because psychosocial factors critical for the effectiveness of homeopathy are nonoperative in such a setting.

Finally, Mark Twain once wrote on homeopathy, "No one doubts—certainly not I—that the mind exercises a powerful influence over the body. From the beginning of time, the sorcerer, the interpreter of dreams, the fortuneteller, the charlatan, the quack, the wild medicine-man, the educated physician, the mesmerist, and the hypnotist, have made use of the client's imagination to help them in their work. They have all recognized the potency and availability of the force. Physicians cure many patients with a bread pill; they know that where the disease is only a fancy, the patient's confidence in the doctor will make the bread pill effective."[32]

It is our firm belief that the study of homeopathy is more properly done by social and experimental psychologists and psychiatrists, in conjunction with medical doctors, rather than by experimental biologists. If homeopathy works through the placebo and doctor–patient effects, and the preparations contain primarily water, alcohol, and trace minerals, then there is no harm in using these techniques to treat mild, non-life-threatening disorders for which no suitable pharmaceutical exists, or in situations around the world where pharmaceuticals are too expensive or not readily available. Indeed, treating patients having a mild case of viral rhinitis with homeopathic remedies would be greatly preferable to frivolously prescribing antibiotics.

Response

There is a lack of internal consistency in arguments concerning the nature of the so-called "signal" in BRAN-type homeopathic preparations. Virtually all theories on possible mechanisms of action in BRAN-type homeopathic preparations refer to "information" being carried in the structure of water, or a water/ethanol mixture. This raises the issue of drying "potentized" solutions on sugar pills, in which case clathrates, and any other structure or "informational content" in the solvent, would be lost. If there are no molecules of the starting material remaining, and if the "vehicle" carries some form of signal, then how is the "vehicle signal" retained when the vehicle is evaporated from the sugar pills? Ad hoc hypotheses about how "imprints" could be passed onto sugar pills have no basis in experimental evidence. It is quite possible that because most pharmaceuticals come in pill form, pills provide a stronger placebo effect for some patients than small volumes of liquid under the tongue.

Many theories of homeopathy invoke concepts of "information," "signal," or a "vital force" being imparted on water as the underlying agent.[33] However, most homeopathic preparations are made in 20% to 100% ethanol to prevent bacterial contamination and because Hahnemann often used brandy as a base for his homeopathic preparations. Water and alcohol have very different physicochemical properties, including much weaker hydrogen bonding among alcohol molecules, which means that homeopathic alcohol/water solutions would be far less "ordered" than those made only in water. Homeopathic theories of water-based "information content" have not taken this fact into account.

With regard to the gene-activation hypothesis of homeopathy, gene activation is a molecular biological event that involves the binding of regulatory ligands to gene response elements, so there is no difference between receptor activation and gene activation at the level of molecular biology. The downstream, effector systems are different, but the basic biological principle of ligand-receptor binding applies.[34-37]

Special considerations for homeopathy have been emphasized by its practitioners, including "mind" and the "general constitution" of the patient, particularly when treating chronic diseases. These issues would be critically operative only if placebo effects were in play in the efficacy of homeopathic preparations. Whereas some double-blind placebo-controlled homeopathic studies have shown an effect slightly greater than placebo, others have shown only a placebo effect. No studies of high quality have shown that BRAN-type homeopathic remedies are even remotely as effective as pharmaceuticals. It is perhaps of interest to contemplate that placebos can be more effective for the patient if the medical practitioner is also receptive to the plausible efficacy of the medication.

Reproducibility is a key hallmark of science but represents a significant challenge to homeopathy research. The issue of the lack of reproducibility of reported homeopathic findings is further compounded by the fact that a great deal of negative data from homeopathy research are never published.[38] Although this problem is not unique to homeopathic research, based on our experience it is a larger issue for homeopathy than for mature sciences in general. This is true in no small part because so much homeopathic dogma is not based on established biological principles that can be used to guide hypothesis formation and experimental design. Many laboratories that have attempted to perform original research into homeopathy or to reproduce previously reported data have failed to find any significant effects, but those data are almost never published in peer-reviewed journals. This renders the negative or nonconfirmatory data unavailable to the scientific community. The hesitancy to publish negative data obtained from homeopathy research provides a skewed dataset wherein positive results can appear to outweigh negative results. Larger, better controlled studies of homeopathy show little or no effect,[39,40] and that places the burden of proof on homeopathy proponents to demonstrate clear efficacy and subsequently to have those results readily reproduced elsewhere.

Experimenter bias is a problem that affects all scientific investigations to some degree, but this issue appears particularly problematic in homeopathy research.[21,39] Many homeopathic studies are done on small groups of humans[41] or in very complex animal models that provide numerous opportunities for unintended bias to affect the final outcomes. When BRAN-type homeopathic preparations are studied in simple, quantitative model biological systems, effects are not apparent. Additional clinical studies into the effects of homeopathy do not appear warranted until basic principles have been determined and repeatedly confirmed in simple model systems.

Rebuttal

Proponents of homeopathy typically claim that the reason why mainstream scientists so often fail to reproduce previous positive results with BRAN-type preparations (termed "potentized drugs" by homeopaths) is their lack of understanding about homeopathy. We disagree. The preliminary studies we reported above were done according to precise instructions given to us by the homeopathic researchers who claimed a 90% success rate in reducing melanoma in the same strain of mice using the same homeopathic remedy from the same manufacturer. We traveled to their laboratory on 3 occasions to observe their methods and techniques so that we could attempt to reproduce them in our laboratory. We could not reproduce their results using the same methods and found no effect whatsoever from the same BRAN-type preparation that was claimed to be most effective in the other laboratory.

We find the Fourier transform infrared spectroscopy (FTIR) study[42] cited by Dr. Khuda-Bukhsh to be of such questionable nature that a detailed critique is warranted. The study by Sukul et al[42] purported to show that specific water-based molecular memory in homeopathic preparations could be transferred to a solid substrate by soaking a powder with the remedy and drying it. The work was based on the dubious assumption that a therapeutic agent was present in the homeopathic preparations that could be added to potassium bromide (KBr) powder and dried, and the powder would retain physical differences that could be examined by solid-state FTIR. Solid-state FTIR is used to study the structure of solids, for example, purified lyophilized proteins. This particular method of infrared spectroscopy is not suitable for studying liquids; that is more properly done by Ramen or liquid-state FTIR spectroscopy. As such, the authors should have used a different infrared spectroscopy method to study homeopathic preparations.

Solid-state FTIR involves thoroughly mixing a powdered solid of interest with powdered KBr and compacting the 2 solids into pellets under great pressure (up to 10 000 psi). One of the most critical aspects of solid-state FTIR is the production of the KBr-sample pellets. The authors did not describe many important details of methods used to prepare the samples, including the ratio of solvent to KBr or the pressure used and the duration of applied pressure to create the KBr pellets. Individual sample pellets were not checked for moisture content, and it is likely that drying the KBr powder without heating would result in pellets with different moisture content. The authors also did not describe many important details of the spectroscopic methods, including the fact that there was no mention of multiple interferograms being

recorded and averaged before Fourier transformation to produce average spectrograms from each sample. No mention was made of how the spectral baseline was corrected, and, indeed, it does not appear that the baseline was corrected for any of the spectra presented.

In their conclusions, Sukul et al[42] stated, "It has long been known in clinical practice that sucrose globules soaked with a liquid potentized [homeopathic] drug retain all the therapeutic properties of the drugs. FTIR spectra of KBr pellets soaked with potentized drugs simply confirm the long-standing clinical observation." The authors suggested that they were observing OH bond bending infrared absorption in the residual water in the KBr pellets. As such, the remaining moisture content in the pellets is a critical factor. The authors remarked: "Because all KBr pellets were prepared under similar conditions, it is quite unlikely that they have different amounts of water in them." Considering how critical a factor the water content of the pellets is under these circumstances, it is highly unusual that the authors made no attempt to determine the moisture content of each sample before spectroscopy. Despite the authors' claims that, after drying, moisture content was probably similar between samples, it seems likely that moisture content was significantly different between samples because the liquid-soaked KBr powder was simply spread out and allowed to dry at room temperature with 50% relative humidity. Slight differences in moisture content between samples would substantially affect the resultant spectra. The spectra presented by Sukul et al appear to reflect artifacts associated with improper pellet production, lack of water content normalization, lack of baseline correction, and a lack of multiple, averaged spectrograms for each sample. Studies of far higher quality will be required to show physicochemical differences between different homeopathic preparations. Subsequently, investigators will have to show that these differences translate to different therapeutic properties if their claims are to be taken seriously.

With regard to the notion that pharmaceuticals have side effects but homeopathic medications have none, we would like to point out that living organisms, which are highly integrated, open, homeostatic systems, always respond to perturbations or inputs with multiple responses at various levels of organization. Feedforward and feedback mechanisms will propagate effects from 1 component or subsystem to another, and the overall behavior of the system will be altered in multiple ways. As such, any drug that has a significant specified effect on living organisms will also have some unwanted or side effects. We contend that homeopathic drugs have no side effects because they have no effects other than the placebo effect (which in and of itself can have substantial influence on the patient's outcome). The burden of proof for demonstrating any beyond-placebo effects of BRAN-type homeopathic preparations remains squarely with homeopathic researchers, who have so far been unable to provide clear evidence for the active agent, mechanism of action, or specificity of action of different preparations.

Homeopathy is a belief system, not a scientific discipline. Belief systems do not require proof; they only require proponents. Homeopaths have not provided any evidence to date that warrants calls for additional funding and further basic research. Until homeopathic proponents demonstrate both the nature of the effective "agent" and the mechanism of action of so-called "potentized" homeopathic preparations, further funding is not justified. Simply claiming that clinical efficacy of BRAN-type homeopathic preparations has been proven, when such has not been rigorously established by reproducible methods, does not meet the criteria of science. Funds for mainstream scientific research are very scarce, and many excellent research programs go unfunded or underfunded because of the scarcity of financing. Allocating a portion of those scarce resources for homeopathy research will not serve science or the public well.

Acknowledgments

This work was supported by grants from the Samueli Institute for Information Biology. We thank Dr Raji Mathew for her technical assistance in the experiments outlined in this work.

References

1. Walach H, Jonas WB, Ives J, Wijk RV, Weingartner O. Research on homeopathy: state of the art. *J Altern Complement Med.* 2005;11:813-829.
2. Weissmann G. Homeopathy: Holmes, Hogwarts, and the Prince of Wales. *FASEB J.* 2006;20:1755-1758.
3. Khuda-Bukhsh AR. Towards understanding molecular mechanisms of action of homeopathic drugs: an overview. *Mol Cell Biochem.* 2003;253:339-345.
4. Jutte R, Riley D. A review of the use and role of low potencies in homeopathy. *Complement Ther Med.* 2005;13:291-296.
5. Dittmann J, Selbach AC, Hentges A, Harisch G. Use of urate oxidase as a test system to characterize the effect of homeopathic potencies and of equally concentrated conventional dilutions. *Pharm Pharmacol Lett.* 1994;4:19-2.
6. Bell IR, Lewis DA, Brooks AJ, Lewis SE, Schwartz GE. Gas discharge visualization evaluation of ultramolecular doses of homeopathic medicines under blinded, controlled conditions. *J Altern Complement Med.* 2003;9:25-38.
7. Anagnostatos GS. Small water clusters (clathrates) in the preparation process of homeopathy. In: Endler PC, Schulte J, eds. *Ultra High Dilution.* Dordrecht, The Netherlands: Kluwer Academic; 1994:121-128.
8. Gregory JK, Clary DC, Liu K, Brown MG, Saykally RJ. The water dipole moment in water clusters. *Science.* 1997;275:814-817.
9. Marotta M, Addabbo I, Kosasi S. The quality control and stability testing of homeopathic preparations. *Boll Chim Farm.* 1998;137:439-441.

10. Anick DJ. High sensitivity 1H-NMR spectroscopy of homeopathic remedies made in water. *BMC Complement Altern Med.* 2004;4:15.
11. Aabel S, Fossheim S, Rise F. Nuclear magnetic resonance (NMR) studies of homeopathic solutions. *Br Homeopath J.* 2001;90:14-20.
12. Milgrom LR, King KR, Lee J, Pinkus AS. On the investigation of homeopathic potencies using low resolution NMR T2 relaxation times: an experimental and critical survey of the work of Roland Conte et al. *Br Homeopath J.* 2001;90:5-13.
13. Demangeat JL, Gries P, Poitevin B et al. Low-field NMR water proton longitudinal relaxation in ultrahighly diluted aqueous solutions of silica-lactose prepared in glass material for pharmaceutical use. *Appl Magn Reson.* 2004;26:465-481.
14. Weingartner O. What is the therapeutically active ingredient of homeopathic potencies? *Homeopathy.* 2003;92:145-151.
15. Rubik B. The biofield hypothesis: its biophysical basis and role in medicine. *J Altern Complement Med.* 2002;8:703-717.
16. Walach H. Entanglement model of homeopathy as an example of generalized entanglement predicted by weak quantum theory. *Forsch Komplementarmed Klass Naturheilkd.* 2003;10:192-200.
17. Roy R, Tiller WA, Bell I, Hoover MR. The structure of liquid water: novel insights from materials research; potential relevance to homeopathy. *Mat Res Innov.* 2005;9:577-608.
18. Roy R. Another look at water and ice. *Science.* 2003;299:44-45.
19. Iler RK. *The Chemistry of Silica: Solubility, Polymerization, Colloid and Surface Properties, and Biochemistry.* New York, NY: Wiley-Interscience; 1979.
20. Pal SK, Peon J, Zewail AH. Biological water at the protein surface: dynamical solvation probed directly with femtosecond resolution. *Proc Natl Acad Sci U S A.* 2002;99:1763-1768.
21. Almeida RM. A critical review of the possible benefits associated with homeopathic medicine. *Rev Hosp Clin Fac Med Sao Paulo.* 2003;58:324-331.
22. Jonas WB, Kaptchuk TJ, Linde K. A critical overview of homeopathy. *Ann Intern Med.* 2003;138:393-399.
23. Mercer SW, Reilly D, Watt GC. The importance of empathy in the enablement of patients attending the Glasgow Homoeopathic Hospital. *Br J Gen Pract.* 2002;52:901-905.
24. Mallick P, Mallick JC, Guha B, Khuda-Bukhsh AR. Ameliorating effect of microdoses of a potentized homeopathic drug, Arsenicum Album, on arsenic-induced toxicity in mice. *BMC Complement Altern Med.* 2003;3:7.
25. Belon P, Banerjee P, Choudhury SC, et al. Can administration of potentized homeopathic remedy, Arsenicum album, alter antinuclear antibody (ANA) titer in people living in high-risk arsenic contaminated areas? I. A correlation with certain hematological parameters. *Evid Based Complement Alternat Med.* 2006;3:99-107.
26. Khuda-Bukhsh AR, Pathak S, Guha B et al. Can homeopathic arsenic remedy combat arsenic poisoning in humans exposed to groundwater arsenic contamination? A preliminary report on first human trial. *Evid Based Complement Alternat Med.* 2005;2:537-548.
27. Murray FJ. A comparative review of the pharmacokinetics of boric acid in rodents and humans. *Biol Trace Elem Res.* 1998; 66:331-341.
28. Witt CM, Ludtke R, Weisshuhn TE, Quint P, Willich SN. The role of trace elements in homeopathic preparations and the influence of container material, storage duration, and potentisation. *Forsch Komplementarmed.* 2006;13:15-21.
29. Rana MA, Douglas RW. The Reaction between glass and water, part 2: discussion of the results. *Physics and Chemistry of Glasses.* 1961;2:196-205.
30. Deshpande A, Narayanan PK, Lehnert BE. Silica-induced generation of extracellular factors increases reactive oxygen species in human bronchial epithelial cells. *Toxicol Sci.* 2002; 67:275-283.
31. Zeidler PC, Roberts JR, Castranova V, et al. Response of alveolar macrophages from inducible nitric oxide synthase knockout or wild-type mice to an in vitro lipopolysaccharide or silica exposure. *J Toxicol Environ Health A.* 2003;66:995-1013.
32. Ober KP. The pre-Flexnerian reports: Mark Twain's criticism of medicine in the United States. *Ann Intern Med.* 1997;126: 157-163.
33. Schwartz GE, Russek LG, Bell IR, Riley D. Plausibility of homeopathy and conventional chemical therapy: the systemic memory resonance hypothesis. *Med Hypotheses.* 2000;54:634-637.
34. Szutorisz H, Dillon N, Tora L. The role of enhancers as centres for general transcription factor recruitment. *Trends Biochem Sci.* 2005;30:593-599.
35. Shuai K, Liu B. Regulation of gene-activation pathways by PIAS proteins in the immune system. *Nat Rev Immunol.* 2005;5: 593-605.
36. Spiegelman BM, Heinrich R. Biological control through regulated transcriptional coactivators. *Cell.* 2004;119:157-167.
37. Roeder RG. Transcriptional regulation and the role of diverse coactivators in animal cells. *FEBS Lett.* 2005;579:909-915.
38. Linde K, Clausius N, Ramirez G, et al. Are the clinical effects of homeopathy placebo effects? A meta-analysis of placebo-controlled trials. *Lancet.* 1997;350:834-843.
39. Shang A, Huwiler-Muntener K, Nartey L, et al. Are the clinical effects of homoeopathy placebo effects? Comparative study of placebo-controlled trials of homoeopathy and allopathy. *Lancet.* 2005;366:726-732.
40. Ernst E. A systematic review of systematic reviews of homeopathy. *Br J Clin Pharmacol.* 2002;54:577-582.
41. Lorenz I, Schneider EM, Stolz P, Brack A, Strube J. Influence of the diluent on the effect of highly diluted histamine on basophil activation. *Homeopathy.* 2003;92:11-18.
42. Sukul NC, Ghosh S, Sukul A, Sinhababu SP. Variation in Fourier transform infrared spectra of some homeopathic potencies and their diluent media. *J Altern Complement Med.* 2005;11:807-812.