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Lecture Series

Immunology and Homeopathy. 3. Experimental Studies on Animal Models

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A search of the literature and the experiments carried out by the authors of this review show that there are a number of animal models where the effect of homeopathic dilutions or the principles of homeopathic medicine have been tested. The results relate to the immunostimulation by ultralow doses of antigens, the immunological models of the 'simile', the regulation of acute or chronic inflammatory processes and the use of homeopathic medicines in farming. The models utilized by different research groups are extremely etherogeneous and differ as the test medicines, the dilutions and the outcomes are concerned. Some experimental lines, particularly those utilizing mice models of immunomodulation and anti-inflammatory effects of homeopathic complex formulations, give support to a real effect of homeopathic high dilutions in animals, but often these data are of preliminary nature and have not been independently replicated. The evidence emerging from animal models is supporting the traditional 'simile' rule, according to which ultralow doses of compounds, that in high doses are pathogenic, may have paradoxically a protective or curative effect. Despite a few encouraging observational studies, the effectiveness of the homeopathic prevention or therapy of infections in veterinary medicine is not sufficiently supported by randomized and controlled trials.

Keywords: homeopathy – immunology – animal models – veterinary homeopathy – similia principle – ultra-high dilutions – isopathy – homeopathic complexes – paradoxical pharmacology

Introduction

Although in recent years we have witnessed a renaissance of interest in homeopathy, the reliability of its main principles, the 'simile' and the 'dilution/dynamization' of medicines, has still to be demonstrated on the experimental ground and few studies have been conducted to understand the underlying mechanism(s). On the other hand, inspection of the literature and experiments carried out by the authors of this review show that the principle of similarity—brought back to its biological meaning, i.e. the inversion of effect of the same or similar compounds—can be found to be operative in various experimental and reproducible phenomena (1–4). Therefore, on the basis of the present knowledge of living systems and of modern techniques of investigations, a scientific reformulation of homeopathy and its action mechanisms can be proposed in order to construct reasonable models, which could be tested at the different levels of biological systems, from cells to animals and to human beings. Our general working hypothesis is that the modern immunological and pathophysiological knowledge should help to clarify at least some mechanisms of action of this traditional medicine.

Over the past few years, there has been an increase in the number of preclinical (*in vitro* and *in vivo*) studies aimed at evaluating the pharmacological activity or efficacy of some homeopathic remedies under potentially reproducible conditions; however, in addition to major differences of experimental models, these studies have also highlighted a series of methodological difficulties and lack of independent replication (5).

Many studies of the efficacy and possible mechanisms of action of homeopathic medicines have been based on tests

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involving experimental animals or isolated organs. They have included various models of the application of 'similia', and attempted to demonstrate the effects of low doses or high dilutions of biologically active compounds. The fields of immunomodulation and inflammation are particularly fertile from this point of view. Immunoallergology represents a bridge between homeopathy and modern medicine insofar as it is a field in which it is easier to apply concepts such as the effect of substances administered on the basis of the logic of the 'similar' and the great sensitivity of living systems to regulations.

As it will become clear from the literature review, there are three different approaches to the exploitation of the homeopathic 'simile':

(i) The concept of 'similia', according to which there is a similitude between the symptoms evoked by the medicine and the symptoms of natural disease; in animal research, this modality has been pursued utilizing both the single medicines and the complex formulations, even if the latter does not follow the classical Hahnemann's rules.
(ii) 'Isopathy', according to which the same substance that causes the disease can be used in low doses or high dilutions to treat the disease; this concept is analog to the hormesis effect that was discussed in a previous article (6); when the preparation is from pathological tissues or microbial products the term 'nosode therapy' is also used.
(iii) 'Iso-endopathy', where therapeutic effects are obtained from highly diluted endogenous molecules (hormones, inflammatory mediators).

In the clinical practice, as in the scientific literature, it is possible to observe that the specificity and magnitude of effects are quite different between these forms of therapy. Therefore, the different concepts should be considered in the evaluation of the evidence coming from experimental studies and in the planning of appropriate experimental design.

Below, we describe a series of experimental studies aimed at verifying the efficacy of homeopathic medicines as immunostimulants and immunoregulators, then we shall examine the effects of homeopathic dilutions—or of other substances utilized according to the 'simile' principle—on models of experimental inflammation. Finally, an overview of main advancements in veterinary homeopathy, i.e. on the fields where homeopathy is used with the aim to cure natural infectious diseases and diseases of immune system, is provided.

All the literature available in Medline, conference proceedings and books was searched, we also report experiments done in our laboratory. As in the previous article reporting studies on cellular models, due to the relative scarcity of literature in this field, the lack of replication articles and the heterogeneity of experiments, we could not perform meta-analysis of data.

Homeopathic Immunostimulation

Bastide's group (7) has shown the immunostimulatory effects in mice of endogenous compounds such as thymic hormones and interferons prepared in high dilutions according to homeopathic procedures. Of the many reported experiments, those describing the effects of high dilutions of α–β interferon ($8–16 \times 10^{-10}$ IU i.p.) and thymic hormones (8×10^{-8} pg i.p.) on parameters of humoral (the number of plaque-forming cells) and cellular immunity (allospecific cytotoxic T cell responses) are particularly interesting (8). The authors suggested that a good therapeutic effect could be obtained in immunodepressed patients by using extremely low doses of these immunity mediators. Another interesting result coming from the studies of this group illustrates one of the most significant problems of homeopathic research: the pathophysiological state of the experimental animal powerfully conditions the results of any given treatment. This prompted the investigators to assess the effect of homeopathic dilutions (from 4c to 12c)[1] of thymus and thymuline on mice of the Swiss strain (which are considered to be immunologically normal) and mice of the New Zealand Black strain, which are considered to be immunologically depressed. The treatment caused significant immunostimulation only in the New Zealand Black mice, whereas the Swiss mice underwent immunodepression (which was particularly marked in the case of the thymus dilutions) (9). Another interesting point has to do with the importance of the chronological factor: a given treatment is perceived differently by the organism depending upon the time of day (circadian rhythm) or the month of the year (circa-annual rhythm) (8,10).

Ultradiluted Antigens Still Prime Immune System

Other immunomodulatory findings worthy of note are those reported by Bentwich's group (11–13). After demonstrating that very small amounts (6c and 7c dilutions) of the protein KLH (hemocyanin) antigen are capable of specifically modulating antibody responses in experimental animals, they repeated and expanded their experiments by showing the immunostimulating effects of homeopathic dilutions of the same antigen in mice. The animals were preconditioned for 8 weeks with i.p. injections of dynamized dilutions of KLH antigen (from 10^{-14} M to 10^{-36} M) or saline (control), and were then regularly immunized with KLH dissolved in complete or incomplete Freund's adjuvant. The serum levels of specific antibodies were determined by means of immunoassay and the results showed a significant increase in specific IgM response at all of the preconditioning dilutions, as well as a significant increase in specific IgG response in the animals pretreated with KLH 10^{-36} M. The authors concluded that extremely small amounts of antigen are enough for specific immunomodulation and, in particular, that homeopathic

[1] 'Homeopathic dilutions', otherwise called 'homeopathic potencies', means solutions of substances diluted and successed ('dynamized' or 'potentized' according to traditional terminology) in 1 : 100 serial dilutions (centesimal, c) or 1 : 10 serial dilutions (decimal, x). According to the Avogadro's law and as an approximate reference value, assuming a 1 mol l^{-1} (1M) concentration of pharmacologically active principles in the starting solution (mother tincture), dilutions higher than 12c or 24x (concentration of $\sim 10^{-24}$ M) should contain >1 molecule per liter of active principle.

dilutions beyond Avogadro's constant still have some effect. However, they also acknowledged that, in view of the vast implications of these findings, the experiments must be rigorously repeated and confirmed.

Neuroendocrine Regulations at High Dilution

The development of the chicken immune system is stimulated by homeopathic dilutions of bursin (7,14,15), a tripeptide (Lys-His-Gly-NH_2) B cell differentiation hormone derived from the bursa fabricii. In these studies, chick embryos underwent bursectomy in order to make them immunodeficient (it is well known that the bursa of *Fabricius* is essential for the development of the B lymphocyte system). The *in ovo* administration of low doses and high dilutions of the hormone bursin (up to 10^{-40} g ml^{-1}), which theoretically no longer contain any molecules of the original substance, restored the immune response as demonstrated by the normal antibody production of the adult animal in response to antigen stimulus (bovine thyreoglobulin). Moreover, an improved response of the pituitary–adrenocortical axis was shown by measuring adrenocorticotropic hormone. Moreover, in early embryonically bursectomized chickens, the plasma melatonin response to immunization by porcine thyroglobulin has found to be abolished (14,16). Conversely, administration of either minute amounts (100 pg, 100 fg) or highly dilute (5×10^{-27} g) bursin, with the exception of a highest dose (100 μg), to bursaless embryos induced recovery of normal antigen-induced melatonin response. The authors suggest that early in embryonic life, the bursa fabricii and its derived signal (bursin) are essential for normal development of pineal synthetic activity and underline the efficacy of very dilute bursin as an informative signal (17).

Minerals and Nosodes

A series of studies examined the action of high dilutions of *silica* on the production of platelet activating factor (PAF) by peritoneal macrophages in the mouse (18). The compound was added to drinking water at a dilution of 9c (corresponding to a theoretical concentration of 1.66×10^{-19} M) for 25 days. The peritoneal macrophages extracted from the mice showed an ability to produce PAF in response to a stimulus with yeast extracts that was 30–60% greater than that of control macrophages (untreated mice, mice treated with NaCl in a 9c dilution or with another homeopathic drug, *Gelsemium* 9c). Lower dilutions (5c) paradoxically had less effect.

For centuries, homeopathic practitioners have claimed that serially agitated dilutions of infectious agents (called 'nosodes') are effective in the prevention of infectious disease. Following this idea, an experimental trial of the immunostimulating power of high dilutions of pathogenic substances was done by a team of American researchers (19,20). They produced the dynamized dilutions from reticuloendothelial tissue of mice infected with *Francisella tularensis*, the microbial agent of tularemia, obtaining three dilutions containing original tissue (3x, 7x and 12x) and three dilutions beyond the presence of original tissue (30c, 200c and 1000c). These preparations were administered orally to a group of mice (0.03 ml, three times per week) for 1 month before and after challenge, whereas another control group was treated with dilutions of ethanol. Animals were challenged with a potentially lethal dose (LD_{50} or LD_{75}) of *F. tularensis*, then evaluated for time of death and total mortality. After 15 experiments the very high homeopathic dilutions brought about a significant increase in survival time and a significant reduction in total mortality compared to controls. Protection rates averaged 22% over controls compared to 100% protection by standard vaccination. A partial protection was thus obtained from a nosode of tularemia, in dilutions below those expected to have protective effects, but not as great as those produced by standard vaccination.

Homeopathic dilutions of *silica* are widely used in homeopathy to treat sores, chronic ulcers and abscesses. An experimental animal model based on induced chronic wounds was used by a group of investigators in Rehovot (Israel) (21), who reported the therapeutic effects of homeopathic silica dilutions on the repair of holes pierced in the ears of mice. The holes were made using dental wire, which was then left hanging from the ear in order to cause persistent mechanical irritation. In each experiment, 3 or 4 groups of 10 mice each were treated by adding homeopathic dilutions of *silica* (10^{-10}, 10^{-60} and 10^{-400}) or saline (10^{-10}) to drinking water for 4–20 days. The size of the holes was measured every second day and/or by means of an objective image analysis system. The results showed that, in 7 out of 11 experiments, the holes in the ears of the silica-treated animals were significantly smaller ($P < 0.05$–0.001) and healed faster than those in the saline-treated animals. The therapeutic effect also progressively increased with increasing silica dilutions.

Interestingly, *silica* is one of the few homeopathic substances that have also been investigated by physical instrumentation: variations in the nuclear magnetic resonance characteristics and, in particular, relaxation times T1 and T2 in highly diluted solutions of *silica* have been measured by a French research team and published in an official journal of physics (22). In brief, it was observed that silica/lactose solutions prepared in centesimal dilutions according to homeopathic methodology led to an increase in T1 and the T1/T2 ratio in comparison with distilled water or diluted and dynamized solutions of NaCl. These changes were also detectable at silica concentrations in the order of 10^{-17} mol l^{-1}. This was therefore the first case in which a physical characteristic was described in a homeopathic remedy whose biological activity was also established experimentally in animals. Unfortunately, these experiments have not yet been replicated by other groups.

Other Homeopathic Medicines

Atropa belladonna and *Echinacea angustifolia* have been tested for their effects on leukocyte migration and macrophage activity induced by experimental peritonitis *in vivo* (23). Mice

were injected (i.p.) with lipopolysaccharide (1.0 mg kg^{-1}) and treated (0.3 ml per 10 g per day, s.c.) with different forms of these medicines. The association of *A. belladonna* and *E. angustifolia* in a formulation containing various potencies produced a significant increase of polymorphonuclear cell migration and a decrease of mononuclear cell percentages. The proportion of degenerate leukocytes was lower in the treated groups, compared to a control group. The treated groups showed increased phagocytosis, mainly in preparations containing high potencies. The authors suggested that *A. belladonna* and *E. angustifolia*, when prepared 'in accord of potencies', modulate peritoneal inflammatory reaction and have a cytoprotective action on leukocytes.

Trichinellosis caused by the gastrointestinal nematode *Trichinella spiralis* occurs in humans, domestic animals and wild animals. A recent study (24) investigated whether potentized homeopathic drugs such as *Cina*, *Santoninum* and *Podophyllum* can affect the muscle phase of the parasite in mice. *Cina* 30 and *Santoninum* 30 were prepared from the mother tincture of the flowering tops of *Artemisia nilagirica* and its active principle santonin, in each case by successive dilution (1 : 100) with 90% ethanol and sonication in 30 steps following the single glass method (30K). Each drug was diluted 1 : 20 with distilled water and administered orally at 0.05 ml per mouse per day. Experimentally infected mice were also treated with an aqueous *Podophyllum* suspension (mother tincture) at 60 mg per kg per day. Each mouse was inoculated with *T. spiralis* larvae and treatment was started on day 7 post-infection and continued for 120 days. After completion of treatment, the mice were sacrificed and the larvae were extracted from muscles by HCl–pepsin digestion. The results showed that *Podophyllum*, *Cina* 30 and *Santoninum* 30 reduced the larval population in the studied mice by 68.14, 84.10 and 81.20%, respectively, as compared to the untreated control group. The homeopathic dilutions were more effective than the mother tincture, an effect which was not due to the medium ethanol. In fact, homeopathic dilutions of ethanol (*Ethanol* 30) achieved no significant reduction in the larval population compared to the untreated control group. The authors suggested that the effect of *Podophyllum* mother tincture might be due to the direct toxic effect of the drug on the larvae, while the homeopathic dilutions of *Cina* and *Santoninum* probably acted through indirect effect, possibly mediated by immunostimulation.

Canova is a homeopathic complex medicine (composed of 19x *Thuya occidentalis*, 18x *Bryonia alba*, 11x *Aconitum napellus*, 19x *Arsenicum album* and 18x *Lachesis muta*) whose effects have been studied in normal and sarcoma 180-bearing mice (25). The mice were examined at daily intervals and the tumors observed histologically. A delay in the development, and a reduction in size of the tumors, and increased infiltration by lymphoid cells, granulation tissue and fibrosis surrounding the tumor were observed with active treatment compared to control. All animals from the treated group survived, 30% of control groups died. In 30% of treated animals, a total regression of the tumor was confirmed using light microscopy, no regression was found in the control groups. Active treatment increased total numbers of leukocytes and lymphocytes, suggesting that protection against experimental sarcoma may result from enhanced immune response of the host.

The major findings of this line of experiments are summarized in Table 1.

Immunoregulation and Regulation of the Inflammatory Processes

The most notable principle of homeopathy means that when a substance is able to induce a series of symptoms in a healthy living system, it would be also able under certain circumstances to cure these symptoms when applied at low doses

Table 1. Models of homeopathic immunostimulation

Animal	Model	Treatment	Key findings	Reference
Mouse	Iso-endopathy	Low doses of thymic hormones and interferons	Immunostimulation	(7,8)
Mouse	Isopathy	High dilutions of antigen	Specific sensitization	(11–13)
Chicken	Iso-endopathy	High dilutions of peptide hormone	Immunostimulation and neuroendocrine regulation	(7,14,15,17)
Mouse	Isopathy	High dilutions of silica	Stimulation of macrophages	(18)
Mouse	Isopathy	High dilutions of silica	Speeds up wound healing	(21)
Mouse	Isopathy (nosode)	Low doses and high dilutions of infectious agent *F. Tularensis*	Protection from specific infection	(19,20)
Mouse	Simile	Various dilutions of *A. belladonna* and *E. angustifolia*	Stimulation of phagocytes	(23)
Mouse	Simile	High dilutions of *Podophyllum*, *Cina* 30 and *Santoninum* 30	Protection from infectious agents	(24)
Mouse	Simile	Complex formulation made by high dilutions of *Thuya*, *Bryonia*, *Aconitum*, *Arsenicum* and *Lachesis*	Protection from tumors, immunostimulation	(25)

('similia similibus curentur'). This concept has a strict relation with modern immunological evidence of non-linear or even opposite responses to antigens, cytokines and other immunoregulatory agents. Therefore, the use of the 'simile' in this modality is aimed to decrease the immune and inflammatory reactions that occur in many conditions ranging from local symptoms of immediate allergy to systemic pathologies associated with chronic autoimmune diseases.

Models of Specific Immunotherapy

Allergen-specific immunotherapy is the only immunomodulatory treatment that may alter the natural course of allergic disease, for example, by preventing the development of asthma in rhinitic patients (26,27). It consists of injecting increasing amounts of offending allergens into sensitive patients with the intention of reducing their level of sensitivity to allergens. The therapy is believed to exert its effects by a combination of means: by the induction of blocking antibodies; a switch from a T helper (Th)2 to a more Th1 allergen-specific immune response; and induction of anergy, probably via the development of allergen-specific regulatory T cells (28). The evolution of new developments, including the sublingual administration of the relevant antigens and new forms of antigen to regulate the immune system, now offers improvements in both the safety and the efficacy of specific immunotherapy (27,29).

In laboratory animals, the secondary immune response to antigens is depressed (state of tolerance) both in animals pretreated with very low doses and in animals receiving high doses of antigen. Pretreatment with intermediate doses, however, cause a priming, revealed by secondary response greater than that of non-pretreated animals (30,31). Similarly, the autoreactivity of T cells is managed by the immune system in at least two different ways that obviously depended on the antigen concentration they encounter. If they come into contact with high concentrations of a self-antigen, they are deleted (killed) but, when given low doses, they undergo a special kind of active inhibition (31–33). The mucosal milieu and the presence of cytokines like IL-4 or IL-10 may enhance this phenomenon (34–37). Other authors have suggested that this type of regulation induced by very low substance concentrations could serve as a model to explain the way in which at least some homeopathic pharmaceuticals mediate their therapeutic effects (38). Therefore, use of sublingual immunotherapy is a typical field in which the boundaries of homeopathy (the so-called isopathic approach) and conventional immunology often overlap.

Adjuvant Cures Adjuvant

A series of studies carried out by our group have considered specific immunomodulation in rat. Briefly, after the injection of Freund's adjuvant (a special non-infective preparation of *Mycobacterium butyricum* in paraffin oil used to stimulate the immune system) in one paw, Lewis rats develop a local inflammatory reaction. Then, after an interval of about 2 weeks, they develop a picture of arthritis in their other limbs that is considered to be a model similar to rheumatoid arthritis. On this type of reaction pharmacologists use to test the conventional anti-inflammatory drugs. We have demonstrated (39–41) that this picture of systemic inflammation is greatly

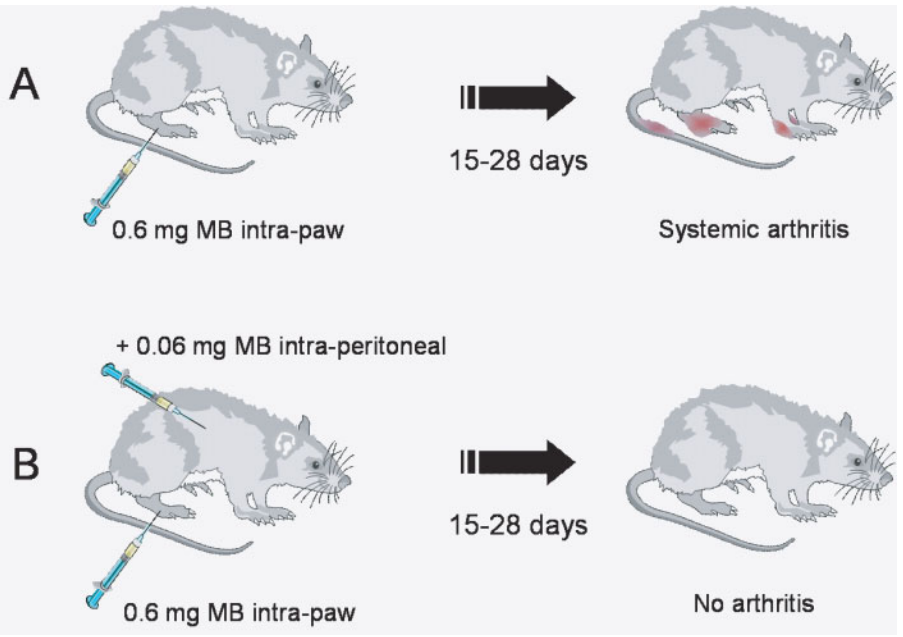

Figure 1. Regulation of adjuvant arthritis by low doses of adjuvant. Rats with adjuvant (killed *M. butyricum* in paraffin oil)-induced arthritis were injected intraperitoneally with the causative antigen at concentrations 10 times lower than the inducing one, on the 3rd and 10th day after arthritis induction. The severity of the disease was assessed on the basis of paw swelling, of general inflammatory involvement (arthritis index) and of biochemical (serum IL-6) parameters (39,40,42).

reduced and slowed by the intraperitoneal injection of small doses of Freund's adjuvant (0.06 mg in two administrations) (Fig. 1). This therapeutic effect was both antigen-specific, because intraperitoneal aspecific inflammation, induced by casein, did not prevent the disease, and long-lasting. The results obtained in this model confirm the possibility of modulating the autoimmune process even when the immunological response is already triggered, suggesting new therapeutic strategies, more suitable than preventive vaccination, in human autoimmune diseases. In a subsequent study (42), we investigated the correlation between the therapeutic effect of low doses of *Mycobacterium* and endogenous mediators of inflammation (nitric oxide and cytokines). The treatment was associated with a significant reduction in IL-6 serum concentrations, a slight decrease in IFN-γ production by peritoneal macrophages and an increase in nitrite/nitrate plasma concentrations, without any apparent differences in peritoneal macrophage nitric oxide production. Our data show that, among the variables investigated, IL-6 seems to be the more representative marker of disease and the effect of treatment, and that nitric oxide may play a role as a modulator rather than a direct mediator in this model of inflammation. The pathological roles of IL-6 have also been clarified in various disease conditions, such as inflammatory, autoimmune and malignant diseases. A new therapeutic approach to block the IL-6 signal using anti-IL-6R antibody for rheumatoid arthritis, Castleman's disease and multiple myeloma has been attempted by various authors (43). Connected with the mechanisms of this immune tolerance, it has been shown that the modulation of adjuvant arthritis can be obtained in Lewis rats by intranasal administration of mycobacterial 10 kDa heat shock protein (hsp10), indicating that molecular mimicry by this protein moiety is responsible of immune tolerance and immune deviation (44).

Tolerance of the ''Simile''

The specific immune tolerance effect is on a borderline connecting some of the themes of modern immunopharmacological research with the most traditional concepts of homeopathy insofar as it is a further example of the induction of immunological tolerance by means of low antigen doses, an immunomodulatory procedure that has been widely revealed over recent years in a large number of human conditions. We can here cite only briefly some examples of therapy that can be considered particular applications of the 'simile' at molecular level (45–50): bacterial endotoxins as immunostimulants, immunoglobulins to treat immune disorders, orally administered myelin in multiple sclerosis, oral collagen in rheumatoid arthritis, bacterial extracts in recurrent bronchitis and locally administered allergens in allergies. Specific immunosuppression and the induction of tolerance by means of microdoses of antigens (also in the field of allergology) are frontiers of modern immunology that apply a principle first proposed by homeopathy in a scientifically controlled manner: the use of a pathogenic substance for therapeutic purposes by exploiting very low doses or particular administration protocols (51,52). Tumor immunotherapy using antigen-pulsed dendritic cells (53) or autologous tumor-derived heat shock protein preparations (54) are also worth mentioning in this context.

Table 2. Treatment of organ-specific autoimmune diseases in animals using the 'simile' substance at very low doses [adapted from ref. (40,49,55)]

Model disease	'Pathogenic' compounds	'Therapeutic' compound
Experimental allergic encephalomyelitis (Lewis rat, guinea pig)	Myelin	Myelin basic protein Oral: 3–10 mg (no effect with 50 mg) Aerosol: 0.005–5 mg
Collagen-induced arthritis (Lewis rat)	Collagen	Collagen type II and type I Oral: 0.003 mg (no effects of 0.3 mg)
Spontaneous autoimmune diabetes (mouse)	Pancreas islet antigens?	Insulin Oral: 1 mg (no effect with 5 mg)
Experimental autoimmune myasthenia gravis (Lewis rat)	Acetylcholine receptor	Acetylcholine receptor Oral: 20 mg
Arthritis induced by mycobacterium extract (Lewis rat)	Mycobacterium extract (heat-shock protein?)	Mycobacterium extract Intraperitoneal: 0.06 mg

Table 2 shows some experimentally induced inflammatory diseases in animals that have been treated by using very low doses of the 'simile' substance, which is the same or similar substance that causes the disease. This is an application by modern immunology of the traditional 'isopathic' method, associating two main principles of homeopathy—the low dose and the similarity between causal and therapeutic agent—that was introduced by ancient homeopaths like Hering and Lux (56). Sometimes, also major scientific journals acknowledge (albeit ironically) that the principles of oral tolerance are connected to homeopathy: 'The oral-tolerance idea, discredited and regarded almost as homeopathy by some, was put into practice to find out whether it works in multiple sclerosis and rheumatoid arthritis...' (57). Needless to say, all these advancements of the ancient isopathic idea in modern immunology do not support the use of homeopathic medicines as immunomodulators in humans, a possibility that should be explored by appropriate clinical studies.

Models of Acute Inflammation

In the previous lecture dealing with the effect of homeopathic dilutions at the cell level (6) we noted that according to several authors high dilutions of *Apis mellifica* (a medicine which is currently used in homeopathy to treat skin manifestations with edema, erythema and itch) inhibit basophil activation, this line of research illustrating a laboratory application of the principle of similarity: a substance known to stimulate inflammation at conventional doses can, at different doses, inhibit the cells responsible for many of the phenomena of

the inflammatory process. Early studies of French researchers reported that high dilutions (7c to 9c) of bee (*A. mellifica*) and its venom (*Apium virus*), have a protective and curative effect on ~50% of X-ray induced erythema in albino guinea pig (58–61). This seems to confirm the principle of reactional similarity underlying homeopathy. It is well known that high doses of bee venom (such as that delivered by a sting) cause edema and erythema, but homeopathic dilutions can cure the edema and erythema caused by another agent. This line of research is quite interesting, but the results await independent confirmation because the methodological quality of the first publications was low (5).

Following a similar experimental idea, our group at the University of Verona (in a line directed by Dr Conforti) studied the effects of homeopathic preparations of *A. mellifica* and *Histamin* on rat paw edema (measured as the swelling due to the accumulation of exudate) induced by the injection of inflammatory doses of histamine. Using this model, it was observed that high dilutions of up to 30x had a small but significant inhibitory effect on the development of edema (62). This was a pilot investigation that unfortunately we had no opportunity to pursue further due to lack of funding.

Complex Formulations

Carrageenin edema, a classical experimental model commonly used to test the activity of anti-inflammatory drugs, was used by our group (63) to evaluate the therapeutic activity of a low-potency mineral complex called *Ultima ratio* (MC), constituted of different salts, at various potencies (2x to 7x): potassium bromide, potassium sulfate, calcium chloride, strontium chloride, barium chloride, manganese sulfate, iron sulfate and others, prepared according to the German pharmacopoeia. The MC was administered in the right plantar surface of albino rats 60 min before, simultaneously and 30 min after the injection of carrageenin, an irritant which causes a local and transient increase in fluid volume. The administration of the MC 60 min before the injection of carrageenin primed an enhanced inflammatory response to the irritant. The administration of MC at the same time as carrageenin did not modify the kinetics or the extent of the edema, whereas the administration of the MC 30 min after the induction of edema significantly reduced the early phase of the inflammatory reaction. This suggests that the therapeutic action of this MC is not due to conventional anti-inflammatory effects but to the activation of endogenous regulatory mechanisms, something that may be regarded as a simple application of the '*similia* rule'.

Our group has also carried out a series of studies on the activity of a homeopathic formulation (*Traumeel-S*, TRS) containing low potencies (4x to 12x) of *Arnica montana* and other plant extracts and minerals (*Calendula officinalis, Hamamelis virginiana, Achillea millefolium, A. belladonna, Aconitum napellum, Hepar sulfuris, Symphytum, Mercurius solubilis, Bellis perennis, Chamomilla, E. angustifolia, Echinacea purpurea, Hypericum*) on an animal model of inflammation (64,65). The main indications of TRS refer to different types of lesions and inflammatory processes involving muscles and joints, sprains and bruises. We tested the *in vivo* effects of TRS on acute and chronic experimental inflammatory conditions caused by the intrapaw injection of carrageenin (carrageenin edema) or Freund's complete adjuvant (adjuvant arthritis). The results suggested that the local administration of TRS reduced the development of edema in a way that is similar to the effect of aspirin at a dose of 30 mg kg^{-1} in the same experimental model. In the adjuvant arthritis model, the i.p. administration of TRS every 2 days led to a significant reduction in acute local inflammation, without affecting the chronic arthritic process.

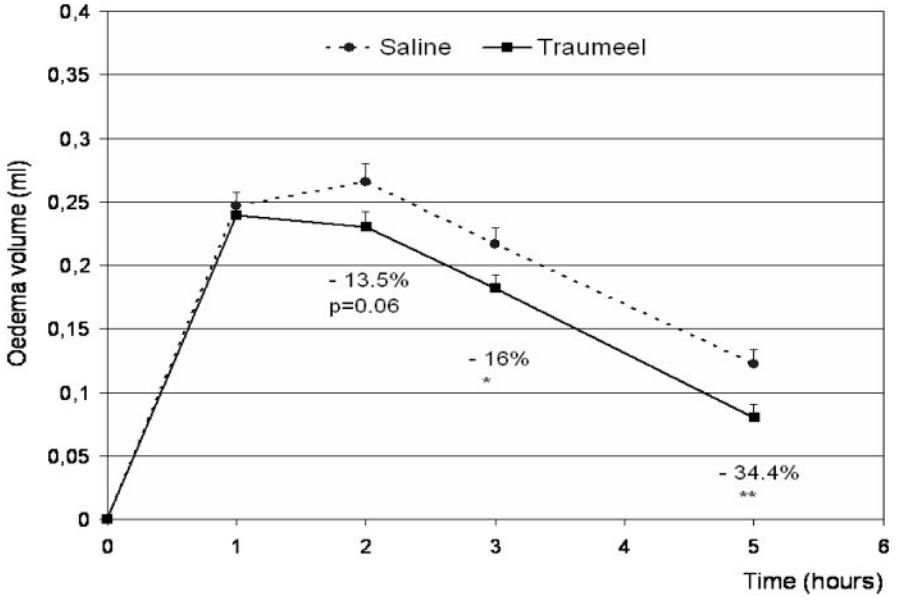

Figure 2. Effect of *Traumeel-S* on the rat paw edema caused by autologous blood injection. Adapted from data of ref. (66). $*P < 0.05$, $**P < 0.01$.

Table 3. Effects of the homeopathic complex *Traumeel-S* or of its single components on experimental models of acute and chronic inflammation

Inflammation inducer	Type of inflammatory reaction	Route of administration	Key effects	Reference
Carrageenin	Acute (1–7 h)	Local injection	Slight inhibition of edema	(64)
Freund's adjuvant	Acute (2–4 days)	Intraperitoneally	Inhibition of local reaction	(64)
	Chronic (14–28 days)	Intraperitoneally	No effect on systemic reaction	(64)
Autologous blood	Acute (1–5 h)	Local injection	Speeds up edema resolution	(66)

Furthermore, we carried out a series of studies aimed at improving the knowledge of the therapeutic action of TRS using a new model represented by the intrapaw injection of a small amount of homologous blood mimicking traumatic blood extravasation, a condition that is usually treated with TRS (66). We also measured the serum level of IL-6, a cytokine known to be involved in inflammatory conditions. The results (Fig. 2) showed a slight but significant decrease in paw edema associated with the process of healing, which was more rapid in the rats treated with TRS ($P < 0.05$ after 3 and $P < 0.01$ after 5 h). Similar effects were also induced by separate injections of most, but not all of the ingredients of TRS. The efficacy of the complete TRS mixture was greater than that of the combination of a selection of active components. The therapeutic effect was associated with a significant decrease in systemic IL-6 production. In conclusion, TRS seems to act by speeding up the healing process instead of blocking the development of edema from the beginning. Moreover, its effect cannot be considered as the 'sum' of its active components but is probably due to synergistic interactions. The main results of our experiments in rat models of inflammation are summarized in Table 3.

Experimental Discrepancies in Homeopathic Doses

We have more recently repeated the tests of anti-inflammatory power using individual homeopathic dilutions in a series of experiments performed in collaboration with the *Istituto Superiore di Sanità* of Italian Health Ministry (A. Conforti, P. Bellavite, S. Bertani, F. Chiarotti, F. Menniti-Ippolito, R. Raschetti, 2006, submitted for publication). Aim of the study was to evaluate, through two randomized controlled animal experiments, the activity of different homeopathic remedies (*A. montana*, *A. mellifica*, *A. belladonna*, *H. virginiana*, *Lachesis* and *Phosphorus*), usually prescribed by homeopaths to treat inflammatory or hemorrhagic manifestations. Two control drugs were used: saline (placebo) and indomethacin (active reference treatment). In experiment A more conditions were tested (six homeopathic remedies, two administration routes—oral and by subplantar injection—and two edema models—carrageenin- and autologous blood-induced edema). Some small, but statistically significant, effects of homeopathic remedies were observed. The most relevant results concerned *Apis* (4x), *Lachesis* (6x) and *Phosphorus* (6x) in the oral treatment of carrageenin-induced edema (volume reduction ranging from 11 to 28%). When retested in experiment B, following a blinding procedure as for treatments allocation and measurement of the outcome, the three homeopathic remedies (both at low and at high dilutions) did not show any anti-inflammatory effect. The activity of indomethacin was reproduced in both experiments. In conclusion, the different results observed in the two experiments make it possible to draw some suggestions useful for designing possible further experiments, among these the discrepancies between single-blind and double-blind methods in animal pharmacological research are noteworthy and should be better investigated, also in non-homeopathic research. In this type of research, several technical factors linked to sensitivity of the tested system, to the type medicines used (e.g. single remedies versus complex formulations, oral administration versus local injection) and even to the type of solvent used for the solutions (water, saline, water/alcohol mixtures) that can affect the drug stability and efficiency (24,67) may explain the observed discrepancies and the lack of reproducibility that we and others have often observed.

The effect of *A. montana* on the classical rat model of paw inflammation was evaluated by others, using acute and chronic models (68). In the acute, carrageenin-induced rat paw edema, the group treated with *Arnica* 6c showed 30% inhibition compared to control group ($P < 0.05$). In nystatin-induced edema (chronic model) the group treated 3 days previously with *Arnica* 6c had reduced inflammation 6 h after the inflammatory agent was applied ($P < 0.05$). When homeopathic treatment was given 6 h after nystatin injection, there was no significant inhibitory effect. In a model based on histamine-induced increase of vascular permeability, pretreatment with *Arnica* 6c blocked the action of histamine.

Homeopathic Dilution of Allopathic Drugs

Special interest for the interpretation of the similia principle may have the study of the effects of homeopathic dilutions of conventional (allopathic) anti-inflammatory medicines. A Brazilian group (69) has evaluated the interaction of dexamethasone 7c and 15c (equivalent to 10^{-17} and 10^{-33} M) with dexamethasone in pharmacological concentrations, using as experimental models based on the acute inflammation induced by carrageenin into the footpad for edema evaluation or in peritoneal cavity for polymorphonuclear cell migration. Animals were treated with the following preparations: (i) phosphate-buffer saline (PBS) solution; (ii) dexamethasone (0.5 mg kg^{-1} for inflammation model or 4 mg kg^{-1} for tumor model) mixed with dexamethasone 7c or 15c; and (iii) dexamethasone (same doses) mixed in PBS. Homeopathic dexamethasone partially blocked the anti-inflammatory effect of pharmacological dexamethasone with regard to paw edema ($P < 0.0008$) and polymorphonuclear cell migration into the peritoneal cavity ($P = 0.0001$). The results demonstrate that

a potentized substance may have opposite effects on its own pharmacological effects and suggest that high dilution effects act also on specific parameters of host response.

Another model showing an experimental proof of the concept of homeopathic simile was recently reported (70). *Causticum* is a homeopathic medicine that when administered in ponderal doses into the hind paw of rats produced an inflammatory reaction with edema formation within the first hour, showing that *Causticum* acts as an edematogenic agent. Carrageenin induced rat paw edema was significantly inhibited in rats treated with *Causticum* 6c, 12c, 30c and 200c dilutions compared to untreated rats.

The anti-inflammatory and antithrombotic effect of high dose acetylsalicylic acid (ASA) is well known, and recently, animal studies hinted the thrombotic effect of ultralow dose of ASA. A laser-induced rat mesenteric vessel thrombosis model was used to evaluate the effect of ASA at ultralow doses in Wistar rats. Compared with placebo, the administration of ASA at ultralow doses (15c diluted and succussed solutions containing a theoretical dose of 10^{-30} mg kg^{-1}) induced an increase in number of emboli and an increase in the duration of embolization (71–73). Since it is well known that aspirin causes an increase in bleeding time at pharmacological doses (50–500 mg), the findings of these studies may possibly be interpreted as a demonstration of the law of similars. Nevertheless, the mechanism whereby this happens is still unclear because, while we know that aspirin at normal doses exerts its action by inhibiting the function of platelets, in the early work by the same group it is reported that homeopathic dilutions of aspirin have no effect on platelet aggregation (74). In further studies (75,76) homeopathic preparations of ASA (15c), injected 2 or 3 h after the administration of aspirin at 100 mg kg^{-1}, inhibited the antithrombotic effect of high-dose ASA. These paradoxical and opposing effects of different doses of aspirin could be of importance for the long-term use of this common drug and possibly for prevention or the treatment of hemorrhagic syndromes.

Application of experimental stress (such as unavoidable footshock) induce gastric erosions and immunological alterations in mice. Low dilutions *of A. belladonna L.*, *Gelsemium sempervirens L.* and *Poumon histamine* showed a significant immunoprotective and gastroprotective effect in mice exposed to this experimental stress (77). Immunological studies were investigated to count white blood cells subpopulations (lymphocytes, neutrophils, monocytes and basophils) by coulter counter. The authors suggested that the immunological protective effects observed were probably induced via a neurotropic effect of the tested medicines.

The main results of this line of experiments are summarized in Table 4.

Other High Dilution Effects in Animal Models

A team of researchers have reported that homeopathic zinc preparations in decimal dilutions (from 4x to 12x, corresponding to amounts of zinc ranging from 0.025 mg to 0.25 pg) administered to rats for seven consecutive days significantly increases the release of histamine by peritoneal mast cells (78).

It is known that beta-2 adrenergic agonists (isoproterenol, salbutamol, tulobuterol) relax the tracheobronchial musculature. It has also been reported (79) that, even at high dilutions (10^{-20} to 10^{-36} M in dynamized decimal dilutions), they are also capable of relaxing basal tone in a model of isolated guinea pig trachea. The fact that visceral smooth muscle may be sensitive to high dilutions of such hormones is interesting because of the close relationships between the immune and neuroendocrine systems, and therefore the possibility that homeopathic medicines may act at the peripheral or central level (which is still unknown) of the dysregulation involved

Table 4. Immunoregulation and regulation of inflammatory processes in animal models

Animal	Model	Treatment	Key findings	Reference
Rat	Isopathy (nosode)	Low doses of Freund's adjuvant	Decrease of reactive arthritis caused by the same agent	(39–42)
Guinea pig	Simile	High dilutions of *A. mellifica* and *Apium virus*	Curative effect on X-ray induced skin erythema	(58–61)
Rat	Isopathy or iso-endopathy	Low doses and high dilutions of *A. mellifica* and *Histamin*	Decrease of histamine-induced paw edema	(62)
Rat	Isopathy or Simile	Low doses of homeopathic mineral complex	Decrease of carrageenin-induced edema	(63)
Rat	Simile	Low doses of the homeopathic complex *Traumeel-S*	Decrease of edema (see Table 3)	(64–66)
Rat	Simile	High dilutions of *A. montana*	Decrease of edema induced by various agents	(68)
Rat	Simile	High dilution of *Causticum*	Decrease of carrageenin-induced edema	(70)
Rat	Iso-endopathy	High dilutions of dexamethasone	Reduces the anti-inflammatory effect of dexamethasone	(69)
Rat	Isopathy	High dilutions of ASA	Prothrombotic effect	(71–73,75,76)

in diseases of the inflammatory and immune systems, such as bronchial asthma and gastroenteritis.

Benveniste's Effects and Further Studies

One important phenomenon of acute inflammation is regulation of the blood flux, which can be assessed in isolated organs. Benveniste and co-workers (80,81) have reported the results obtained using an experimental model of isolated and perfused guinea pig heart (Langendoff's system). Coronary flow increased with the infusion of very high dilutions of histamine (>30x) as normally happens with the usual low dilutions. The blind infusion of buffer alone or a high dilution of the methyl-histamine analog had no effect on coronary flow. The vasodilating activity of highly diluted histamine was abolished by treatment at 70°C for 30 min or exposure to a 50 Hz magnetic field for 15 min (82). The authors concluded that the water, deprived of solute by means of serial dilutions, retains a specific activity that can be suppressed by physical treatments that do not have any effect on the solute itself.

The model of isolated and perfused guinea pig heart seems to be effective in providing reliable results. In two subsequent publications (83,84), the same group reported that the system is also sensitive to immunization-dependent activation. By immunizing guinea pigs with ovalbumin and removing the heart after between 9 and 20 days, it was possible to obtain an increase in coronary flow at extremely high ovalbumin dilutions (10^{-31} to 10^{-41} M). The authors have opened a website in which they claim to have pursued their research lines with significant results (http://www.digibio.com).

The homeopathic medicine *A. belladonna* was tested on the *in vitro* contraction of isolated duodenum (85). It is noteworthy that low dilutions of *Belladonna* (1c and 5c) showed inhibitory effects, as expected from the spasmolytic effect of atropin, while highly diluted and dynamized solutions (30c and 45c) showed stimulatory effects. Non-dynamized solutions were inactive.

Veterinary Homeopathy and Cure of Infections

Veterinarians are showing more interest in alternative methods of farming and of cure, but information about the extension of homeopathy to veterinary medicine is limited, as good records of the use of these treatments do not exist. In the treatment of farm animals, alternative therapies have been in focus mainly in relation to organic farming, because of the emphasis on natural methods and medicines in the organic standards and the general intention to reduce use of chemical substances (86–90).

Preliminary Evidence

The prevalence of resistant bacteria is rising, and organisms resistant to almost all antibiotics have been identified. The main causes are indiscriminate prescribing and the use of antibiotics in animal feeds and other agricultural applications. Policies to restrict use of antibiotics have had limited success. Homeopathy may have a role to play in combating the infectious diseases (91) and development of antibiotic resistance (92). In the pig, the value of using homeopathy (a complex of *Caulophyllum* 30c, *Sepia* 6x and *Cocculus* 6x) on the delivery process and on the viability of piglets has also been suggested (93).

As reported in a preliminary communication, *Baptisia tinctoria* 30c was tested with positive results to cure salmonellosis in broiler chickens (94). An experimental study sought to determine the efficacy of isopathic and pluralist homeopathic treatment of colibacillosis in broiler chickens (95). Three groups of broilers, infected intratracheally at 8 days of age with *Escherichia coli* (O78:K80), were treated with different combinations of homeopathic remedies. Control groups and an infected, doxycyline-treated group were included. Efficacy of treatment was evaluated based on the parameters mortality, body weight gain and colibacillosis lesions. In both experiments doxycyline prevented mortality and reduced *E. coli* lesions and stunting. None of the homeopathically treated groups differed significantly with respect to any of the parameters from the non-medicated, infected control group. It is concluded that the results of this study do not justify use of these homeopathic remedies for treatment of colibacillosis in broilers.

Mastitis is the major problem of dairy animals despite a number of preventive and therapeutic approaches. Treatment is costly and out of reach of farmers of developing countries. 'Encouraging' results in cows with acute mastitis were reported, in uncontrolled studies, with low-potency homeopathic medicines like *Aconitum* 4x, *Phytolacca* 1x and *Bryonia* 4x (96). Similar results were reported also by others (97). More recently, two observational studies investigated the treatment economics of homeopathic drugs and conventional drugs for the management of bovine mastitis (98,99). In the second one (99), a reference group (non-randomized) that was treated with antibiotics was also included in the study. In the homeopathy group, 96 mastitic quarters (non-fibrosed 67 and fibrosed 29) were treated with the homeopathic combination medicine *Healwell VT-6* (consisting of *Phytolacca* 200c, *Calcarea fluorica* 200c, *Silicea* 30c, *Belladonna* 30c, *Bryonia* 30c, *Arnica* 30c, *Conium* 30c and *Ipecacuanha* 30c). The overall effectiveness of homeopathic combination medicine in the treatment of acute non-fibrosed mastitis was 86.6% with a mean recovery period of 7.7 days (range 3–28), and total cost of therapy as €0.39, US$ 0.47. The corresponding cure rate for the antibiotic group was 59.2% with a mean recovery period of 4.5 days (range 2–15) and an average treatment cost of €2.69, US$ 3.28. In synthesis, the homeopathic complex medicine may be effective and economical in the management of bovine mastitis, but definitive conclusions are premature due to the design of the studies that did not include radomization and blinding.

Few Randomized Trials

A veterinary randomized clinical trial was carried out in order to evaluate the efficacy of homeopathy in treatment of clinical

mastitis in dairy cows (100). A three-armed, stratified, semi-crossover design comparing classical (individualized) homeopathy, placebo and a standardized antibiotic treatment was used. Significant reductions in mastitis signs were observed in all treatment groups. Two-thirds of the cases both in the homeopathy and placebo groups responded clinically within 7 days. However, homeopathic treatment was not statistically different from either placebo or antibiotic treatment. The latter treatment was significantly better than placebo. Evidence of efficacy of homeopathic treatment beyond placebo was not found in this study, but authors suggested that the design can be useful in subsequent larger trials on individualized homeopathic treatment. Furthermore, the use of a homeopathic nosode in the treatment of bovine mastitis was unsuccessful in a randomized trial (101).

Another study conducted in pigs showed that various combinations of *Lachesis*, *Pulsatilla* and *Sabina*, or *Lachesis*, *Echinacea* and *Pyrogenium*, associated with *Caulophyllum* (all in low dilutions, from 1x to 6x) have prophylactic and therapeutic effects on infections (metritis and mastitis) of sows and on diarrhea of piglets (102). A double-blind, placebo-controlled clinical trial of a homeopathic treatment of neonatal calf diarrhea was performed (103). Calves with spontaneously derived diarrhea were treated with either the homeopathic remedy *Podophyllum* 30x or a placebo. No clinically or statistically significant difference between the two groups was demonstrated in the duration of diarrhea, inappetence and fever.

Knowledge of non-invasive treatments with few or no side effects that have the potential to heal animals should be welcomed, and homeopathy, as well as other complementary therapies, fits this description (104). Homeopathy may offer great advantages in animal farming, particularly for its potential immunostimulating effects, which in turn would reduce the need of chemical treatments, but documentation of these effects is largely insufficient.

Discussion

As in conventional research, animal models are also used in the field of homeopathy both for testing the principle of dilution/dynamization and for studying the possible mechanism of action of homeopathic remedies in a thorough and repeatable manner, as well as for discovering remedies to be used in the veterinary context. The aim of this type of experimental work is to provide a rational approach to the investigation of the various aspect of the 'similia similibus curentur' principle and of the use of high dilutions, in order to construct a plausible framework of ideas capable of facilitating further basic and clinical research into these historical yet also modern medical principles. Acceptance of the homeopathic claims requires supporting evidence of plausible mechanisms and high quality studies exploring its effectiveness in experimental settings.

The Mosaic of Homeopathic Research

The problems raised by homeopathy are very wide and complex, so it is not the matter of a single experiment that can clarify everything of homeopathic theories. 'There is not a single point whose clarification will explain everything, there is no a single ''Nobel-prize''-experiment that could ''prove'' homeopathy.' Instead, the picture of homeopathy is at present going to be clarified like a puzzle or like a mosaic whose pieces are progressively going in the right place, where all the little pieces are important for the realization of the whole image.

The studies referred to here do not cover all the homeopathic literature of animal research but only those dealing with immunity and inflammation. As in all fields of research it is important to establish the goals of experimental studies. Animal studies in homeopathy refer to three main goals, namely (i) preclinical research to test in animals the medicines before their utilization in human beings; (ii) finding new, non-toxic therapies for animals in veterinary research; and (iii) exploring in controlled and reproducible settings specific aspects and mechanisms of action of homeopathic approach. All these goals appear very important and urgent, but none of them can answer the question of whether homeopathy is an effective cure for humans.

Starting from the first point, the preclinical research in homeopathy has virtually never pursued and the situation is inverted with respect to conventional pharmacological research. In fact, homeopathy is founded on human 'provings' and medicines have been employed in humans for two centuries without verification of their effects in animal models. Only in the recent decades, some groups have begun testing in animal models some already commercially available homeopathic medicines, especially low-dose complex formulations. The main studies in immunological and immunopharmacological fields have been here reported and gave, in general, positive results. Even if these medicines are already in the human use, pursuing this line of research in animals could be useful to better identify new fields of pathologies where those medicines could be potentially employed and to improve formulations as regards their constituents and/or their grade of dilution.

As the veterinary research is concerned, the authors of this review have searched literature regarding immunostimulating effects of homeopathy and/or protection from infectious diseases. In addition to Internet databases, books and conference proceedings, we have also directly asked homeopathic veterinary associations and private foundations to provide available literature. Unfortunately, we were able to find only a few articles on these topics. The Carstens Foundation kindly provided a list of veterinary studies but most of them were unpublished university dissertations. So, to the best of our knowledge, the question of the potential utilization of homeopathy in the infectious diseases of domestic animals or in natural farming is still largely unexplored, or unpublished.

The study of the mechanisms of therapeutic action would be the main goal of basic research in homeopathy. Here, two major themes are under investigation, namely, (i) the 'high dilution' effects, i.e. the question whether and how substances diluted (and dynamized) in away that few or no molecules of

active principle may have pharmacological effects; and (ii) the mode of action of the 'simile' effect, i.e. how a substance known to have pathogenetic effects in healthy organisms (cells, animals and humans) may turn to become a therapeutic agent in diseased organisms. These two themes may be in turn split in a number of minor questions regarding the methods of dilution, the most useful model to show the effect, the type of solvent used (distilled water, saline solutions, water/ethanol mixtures) and other technical details that may often affect the final outcomes.

An important point is the distinction between very low dose and high-dilution effects. Both fields fully belong to homeopathy, provided that the medicine is prescribed according to the similia principle and according to a holistic clinical approach. From a scientific standpoint, the mechanism(s) underlying the 'similia law' and the effect of microdoses of drugs can be investigated and understood independently of the so-called 'high dilution' or 'high potency' effect. The similia principle is the foundation of the homeopathy and the conditio-sine-qua-non of its definition. Hahnemann started its system by utilizing low doses of drugs that were available at his time. The principle of 'dynamization' or 'potentization' was established in subsequent years, after a number of experimental trials. Moreover, even now the major portion of the market of homeopathic medicines in Europe is represented by low-dilution medicines containing very low doses of active principles in the molecular range. It is worth noting that homeopathic dilutions in the range of 10x to 20x (5c to 10c) may theoretically contain molecules in a range of extremely low concentrations (10^{-10} to 10^{-20} mol l^{-1}) that have been shown to be biologically active in 'conventional' biochemistry and immunology (2).

Aims and Limits of Animal Models

As noted in the case of *in vitro* studies (6), also the animal research in laboratory is by its nature a reductionistic one: while it should allow better definition of single problems, it loses the ability of evaluating a therapeutic approach in all its integrity. In classical homeopathy, as well as in most other alternative approaches, a holistic view of disease is emphasized, and individual judgement and treatment is important. This implies that when choosing the homeopathic remedy, attention is focused on the totality of the organism as a whole, including personality and behavior, and not merely on symptoms related to the affected organ system. As a consequence, any reductionistic experimental approach must be taken for the scientific advancements that may allow, but not as a 'proof' or a 'prove to be wrong' of homeopathy itself. Therefore, care should be always applied in the interpretation of results from animal studies with respect to possible mechanisms of action of homeopathy. Finding a direct effect of a homeopathic dilution in a cell model or in a animal model of inflammation (e.g. foot edema, skin rash, etc.) may indicate a 'local' effect of the medicine, for example, at the receptor level. However, inflammation has always a complex neurohumoral regulation and indirect or systemic pathways of regulation cannot be excluded.

Most of the studies reported here, including ours, have explored what is conventionally denoted as pharmacological 'activity' on one symptom (e.g. foot swelling). While conventional anti-inflammatory drugs are designed to suppress the symptom pointing to the underlying enzymatic mechanisms (e.g. prostaglandin production), homeopathic treatment is supposed to regulate the pathological excess of inflammation because the phenomenon by itself is seen as an expression of natural healing dynamics (the so-called Hahnemann's 'life force'). According to classical homeopathic theory, an 'anti-edema' effect could not reflect the full potential of the homeopathic treatments of inflammatory diseases.

For example, high dilutions of histamine may act either on basophil regulation of local blood supply or vascular permeability, as shown in above mentioned reports (105), or as a neurotropic drug and central neurotransmitter, as recently reported by others (77,106). The latter report showed that ultradiluted histamine (30c) increases arousal of Wistar rats via H1 receptors and in the EEG lowers the mean delta band spectral density as compared to the control group. Significant differences between histamine 30c and baseline during the first 2 h imply an immediate effect.

High Dilution Effects

As far as the proof of effects of solutions diluted beyond the Avogadro constant are concerned, the studies on animal models appear to parallel those on isolated cells (6): as a matter of facts, there are several reports claiming to have demonstrated significant modifications of some physiological parameter or vitality of animals treated with high potencies of homeopathic medicines or with high dilutions of other pharmacologically active compounds. On the other hand, the state of the art illustrates the preliminary nature of most reports and the lack of independent replications, so that definite conclusions on the efficacy of highly diluted homeopathic medicines in specific conditions would be premature.

Finding a rationale and systematic approach to the high dilution and dynamization phenomenon would also require more knowledge of the physicochemical state of the solution employed. There is a number of theoretical and experimental advances suggesting the existence of peculiar physicochemical states in water, compatible with the hypothesis that structure (and information) can be stored in liquid water (22,67,107–116), while others studies carried out with magnetic resonance spectroscopy could not confirm these models (117,118). Therefore, proofs of the presence of stable structures in water are not so consistent and reproducible as it should be for a general acceptation by the scientific community. Skeptics are not convinced by the available evidence. On the other hand, people with more 'open-minded' position are reinforced in their belief that 'anomalous states' of water and 'condensed matter physics' are giving to high-dilution/high potency homeopathy an increased credibility (119).

Our belief is that many 'high-dilution' experiments point out elusive physicochemical and biological phenomena that really occur in nature, associated with the structural and dynamical properties of water and water/alcohol solution, but they are difficult to be reproduced in laboratory settings (6) because the subtle information possibly associated with ultra-high dilutions can theoretically affect only highly sensitive, far-from equilibrium and unstable systems. As a consequence, they are markedly affected by minimal technical differences and conditions, as postulated by the dynamic systems theory on chaotic phenomena (3,120). Homeopathy used with ultradiluted drugs may be regarded as a new paradigm of communicating biological information in complex systems, a paradigm that has been also designed as 'the body signifier' (121,122). The potential strength of the method consists in the fact that, by using an analogical reasoning (similitude) and information-specific long-range signals of biophysical nature, it attempts to achieve a systemic level of regulation of the body.

The Extensive Phenomenon of Biological 'Similitude'

Several lines of evidence indicate that the above-presented models of 'simile' effects in biological systems are consistent with a large series of experimental data emerging from various fields of modern biomedical research. Both in homeopathic literature and in 'mainstream' immunology, there are a number of examples, both at cellular and at systemic level where the effect of a certain compound can be either positive (e.g. stimulating or priming) or negative (e.g. inhibiting or blocking) depending on the doses employed and on the conditions of the experiments. However, this is only a general indication, because the clarification of the mechanism(s) underlying most of the paradoxical effects reported requires further investigation.

Although many results obtained in animal models are of provisional nature and wait for confirmation, the overall body of literature suggests that the scientific re-evaluation of the principle of similarity is worthy of increasing attention (1,2,123,124). The first reason is that this concept may represent a broad overall frame of reference for theoretical models explaining both a body of empirical observations emerging from old medical literature and the increasing experimental evidence of paradoxical results or of apparently opposite results described by different investigators in fields ranging from molecular biology to immunology and neurobiology. If this general frame of reference gains credibility and is increasingly documented experimentally, some apparent contrasts between empirical medical approaches and mainstream medicine approaches could be reconciled in a rational way.

The second reason why a reappraisal of the principle of similarity appears to be worthy of attention is that it can be used as a 'heuristic principle', i.e. a driving force on the basis of which new experimental ideas are generated in intellectually curious medical investigators. Every investigator dealing with a specific field could design new experiments based on the principle of similarity. A knowledge of the principle of similarity/inverse effects phenomena should encourage a positive and fruitful reappraisal of certain experimental results that may appear at variance with or even opposed to the starting hypothesis. Finding unexpected, controversial and paradoxical results is a common experience in science, but often these results are ignored and discarded because they do not fit the main theories (125). The occurrence of inverse effects according to the general principle of similarity could help and stimulate scientists to subject these data to a positive re-think: they will appear as an expression of the self-recovery phenomena which are typical of complex biological systems. A very recent paper demonstrated that the isopathic approach may have further promising applications also in farming: chickens receiving a treatment made of 30× potency of *Salmonella enterica* were protected from salmanellosis as compared to untreated controls (126).

Finally, the principle of similarity could be re-evaluated as a way of designing therapeutic strategies, according to two main lines, i.e. either by administering the 'simile' as a substance belonging to a known pathogenetic mechanism of the disease or administering the 'simile' as a compound that causes similar symptoms. The first line corresponds to the approach which historically has been called 'isopathy' or 'therapy by nosodes' and whose current updating consists in the utilization of a series of agents that are pathogenic when used at high doses in healthy people and therapeutic when used at low doses in sick people: cytokines, bacterial products, specific antigens, nitric oxide, cancer cells modified by genetic engineering, etc. The second line is followed by classical, individualized, homeopathy. The effectiveness of these approaches in human medicine is under increasing debate and their applications in the immunopharmacological field will be the object of a further publication of this series.

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References

1. Bellavite P, Andrioli G, Lussignoli S, Signorini A, Ortolani R, Conforti A. A scientific reappraisal of the 'principle of similarity'. *Med Hypotheses* 1997;49:203–12.
2. Eskinazi D. Homeopathy re-revisited: is homeopathy compatible with biomedical observations? *Arch Intern Med* 1999;159:1981–7.
3. Bellavite P, Signorini A. The Emerging Science of Homeopathy. Berkeley (CA): North Atlantic, 2002.
4. Teixeira MZ. 'Paradoxical strategy for treating chronic diseases': a therapeutic model used in homeopathy for more than two centuries. *Homeopathy* 2005;94:265–6.
5. Vickers AJ. Independent replication of pre-clinical research in homeopathy: a systematic review. *Forsch Komplementarmed* 1999;6:311–20.
6. Bellavite P, Conforti A, Pontarollo F, Ortolani R. Immunology and homeopathy. 2. Cells of the immune system and inflammation. *Evid Based Complement Alternat Med* 2006;3:13–24.

7. Bastide M. Immunological examples on ultra high dilution research. In: Endler PC, Schulte J (eds). *Ultra High Dilution.* Dordrecht: Kluwer Academic Publishers, 1994, 27–33.
8. Bastide M, Doucet-Jaboeuf M, Daurat V. Activity and chronopharmacology of very low doses of physiological immune inducers. *Immunol Today* 1985;6:234–5.
9. Bastide M, Daurat V, Doucet-Jaboeuf M, Pelegrin A, Dorfman P. Immunomodulatory activity of very low doses of thymulin in mice. *Int J Immunother* 1987;3:191–200.
10. Doucet-Jaboeuf M, Pelegrin A, Cot MC, Guillemain J, Bastide M. Seasonal variations in the humoral immune response in mice following administration of thymic hormones. In: Reinberg A, Smolensky Labrecque M (eds). *Annal Review of Chronopharmacology.* Oxford: Pergamon Press, 1984, 231–4.
11. Weisman Z, Topper R, Oberbaum M, Bentwich Z. . *Proceedings of 5th GIRI Meeting*. Paris, 1991.
12. Bentwich Z, Weisman Z, Topper R, Oberbaum M. Specific immune response to high dilutions of KLH; transfer of immunological information. In: Bornoroni C (ed). *Omeomed92.* Bologna: Editrice Compositori, 1993, 9–14.
13. Weisman Z, Oberbaum M, Topper R, Harpaz N, Bentwich Z. High dilution of antigens modulate the immune response to KLH. In: Bastide M (ed). *Signals Images.* Dordrecht: Kluwer Academic Publishers, 1997, 179–89.
14. Youbicier-Simo BJ, Boudard F, Mekaouche M, Bastide M, Bayle JD. Effects of embryonic bursectomy *in ovo* administration of highly diluted bursin on adenocorticotropic immune responses of chickens. *Int J Immunother* 1993;9:169–80.
15. Youbicier-Simo BJ, Boudard F, Mekaouche M, Bayle JD, Bastide M. Specific abolition reversal of pituitary-adrenal activity and control of the humoral immunity in bursectomized chickens through highly dilute bursin. *Int J Immunopathol Pharmacol* 1996;9:43–51.
16. Youbicier-Simo BJ, Boudard F, Mekaouche M, Bayle JD, Bastide M. A role for bursa fabricii and bursin in the ontogeny of the pineal biosynthetic activity in the chicken. *J Pineal Res* 1996;21:35–43.
17. Youbicier-Simo BJ, Boudard F, Guellati M, Mekaouche M, Bayle JD, Bastide M. The role of the bursa of Fabricius and highly diluted bursin in immunoendocrine interactions in the chicken. In: Bastide M (ed). *Signals and Images.* Dordrecht: Kluwer Academic Publishers, 1997, 121–47.
18. Davenas E, Poitevin B, Benveniste J. Effect on mouse peritoneal macrophages of orally administered very high dilutions of silica. *Eur J Pharmacol* 1987;135:313–9.
19. Jonas WB, Dillner D. Protection of mice from Tularemia infection with ultra-low, serial agitated dilutions prepared from *Francisella tularensis*-infected tissue. *J Scient Explor* 2000;14:35–52.
20. Jonas WB. Do homeopathic nosodes protect against infection? An experimental test. *Altern Ther Health Med* 1999;5:36–40.
21. Oberbaum M, Weisman Z, Kalinkovich A, Bentwich Z. Healing chronic wounds performed on mouse ears using silica (SiO2) as a homeopathic remedy. Pharmacological study of homeopathic high dilutions. In: Bastide M (ed). *Signals Images.* Dordrecht: Kluwer Academic Publishers, 1997, 191–9.
22. Demangeat JL, Demangeat C, Gries P, Poitevin B, Constantinesco A. Modifications des temps de relaxation RMN a 4 MHz des protons du solvant dans les trés hautes dilutions salines de Silice/Lactose. *J Med Nucl Biophy* 1992;16:135–45.
23. Pedalino CM, Perazzo FF, Carvalho JC, Martinho KS, Massoco CO, Bonamin LV. Effect of *Atropa belladonna* and *Echinacea angustifolia* in homeopathic dilution on experimental peritonitis. *Homeopathy* 2004;93:193–8.
24. Sukul NC, Ghosh S, Sinhababu SP. Reduction in the number of infective *Trichinella spiralis* larvae in mice by use of homeopathic drugs. *Forsch Komplementarmed Klass Naturheilkd* 2005;12:202–5.
25. Sato DY, Wal R, de Oliveira CC, Cattaneo RI, Malvezzi M, Gabardo J, et al. Histopathological and immunophenotyping studies on normal and sarcoma 180-bearing mice treated with a complex homeopathic medication. *Homeopathy* 2005;94:26–32.
26. Malling HJ. Comparison of the clinical efficacy and safety of subcutaneous and sublingual immunotherapy: methodological approaches and experimental results. *Curr Opin Allergy Clin Immunol* 2004;4:539–42.
27. Senti G, Johansen P, Martinez GJ, Prinz Varicka BM, Kundig TM. Efficacy and safety of allergen-specific immunotherapy in rhinitis, rhinoconjunctivitis, and bee/wasp venom allergies. *Int Rev Immunol* 2005;24:519–31.
28. Wheeler AW, Woroniecki SR. Allergy vaccines—new approaches to an old concept. *Expert Opin Biol Ther* 2004;4:1473–81.
29. Canonica GW, Compalati E, Fumagalli F, Passalacqua G. Sublingual and oral immunotherapy. *Immunol Allergy Clin North Am* 2004;24: 685–704.
30. Friedman A, Weiner HL. Induction of anergy or active suppression following oral tolerance is determined by antigen dosage. *Proc Natl Acad Sci USA* 1994;91:6688–92.
31. Weiner HL. Oral tolerance: immune mechanisms and treatment of autoimmune diseases. *Immunol Today* 1997;7:336–43.
32. Zhang X, Izikson L, Liu L, Weiner HL. Activation of CD25(+)CD4(+) regulatory T cells by oral antigen administration. *J Immunol* 2001;167: 4245–53.
33. Weiner HL. Oral tolerance: immune mechanisms and the generation of Th3-type TGF-beta-secreting regulatory cells. *Microbes Infect* 2001;3: 947–54.
34. Inobe J, Slavin AJ, Komagata Y, Chen Y, Liu L, Weiner HL. IL-4 is a differentiation factor for transforming growth factor-beta secreting Th3 cells and oral administration of IL-4 enhances oral tolerance in experimental allergic encephalomyelitis. *Eur J Immunol* 1998;28: 2780–90.
35. Weiner HL. The mucosal milieu creates tolerogenic dendritic cells and T(R)1 and T(H)3 regulatory cells. *Nat Immunol* 2001;2:671–2.
36. Slavin AJ, Maron R, Weiner HL. Mucosal administration of IL-10 enhances oral tolerance in autoimmune encephalomyelitis and diabetes. *Int Immunol* 2001;13:825–33.
37. Ciprandi G, Fenoglio D, Cirillo I, Vizzaccaro A, Ferrera A, Tosca MA, et al. Induction of interleukin 10 by sublingual immunotherapy for house dust mites: a preliminary report. *Ann Allergy Asthma Immunol* 2005;95: 38–44.
38. Heine H, Schmolz M. Immunoregulation via 'bystander suppression' needs minute amounts of substances—a basis for homeopathic therapy? *Med Hypotheses* 2000;54:392–3.
39. Conforti A, Lussignoli S, Bertani S, Ortolani R, Verlato G, Bellavite P. Intraperitoneal administration of adjuvant inhibits the development of adjuvant arthritis in rats. *Int J Immunopathol Pharmacol* 1995;8:113–21.
40. Conforti A, Lussignoli S, Bertani S, Verlato G, Ortolani R, Bellavite P, et al. Specific and long-lasting suppression of rat adjuvant arthritis by low-dose *Mycobacterium butyricum*. *Eur J Pharmacol* 1997;324: 241–7.
41. Conforti A, Lussignoli S, Bertani S, Ortolani R, Brendolan A, Cestari T, et al. Suppression of adjuvant arthritis in rats by intraperitoneal *Mycobacterium butyricum*. *J Chemother* 1998;10:169–72.
42. Conforti A, Lussignoli S, Bertani S, Ortolani R, Cuzzolin L, Benoni G, et al. Cytokine and nitric oxide levels in a rat model of immunologic protection from adjuvant-induced arthritis. *Int J Immunopathol Pharmacol* 2001;14:153–60.
43. Naka T, Nishimoto N, Kishimoto T. The paradigm of IL-6: from basic science to medicine. *Arthritis Res* 2002;4: Suppl 3:S233–42.
44. Agnello D, Scanziani E, Di Giancamillo M, Leoni F, Modena D, Mascagni P, et al. Preventive administration of *Mycobacterium tuberculosis* 10-kDa heat shock protein (hsp10) suppresses adjuvant arthritis in Lewis rats. *Int J Immunopharmacol* 2002;2:463–74.
45. Scadding GK, Brostoff J. Low dose sublingual therapy in patients with allergic rhinitis due to house dust mite. *Clin Allergy* 1986;16: 483–91.
46. Trentham DE, Dynesius-Trentham RA, Orav EJ, Combitechi D, Lorenzo C, Sewell KL, et al. Effects of oral administration of type II collagen on rheumatoid arthritis. *Science* 1993;261:1727–30.
47. MacDonald TT. Eating your way towards immunosuppression. *Curr Biol* 1994;4:178–81.
48. Malling HJ. Sublingual immunotherapy. *Clin Exp Allergy* 1996;26: 1228–31.
49. Garcia G, Komagata Y, Slavin AJ, Maron R, Weiner HL. Suppression of collagen-induced arthritis by oral or nasal administration of type II collagen. *J Autoimmun* 1999;13:315–24.
50. Komagata Y, Weiner HL. Oral tolerance. *Rev Immunogenet* 2000;2: 61–73.
51. Teklenburg G, Albani S. The role of immune tolerance in preventing and treating arthritis. *Curr Rheumatol Rep* 2004;6:434–41.
52. Larche M, Wraith DC. Peptide-based therapeutic vaccines for allergic and autoimmune diseases. *Nat Med* 2005;11:S69–76.
53. Escobar A, Lopez M, Serrano A, Ramirez M, Perez C, Aguirre A, et al. Dendritic cell immunizations alone or combined with low doses of

interleukin-2 induce specific immune responses in melanoma patients. *Clin Exp Immunol* 2005;142:555–68.
54. Tamura Y, Peng P, Liu K, Daou M, Srivastava PK. Immunotherapy of tumors with autologous tumor-derived heat shock protein preparations. *Science* 1997;278:117–20.
55. Weiner HL, Friedman H, Miller A, Khoury SJ, Al Sabbagh A, Santos L, et al. Oral tolerance: immunologic mechanisms and treatment of animal and human organ-specific autoimmune diseases by oral administration of autoantigens. *Annu Rev Immunol* 1994;12:809–37.
56. Bellavite P, Conforti A, Piasere V, Ortolani R. Immunology and homeopathy. 1. Historical background. *Evid Based Complement Alternat Med* 2005;2:441–52.
57. Bosch X. No easy answers. *Nature* 2001;413:457–8.
58. Bastide P, Aubin B, Baronnet S. Etude pharmacologique d'une preparation d'Apis mel.(7CH) vis-a-vis de l'erytheme aux rayons U.V. chez le cobayes albinos. *Ann Homeopath Fr* 1975;3:289–94.
59. Bildet J, Guyot M, Bonini F, Grignon MC, Poitevin B, Quilichini R. Mise en évidence des effets de dilutions d'Apis mellifica et d'Apium virus vis-à-vis de l'erythème provoqué par un rayonnement U.V. chez le cobaye (The effect of dilutions of *Apis mellifica* and *Apium virus* on ultraviolet light-induced erythema in the guinea pig). *Ann Pharm Fr* 1989;47:24–32.
60. Bildet J, Guyot M, Bonini F, Grignon MC, Poitevin B, Quilichini R. Demonstrating the effects of *Apis mellifica* and *Apium virus* dilutions on erythema induced by U.V. radiation on guinea pigs. *Berl J Res Hom* 1990;1:28–33.
61. Poitevin B. Est-il possible d'evaluer l'homéopathie. *Homeopathie Franc* 1988;76:93–100.
62. Conforti A, Signorini A, Bellavite P. Effects of high dilutions of histamin and other natural compounds on acute inflammation in rats. In: Bornoroni C (ed). *Omeomed92.* Bologna: Editrice Compositori, 1993, 163–9.
63. Bertani S, Lussignoli S, Andrioli G, Bellavite P, Conforti A. Dual effects of a homeopathic mineral complex on carrageenan-induced oedema in rats. *Br Homeopath J* 1999;88:101–5.
64. Conforti S, Bertani S, Metelmann H, Chirumbolo S, Lussignoli S, Bellavite P. Experimental studies on the anti-inflammatory activity of a homeopathic preparation. *Biomed Ther* 1997;15:28–31.
65. Conforti A, Bertani S, Lussignoli S, Bellavite P. Wirkungen Anti-homotoxischer Präparate auf akute und chronische Entzndungen. *Biologische Medizin* 1998;27:63–6.
66. Lussignoli S, Bertani S, Metelmann H, Bellavite P, Conforti A. Effect of Traumeel S, a homeopathic formulation, on blood-induced inflammation in rats. *Complement Ther Med* 1999;7:225–30.
67. Sukul NC, Sukul A. High Dilution Effects: Physical and Biochemical Basis. Dordrecht: Kluwer Academic Publishers, 2003.
68. Macedo SB, Ferreira LR, Perrazzo LL, Tavarea-Carvalho JC. Anti-inflammatory activity of *Arnica montana* 6cH. Preclinical study in animals. *Homeopathy* 2004;93:84–7.
69. Bonamin LV, Martinho KS, Nina AL, Caviglia F, Do Rio RG. Very high dilutions of dexamethasone inhibit its pharmacological effects *in vivo*. *Br Homeopath J* 2001;90:198–203.
70. Prado Neto JA, Perazzo FF, Cardoso LG, Bonamin LV, Carvalho JC. Action of causticum in inflammatory models. *Homeopathy* 2004;93: 12–6.
71. Doutremepuich C, Aguejouf O, Pintigny D, Sertillanges MN, De Seze O. Thrombogenic properties of ultra-low-dose of acetylsalicylic acid in a vessel model of laser-induced thrombus formation. *Thromb Res* 1994;76:225–9.
72. Doutremepuich C, Aguejouf O, Belon P. Effects of ultra-low-dose aspirin on embolization in a model of laser-induced thrombus formation. *Semin Thromb Hemost* 1996;22: Suppl 1:67–70.
73. Belougne-Malfatti E, Aguejouf O, Doutremepuich F, Belon P, Doutremepuich C. Combination of two doses of acetyl salicylic acid: experimental study of arterial thrombosis. *Thromb Res* 1998;90:215–21.
74. Lalanne MC, Doutremepuich C, De Seze O, Belon P. What is the effect of acetylsalicylic acid at ultra low dose on the interaction platelets/vessel wall? *Thromb Res* 1990;60:231–6.
75. Aguejouf O, Malfatti E, Belon P, Doutremepuich C. Time related neutralization of two doses acetyl salicylic acid. *Thromb Res* 2000;100: 317–23.
76. Eizayaga FX, Aguejouf O, Belon P, Doutremepuich C. Platelet aggregation in portal hypertension and its modification by ultra-low doses of aspirin. *Pathophysiol Haemost Thromb* 2005;34:29–34.
77. Bousta D, Soulimani R, Jarmouni I, Belon P, Falla J, Foment N, et al. Neurotropic, immunological and gastric effects of low doses of *Atropa belladonna* L., *Gelsemium sempervirens* L. and Poumon histamine in stressed mice. *J Ethnopharmacol* 2001;74:205–15.
78. Harish G, Kretschmer M. Smallest zinc quantities affect the histamine release from peritoneal mast cells of the rat. *Experientia* 1988;44:761–2.
79. Callens E, Debiane H, Santais MC, Ruff F. Effects of highly diluted beta2-adrenergic agonists on isolated guinea pig trachea. *Br Homeopath J* 1993;82:123.
80. Hadji L, Arnoux B, Benveniste J. Effect of dilute histamine on coronary flow of isolated guinea-pig heart. *FASEB J* 1991;5:A1583.
81. Benveniste J. Further biological effects induced by ultra high dilutions. Inhibition by a magnetic field. In: Endler PC, Schulte J (eds). *Ultra High Dilution.* Dordrecht: Kluwer Academic Publishers, 1994, 35–8.
82. Benveniste J. Memory of water revisited (letter). *Nature* 1994;370:322.
83. Benveniste J, Arnoux B, Hadji L. Highly dilute antigen increases coronary flow of isolated heart from immunized guinea pigs. *FASEB J* 1992;6:A1610.
84. Litime MH, Aissa J, Benveniste J. Antigen signaling at high dilution. *FASEB J* 1993;7:A602.
85. Cristea A, Nicula S, Dare V. Pharmacodynamic effects of very high dilutions of Belladonna on the isolated rat duodenum. In: Bastide M (ed). *Signals Images.* Dordrecht: Kluwer Academic Publishers, 1997, 161–70.
86. Keatinge R, Gray D, Thamsborg SM, Martini A, Plate P. EU Regulation 1804/1999—the implications of limiting allopathic treatment. In: Hovi M, Trujillo RG (eds). *Diversity of Livestock Systems and Definition of Animal Welfare. Proceedings of the Second NAHWOA Workshop, Córdoba*. Reading (UK): University of Reading 2000. 92–8.
87. Loken T. Alternative therapy of animals—homeopathy and other alternative methods of therapy. *Acta Vet Scand Suppl* 2001;95:47–50.
88. Hammarberg KE. Animal welfare in relation to standards in organic farming. *Acta Vet Scand Suppl* 2001;95:17–25.
89. Hektoen L. Investigations of the motivation underlying Norwegian dairy farmers' use of homoeopathy. *Vet Rec* 2004;155:701–7.
90. Hektoen L. Review of the current involvement of homeopathy in veterinary practice and research. *Vet Rec* 2005;157:224–9.
91. Albrecht H, Schutte A. Homeopathy versus antibiotics in metaphylaxis of infectious diseases: a clinical study in pig fattening and its significance to consumers. *Altern Ther Health Med* 1999;5:64–8.
92. Viksveen P. Antibiotics and the development of resistant micro-organisms. Can homeopathy be an alternative?. *Homeopathy* 2003;92: 99–107.
93. Grandmontagne Y, Riaucourt A. The value of using homeopathy at the end of gestation in the dog pig. In: Bastide M (ed). *Signals Images.* Dordrecht: Kluwer Academic Publishers, 1997, 227–35.
94. Sandoval CH, Morfin LL, Lopez BB. Preliminary research for testing *Baptisia tinctoria* 30c effectiveness against salmonellosis in first and second quality broiler chickens. *Br Homeopath J* 1998;87: 131–7.
95. Velkers FC, te Loo AJ, Madin F, van Eck JH. Isopathic and pluralist homeopathic treatment of commercial broilers with experimentally induced colibacillosis. *Res Vet Sci* 2005;78:77–83.
96. Merck CC, Sonnenwald B, Rollwage H. The administration of homeopathic drugs for the treatment of acute mastitis in cattle. *Berl Munch Tierarztl Wochenschr* 1989;102:266–72.
97. Searcy R, Reyes O, Guajardo G. Control of subclinical bovine mastitis: utilization of a homoeopathic combination. *Br Homeopath J* 1995;84: 67–70.
98. Varshney JP, Naresh R. Evaluation of a homeopathic complex in the clinical management of udder diseases of riverine buffaloes. *Homeopathy* 2004;93:17–20.
99. Varshney JP, Naresh R. Comparative efficacy of homeopathic and allopathic systems of medicine in the management of clinical mastitis of Indian dairy cows. *Homeopathy* 2005;94:81–5.
100. Hektoen L, Larsen S, Odegaard SA, Loken T. Comparison of homeopathy, placebo and antibiotic treatment of clinical mastitis in dairy cows—methodological issues and results from a randomized-clinical trial. *J Vet Med A Physiol Pathol Clin Med* 2004;51:439–46.
101. Holmes MA, Cockcroft PD, Booth CE, Heath MF. Controlled clinical trial of the effect of a homoeopathic nosode on the somatic cell counts in the milk of clinically normal dairy cows. *Vet Rec* 2005;156: 565–7.

102. Both G. Zur prophylaxe und Therapie des metritis-mastitis-agalactie (MMA)—komplexes des schweines mit biologischen arzneimitteln. *Biologische Tiermedizin* 1987;4:39–65.
103. de Verdier K, Ohagen P, Alenius S. No effect of a homeopathic preparation on neonatal calf diarrhoea in a randomised double-blind, placebo-controlled clinical trial. *Acta Vet Scand* 2003;44: 97–101.
104. Vockeroth WG. Veterinary homeopathy: an overview. *Can Vet J* 1999;40:592–4.
105. Belon P, Cumps J, Ennis M, Mannaioni P, Roberfroid M, Sainte-Laud J, et al. Histamine dilutions modulate basophil activation. *Inflamm Res* 2004;53:181–8.
106. Ruiz-Vega G, Poitevin B, Perez-Ordaz L. Histamine at high dilution reduces spectral density in delta band in sleeping rats. *Homeopathy* 2005;94:86–91.
107. Schulte J. Conservation of structure in aqueous ultra high dilutions. In: Endler PC, Schulte J (eds). *Ultra High Dilutions.*. Dordrecht: Kluwer Academic Publishers, 1994, 105–15.
108. Arani R, Bono I, Del Giudice E, Preparata G. QED coherence and the thermodynamics of water. *Int J Mod Phys* 1995;B9:1813–41.
109. Del Giudice E, Galimberti A, Gamberale L, Preparata G. Electrodynamical coherence in water. A possible origin of the tetrahedral coordination. *Mod Phys Lett* 1995;B9:953–61.
110. Schiff M. *The Memory of Water. Homeopathy and the Battle of Ideas in the New Science*. London: Thorsons, 1995.
111. Del Giudice E, Preparata G. Coherence electrodynamics in water. In: Schulte J, Endler C (eds). *Fundamental Research in Ultrahigh Dilution and Homeopathy.* Dordrecht: Kluwer Academic Publishers, 1998, 89–100.
112. Schulte J. Effects of potentization in aqueous solutions. *Br Homeopath J* 1999;88:155–60.
113. Elia V, Niccoli M. Thermodynamics of extremely diluted aqueous solutions. *Ann N Y Acad Sci* 1999;879:241–8.
114. Widakowich J. Pharmacodynamic principles of homeopathy. *Med Hypotheses* 2000;54:721–2.
115. Elia V, Baiano S, Duro I, Napoli E, Niccoli M, Nonatelli L. Permanent physico-chemical properties of extremely diluted aqueous solutions of homeopathic medicines. *Homeopathy* 2004;93:144–50.
116. 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.
117. Aabel S, Fossheim S, Rise F. Nuclear magnetic resonance (NMR) studies of homeopathic solutions. *Br Homeopath J* 2001;90:14–20.
118. Anick DJ. High sensitivity 1H-NMR spectroscopy of homeopathic remedies made in water. *BMC Complement Altern Med* 2004;4:15.
119. Vallance AK. Can biological activity be maintained at ultra-high dilution? An overview of homeopathy, evidence, and Bayesian philosophy. *J Altern Complement Med* 1998;4:49–76.
120. Bellavite P. Complexity science and homeopathy. A synthetic overview. *Homeopathy* 2003;92:203–12.
121. Bastide M, Lagache A. A communication process: a new paradigm applied to high-dilution effects on the living body. *Altern Ther Health Med* 1997;3:35–9.
122. Bonamin LV. Experimental illustrations of body signifiers theory. In: Halm RP (ed). *Les Médecines Non-conventionelles: une nouvelle approche de la santé.*. Monaco: Les Entretiens Internationaux, 2005, 195–207.
123. Van Wijk R, Wiegant FA. The similia principle as a therapeutic strategy: a research program on stimulation of self-defense in disordered mammalian cells. *Altern Ther Health Med* 1997;3:33–8.
124. Teixeira MZ. Similitude in modern pharmacology. *Br Homeopath J* 1999;88:112–20.
125. Bond RA. Is paradoxical pharmacology a strategy worth pursuing? *Trends Pharmacol Sci* 2001;22:273–6.
126. Berchieri A, Turco WCP, Paiva JB, Oliveira GH, Sterzo EV. Evaluation of isopathic treatment of Salmonella enteritidis in poultry. *Homeopathy* 2006;95:94–97.

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