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The history of the Memory of Water

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'Homeopathic dilutions' and 'Memory of Water' are two expressions capable of turning a peaceful and intelligent person into a violently irrational one,' as Michel Schiff points out in the introduction of his book 'The Memory of Water'. The idea of the memory of water arose in the laboratory of Jacques Benveniste in the late 1980s and 20 years later the debate is still ongoing even though an increasing number of scientists report they have confirmed the basic results.

This paper, first provides a brief historical overview of the context of the high dilution experiments then moves on to digital biology. One working hypothesis was that molecules can communicate with each other, exchanging information without being in physical contact and that at least some biological functions can be mimicked by certain energetic modes characteristics of a given molecule. These considerations informed exploratory research which led to the speculation that biological signaling might be transmissible by electromagnetic means. Around 1991, the transfer of specific molecular signals to sensitive biological systems was achieved using an amplifier and electromagnetic coils. In 1995, a more sophisticated procedure was established to record, digitize and replay these signals using a multimedia computer. From a physical and chemical perspective, these experiments pose a riddle, since it is not clear what mechanism can sustain such 'water memory' of the exposure to molecular signals. From a biological perspective, the puzzle is what nature of imprinted effect (water structure) can impact biological function. Also, the far-reaching implications of these observations require numerous and repeated experimental tests to rule out overlooked artifacts. Perhaps more important is to have the experiments repeated by other groups and with other models to explore the generality of the effect. In conclusion, we will present some of this emerging independent experimental work. *Homeopathy* (2007) **96,** 151–157.

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Historical overview: the early history of high dilution experiments

Presenting a brief history of what is known as the 'Memory of Water' is not an easy task mainly because one of the main actors, Jacques Benveniste, is no longer with us (Figure 1). There are always many controversies around cutting edge science, and especially with those whose lives have been spent pursuing unorthodox trails.

I first met Benveniste during a FASEB meeting in Atlanta in 1981 and joined his laboratory a few years later to set up my own Immunology team. I had the good fortune of being able to collaborate with him for over 16 years. At that time, he was at the top of his fame and gained an international reputation as a specialist on the mechanisms of allergies and inflammation with his discovery of the 'Platelet Activating

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Figure 1 Jacques Benveniste 1935–2004.

Factor' (paf-acether) in 1970.[1,2] Throughout his long career, working both in the US and in France, he was responsible for the development of new ways of approaching inflammation including the patenting by the French National Institute of Health and Medical Research (INSERM) of his innovative allergy test using blood cells called basophils (FR-patent-7,520,273). Jacques' research into allergies took him deep into the mechanisms which create such responses: understanding how the smallest amount of a substance affects the organism. The life and work of Jacques Benveniste was not only written in water.

In the early 1980s, while heading up the unit INSERM 200, Jacques took a new member onto his staff, a young medical doctor, Bernard Poitevin, whose side-interest was homeopathy. 'He asked me if he could try my basophil degranulation test on some homeopathic preparations', Jacques recalled, 'and I remember distinctly saying "OK, but all you will be testing is water".' Thus, Jacques expressed his skepticism but accepted the proposal.

After 5 years of research they empirically observed that highly dilute (i.e., in the absence of any physical molecule) biological agents nevertheless triggered the relevant biological systems. Intrigued but cautious, Jacques was a man who adhered to the facts. He ordered a two-year long series of retests, but the same results kept recurring. Finally, Poitevin and Benveniste submitted two papers which were published in peer review journals.[3,4] Here, the work was treated as conventional research like many other manuscripts from peer-reviewed journals which can be found in the scientific literature on the effect of high dilutions (HD) (review in[5,6]).

Following accepted scientific practice, Jacques then asked other laboratories to try to replicate the findings. In 1988, scientists from six laboratories in four countries (France, Canada, Israel and Italy) co-authored an article showing that highly diluted antibodies could cause basophil degranulation. This was established under stringent experimental conditions such as blind double-coded procedures. Further, the experimental dilution (anti-IgE) and the control one (anti-IgG) were prepared in exactly the same manner, with the same number of dilution and agitation sequences. The article was submitted to *Nature*.[7] *Nature*'s referees could not fault Benveniste's experimental procedures but could not comprehend his results. How can a biological system respond to an antigen when no molecules of it can be detected in the solution? It goes against the accepted 'lock-and-key' principle, which states that molecules must be in contact and structurally match before information can be exchanged. In the paper, Jacques suggested that specific information must have been transmitted during the dilution/shaking process via some molecular organization occurring in the water.

Finally, the editor of the journal, John Maddox agreed to publication, on condition that a 'committee' could verify Benveniste's laboratory procedures. In July 1988, after two weeks after publication, instead of sending a committee of scientific experts, Maddox recruited—James Randi, a magician, and Walter Stewart, a fraud investigator. The three of them spent 5 days in the laboratory. Well, you all know what followed. *Nature*'s attempted debunking exercise failed to find any evidence of fraud. Nevertheless, they concluded that Benveniste had failed to replicate his original study.[8] This marked the beginning of the 'Water Memory' war, which placed him in a realm of 'scientific heresy'. As Michel Schiff later remarked in his book: 'INSERM scientists had performed 200 experiments (including some fifty blind experiments) before being challenged by the fraud squad. The failure to reproduce[8] only concerned two negative experiments'.[9] Benveniste replied to *Nature*[10] and reacted with anger, 'not to the fact that an inquiry had been carried out, for I had been willing that this be done… but to the way in which it had been conducted and to the implication that my team's honesty and scientific competence were questioned. The only way definitely to establish conflicting results is to reproduce them. It may be that we are all wrong in good faith. This is not crime but science…'.

As a consequence of the controversy that ensued, Jacques became increasingly isolated. Nonetheless the team repeated the work on a larger scale, entirely designed and run under the close scrutiny of independent statistical experts, and confirmed the initial findings in *Nature*.[11] These further experiments have been coolly received or ignored by most scientists at least partly because, given Jacques' now-acrimonious relationship with *Nature*, they were published in a less renowned journal.

To date, since the *Nature* publication in 1988, several laboratories have attempted to repeat Benveniste's original basophil experiments. Most importantly, a consortium of four independent research laboratories in France, Italy, Belgium, and Holland, led by M. Roberfroid at Belgium's Catholic University of Louvain in Brussels, confirmed that HD of histamine modulate basophil activity. An independent statistician analyzed the resulting data. Histamine solutions and controls were prepared independently in three different laboratories. Basophil activation was assessed by flow-cytometric measurement of CD63 expression (expressed on cytoplasmic granules and on the external membrane after activation). All experiments were randomized and carried out under blind conditions. Not much room, therefore, for fraud or wishful thinking. Three of the four labs involved in the trial reported statistically significant inhibition of the basophil degranulation reaction by HD of histamine as compared to the controls. The fourth lab gave a result that was almost significant. Thus, the total result over all four labs was positive for histamine HD solutions.[12,13] 'We are,' the authors say in their paper, 'unable to explain our findings and are reporting them to encourage others to investigate this phenomenon'.

Different attempts have been made to substantiate the claim that serial dilution procedures are associated with changes in the water's physical properties ([14,15]and see Louis Rey contribution in this issue pages 170–174). Yet, the challenge of understanding the mechanisms of how HDs work, and the role of water in them, is a difficult one to say the least. Several possible scenarios have been suggested. One proposed by Giuliano Preparata and Emilio Del Giudice, is that long range coherent domains between water molecules (quantum electrodynamics, QED) gives high dilution laser-like properties.[16,17] When the field matches the kinetic of the reaction, the latter becomes functional as the optimal field strength as for a radio receiver. It was to a scientific meeting in Bermuda that took place a few months before the *Nature* 'affair' erupted that these two physicists working at Milan University brought the theoretical basis for the memory of water. Another scenario predicts changes in the water structure by forming more or less permanent clusters.[18] Other hypotheses will be discussed in this issue. High dilution experiments and memory water theory may be related, and may provide an explanation for the observed phenomena. As M. Schiff points out, only time and further research will tell, provided that one gives the phenomena a chance.[9]

Exploring the physical nature of the biological signal

Despite the difficulties after the *Nature* fracas, Jacques and his now-depleted research team continued to investigate the nature of the biological activity in high dilutions and aimed at understanding the physical nature of the biological signal. In his *Nature* paper, Jacques reasoned that the effect of dilution and agitation pointed to transmission of biological information via some molecular organization going on in the water. The importance of agitation in the transmission of information was explored by pipetting dilutions up and down ten times and comparing with the usual 10-s vortexing. Although the two processes resulted in the same dilution, basophil degranulation did not occur at HD after pipetting. So transmission of the information depended on vigorous agitation, possibly inducing a submolecular organization of water or closely related liquids (ethanol and propanol could also support the phenomenon). In contrast, dilutions in dimethylsulphoxide did not transmit the information from one dilution to the other. In addition, heating, freeze-thawing or ultrasonication suppressed the activity of highly diluted solutions, but not the activity of several active compounds at high concentrations. A striking feature was that molecules reacted to heat according to their distinctive heat sensitivity, whereas all highly diluted solutions ceased to be active between 70 and 80 °C. This result suggested a common mechanism operating in HDs, independent of the nature of the original molecule. In addition, in 1991 and in collaboration with an external team of physicists (Lab. Magnetisme C.N.R.S.-Meudon Bellevue, France), it was shown in twenty four blind experiments that the activity of highly dilute agonists was abolished by exposure to a magnetic field (50 Hz, 15×10^{-3} T, 15 min) which had no comparable effect on the genuine molecules. Moreover, it is worth pointing out that a growing number of observations suggest the susceptibility of biological systems or water to electric and low-frequency electromagnetic fields.[19–21] In addition, what is suggested from the literature is a possible role of electromagnetic fields regarding informational process in cell communication.[22–24]

At this stage, Jacques hypothesized that transmission of this ordering principle was electromagnetic in nature and move on to the idea that molecules could communicate via specific electromagnetic waves. If so, what molecule vibration modes are efficient and how can these signals be used to mimic some of the biological functions of a molecule without its physical presence?

From high dilution to digital biology

It was at the beginning of the nineties that a homeopathic physician, E. Attias convinced Jacques to try out an electrical device that he claimed transmitted chemical information. After a few positive trials with this machine, Jacques had another one built, which was used for later experiments. This second

device was essentially a standard audio amplifier that, when connected to another coil, behaves as an audio-frequency oscillator. Between 1992 and 1996, we performed a number of experiments showing that we could transfer, in real time, molecular signals indirectly to water or directly to cells. Briefly, cells were placed in a 37 °C humidified incubator on one coil attached to the oscillator, while an agonist (or vehicle as control) was placed on another coil at room temperature. Here, the transfer was not a two step-process, as when water acts as an intermediary recipient of the molecular signal. In one such exploration, we showed that molecular signals associated with a common phorbol ester (phorbol-myristate-acetate) could be transmitted by physical means directly to human neutrophils to modulate reactive oxygen metabolite production. In 1996, I submitted an article about these experiments to several prestigious journals. The article was flatly rejected each time, on the grounds that we could not explain the underlying mechanism, in spite of the referees' general opinions that our work was 'state-of-the-art' and was 'provocative and intriguing and we have gone to great lengths to try to eliminate any biological variables that could bias our results.' It was finally published in 2000.[25] Appended to this article were two affidavits, one from a French laboratory (F. Russo Marie, INSERM U332, Paris, France) testifying that they supervised and blinded the experiments we did in this laboratory; the other from an US laboratory (W. Hsueh, Department of Pathology, Northwestern University, Chicago) testifying that they did some preliminary experiments similar to ours, without any physical participation on our part, and detected the same effect.

Because of the material properties of the oscillator and the limitations of the equipment used, it is most likely that the signal is carried by frequencies in the low kilohertz range.[26] These considerations led to the establishment in 1995 of a more sophisticated procedure for the recording and retransmission of the molecular signals. DigiBio, a company that Jacques had set up in 1997 to finance his research, obtained in 2003 an approval for one of his French patents by the *US Patent Office* (6,541,978: method, system and device for producing signals from a substance biological and/or chemical activity). The characteristics of the equipment are described in Figure 2 and in.[26] Briefly, the process is to first capture the electromagnetic signal from a biologically active solution using a transducer and a computer with a sound card. The digital signals are stored (Microsoft sound files *.wav). The signal is then amplified and 'played back', usually for 10 min, from the computer sound card to cells or organs placed within a conventional solenoid coil. The digitally recorded signals can also be played back into untreated water, which thereafter will act as if the actual substance was physically present.

From 1995 to the present, several biologically active molecules (eg histamine, acetylcholine, caffeine, PMA, Melagatran... even homeopathic medicines such as *Arnica montana*) have been recorded, digitized and replayed to biological systems sensitive to the original molecular substance. Several biological models were used. The first one was a commonly used system by pharmacologists, called the Langendorff preparation. By injecting different vasoactive substances into the coronary artery of an isolated, perfused guinea pig

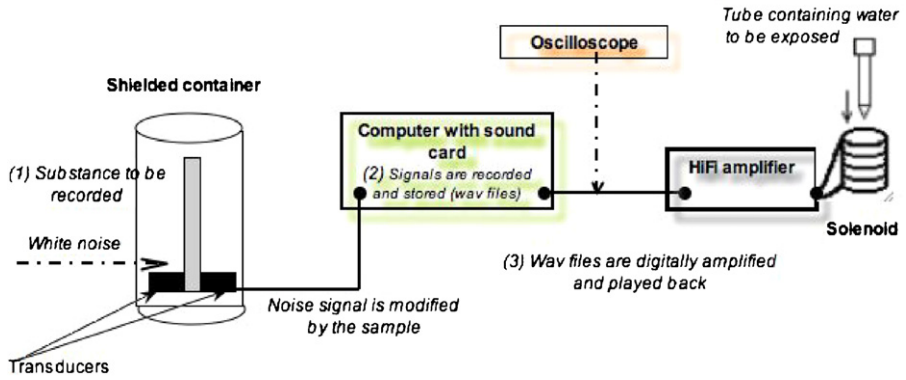

Figure 2 Schematic drawing of the computer-recorded signals: capture, storage and replay:

- *Shielded cylindrical chamber*: Composed of three superposed layers: copper, soft iron, permalloy, made from sheets 1 mm thick. The chamber has an internal diameter of 65 mm, and a height of 100 mm. A shielded lid closes the chamber.
- *Transducers*: Coil of copper wire, impedance 300 Ω, internal diameter 6 mm, external diameter 16 mm, length 6 mm, usually used for telephone receivers.
- Multimedia computer (Windows OS) equipped with a sound card (5–44 KHz in linear steps), (Sound Blaster AWE 64, CREATIVE LABS).
- HiFi amplifier 2 × 100 watts with an 'in' socket, an 'out' socket to the speakers, a power switch and a potentiometer. Pass band from 10 Hz to 20 kHz, gain 1–10, input sensitivity +/− V.
- *Solenoid coil*: Conventionally wound copper wire coil with the following characteristics: internal diameter 50 mm, length 80 mm, $R = 3.6\,\Omega$, three layers of 112 tums of copper wire, field on the axis to the centre 44×10^{-4} T/A, and on the edge 25×10^{-4} T/A.

All links consist of shielded cable. All the apparatus is earthed.

heart and measuring the coronary flow, you can quantify the vasoconstricting or vasodilating effect of the agent. In typical experiments, the signal of acetylcholine (or water as control), a classical vasodilating molecule was recorded and digitized. The signal was then amplified and 'played' back onto water. The signal-carrying water is then injected into the isolated heart, and consequently the coronary flow increased. Interestingly, atropine, an acetylcholine inhibitor, inhibited both the effects of the molecular acetylcholine as well as the digital signal of acetylcholine. Of note, the order of the conditions and their repetitions was always randomized and blinded. Other models include: human neutrophil activation; detection of the recorded signal of bacteria (*E. Coli* and *Streptococcus*) by playing them to a biological system specific to the bacterial signal and; the inhibition of fibrinogen coagulation by a Direct Thrombin Inhibitor. Further details of three of these salient biological models have been previously described.[26] Together, these results suggested that at least some biologically active molecules emit signals in the form of electromagnetic radiation at a frequency of less than 44 kHz that can be recorded, digitized and replayed directly to cells or to water, in a manner that seems specific to the source molecules.[26]

Assuming that we give credence to the phenomena described, one question naturally springs to mind: what do molecule vibration modes sound like? Can measurable signals been identified in the form of low frequency spectral components? Didier Guillonnet, an engineer in computer science, and at the time, a close collaborator of Jacques Benveniste admitted, 'When we record a molecule such as caffeine, for example, we should get a spectrum, but it seems more like noise. We are only recording and replaying; at the moment we cannot recognize a pattern although the biological systems do.' Jacques called this matching of broadcast with reception 'co-resonance,' and said it works like a radio set.

Among the various theoretical problems associated with such a signal, two appear particularly relevant. First, how is such information using water as an intermediary detected amongst much electromagnetic noise? In fact, it has been suggested that stochastic resonance is an important mechanism by which very weak signals can be amplified and emerge from random noise.[27] Second, the limitations of the equipment used here, suggest that the signal is carried by frequencies in the low kilohertz range, many orders of magnitude below those generally associated with molecular spectra (located in the infrared range). However, molecules may also produce much lower 'beat' frequencies (Hz to kHz) specific for every different molecule. The 'beat frequency' phenomenon may explain this discrepancy, since a detector, for instance a receptor, will 'see' the sum of the components of a given complex wave.[28] Clearly, more experimental and theoretical work is needed in order to unveil the physical basis of the transfer (and storage?) of specific biological information either between interacting molecules or via an electronic device.

Replicability: Although since the very beginning we have placed a great deal of emphasis on carrying out our work under the highest standards of methodology and that great effort has been made to isolate it from environmental artifacts, attempts to replicate these data in other laboratories yielded mixed results. For instance, in 1999, Brian Josephson, Nobel Laureate for Physics in 1973 invited Benveniste to the Cavendish Laboratory in Cambridge. He said, 'We invited him to learn more about the research which seems both scientifically interesting and potentially of considerable practical importance. Jacques definitely recognized there was a problem with reproducing the effect. The situation seemed to be that in some circumstances you had reproduction and in others you didn't; but the overall results were highly significant.' We then realized the difficulty in 'exporting' a method, which is very far from conventional biology. There are many key variables that might be involved like, water purification, the container shape and material being used, the purity of chemicals, atmospheric conditions…. Only if these underlying variables are known could the experiments be reproducible. When the transfer is a two-step process using water as an intermediary support for transmitted molecular signals, it takes even more stringent conditions for the experiments to be repeatable. The digital signal is replayed onto the water, which may take or not take the signal depending, for instance, upon the local electromagnetic conditions. In this regard, it is interesting to note that the 'informed water' as in the HD experiments, loses its activity after heating or being exposed to magnetic fields.

More surprising and mysterious was the fact that in some cases certain individuals (not claiming special talents) consistently get digital effects and other individuals get no effects or perhaps block those effects (particularly when handling a tube containing informed water). The inhibition of fibrinogen–thrombin coagulation by a digitized thrombin inhibitor is a model particularly sensitive to experimenter effects and therefore may account for the difficulty in consistently replicating this experimental system. Despite the precautions taken to shield the information transfer equipment from magnetic or electromagnetic pollution, very little concern has been given to possible subtle human operator effects.[29] We dealt with this problem in some of our own studies and also in the course of one independent replication.[30]

The present situation

Now that Jacques Benveniste is no longer with us, the future of the 'digital biology' is in the hands of those who have been convinced of the reality of the

basic phenomena. It is up to them to explore with other models the generality of the effect. Most likely they will succeed if they combine full biological and physical skills to understand the nature of the biological signals.

In this regard, since June 2005, Luc Montagnier, the co-discoverer of HIV, is conducting experiments (detection of the recorded signals of various micro-organisms derived from human pathologies) which, confirm and extend the original finding. In 2006, he set up a company called Nanectis. Perhaps the most impressive emerging data is from a US group located in La Jolla, CA.

In barely four years, they have conducted novel research programs and expanded the original technology into a series of potential industrial applications. Since 2004, they have obtained several *US patents* (6,724,188; 6,952,652; 6,995,558; 7,081,747) and applied for *International Patents* (WO 06/015038: system and method for collecting, storing, processing, transmitting and presenting very low amplitude signals; WO 06/073491: system and method for producing chemical or biochemical signals). They can improve the molecular signal recording in particular by using both magnetic and electromagnetic shielding coupled to a superconducting quantum interference device (SQUID). The system records a time-series signal for a compound; the wave form is processed and optimized (selected noise amplitude, power setting...) to identify low-frequency peaks that are characteristic of the molecule being interrogated (Molecular Data Interrogation System, MIDS). The optimized signal is played back for various periods of time to sensitive biological systems. For instance, they describe one interesting model particularly relevant to the specificity of the molecular signal transmission effect. The arabinose-inducible bacterial system with a lac operon is inducible by signals from the L (+) arabinose form but not from the D (−) arabinose inactive isomer or the white noise control. Other systems include digital herbicides and plant growth regulator as well as pharmaceutical compounds such as Taxol ®, a prototype for a class of anticancer drugs. For instance, in a classic *in vivo* mouse xenograft model, the digital Taxol was assessed by the growth inhibitory potential of a human breast tumor. The results revealed that tumor growth, by day 36, was as statistically significantly inhibited in the group treated with the Taxol signal, as it was in the control group treated with actual molecular Taxol. If these new experimental observations can be validated, we will have added yet another valuable piece to the puzzle.

Although a theoretical explanation of how the memory of water might work must still be explored, the fact that the effective transmission of molecular signals has now been observed by independent teams using different biological systems, provides a strong additional basis to suggest that the phenomena observed by Jacques were not due simply to laboratory artefacts.

Whatever knowledge ongoing and future investigation may bring, the difficult road that Jacques travelled by opposing the automatic acceptance of received ideas, will have contributed to sustaining freedom in scientific research and putting the emphasis back where it belongs, on observable fact.

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