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BIOLOGICAL EFFECTS OF PHYSICALLY CONDITIONED WATER

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Abstract—Physically conditioned water, made by passage between the poles of a magnet or by injecting a weak electrical signal, is used for the prevention and removal of lime-scale, but also has diverse biological effects. In this investigation, we have shown both stimulations and inhibitions of the multiplication of yeast cells, depending on the degree of conditioning. Weakly conditioned water is stimulatory, but strongly conditioned water is inhibitory. Conditioned water also increases the toxicity of heavy metal ions such as copper and cobalt. We suggest that these effects are due to colloidal impurities, which have been activated by the conditioning process, interacting with structural calcium in the cell membranes to make them more permeable. The stimulations may be due to the inward leakage of small amounts of calcium to stimulate metabolism by acting as a "secondary messenger". The inhibitions may be due to more severe damage to the membranes allowing the entry of larger and more toxic quantities of calcium and, if present, other noxious materials. This is discussed in relation to the beneficial and adverse effects of conditioned water on the growth and well-being of higher organisms. Possible applications include stimulating the growth of organisms at optimum levels of conditioning, inhibiting unwanted microorganisms at higher levels and increasing the efficacy of biocides. Attention is also drawn to a similarity between the biological effects of conditioned water and those of weak electromagnetic fields. We discuss the possibility that some of the effects of electromagnetic fields on living organisms are due to their interacting with colloidal cell components and membrane surfaces by a mechanism analogous to the conditioning of water. This is discussed in relation to ion cyclotron resonance phenomena and an explanation based on our proposals given for the hitherto unexplained differences in biological responses to the resonant frequencies for calcium and potassium ions. © 1999 Elsevier Science Ltd. All rights reserved

Key words—biocides, calcium, cyclotron resonance, electromagnetic-fields, growth, membranes, permeability, water-treatment, yeast

INTRODUCTION

Magnetic water conditioners

Physically conditioned water has often been reported to reduce or prevent lime-scale forming in boilers and pipes and to soften and remove scale which has already formed (Donaldson and Grimes, 1988). Much of the original work was done in the Soviet Union, but met initially with skepticism among Western scientists because it involved no more than making the water flow between the poles of a magnet, the energy imparted was orders of magnitude less than its thermal energy (kT), and there was no obvious mechanism for the effect. Nevertheless, the treatment often seemed to work and a thriving industry grew up manufacturing conditioning devices. There has since been a considerable amount of scientific investigation, and there is now a large body of literature on the subject which has been comprehensively reviewed by Baker and Judd (1996). They compare the various designs of conditioner and the effects that conditioning has on the physico-chemical properties of the water, which include changes in the ζ-potential and rate of coagulation of suspended colloids, as well as its ability to reduce scale-formation. They conclude that although there is considerable variation in the efficacy of different conditioners, there is little doubt that at least some of them work when used under the right conditions. However, not all water supplies are amenable to conditioning and the effects of the conditioning process on them may be variable. Present evidence suggests that this is because it is not the water itself which is being conditioned, but the colloids and ions which are present as impurities. Since these vary in different water supplies, it is best to regard conditioned water as a family of substances whose physico-chemical properties have been changed by the conditioning process, but which are not necessarily identical.

The mechanism by which conditioners work is still controversial. Baker and Judd (1996) list several alternatives, most of which involve some form

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of activation of suspended colloids to make them better nuclei for the crystallisation of the salts which normally form lime-scale. When the water is heated, these salts crystallise on the activated particles rather than on the heated surface and remain in suspension instead of forming hard scale. These hypotheses explain the apparent requirement for a "two-phase" system containing colloidal impurities to give convincing scale-reduction (Baker and Judd, 1996) and why there are changes in the crystal structure of the precipitated scale when the water has been conditioned (Donaldson and Grimes, 1988).

How, in this case, are the colloids activated? Probably the most plausible explanation was given by Gamayunov (1983). He calculated that when water is made to flow at 2 m/s through a 0.1 T field (as in a typical magnetic conditioner) the Lorentz forces generated are sufficient to cause a distortion of the electrical double layer of ions which normally surrounds suspended colloidal particles. These forces, which act orthogonally to the direction of flow and the direction of the magnetic field, drive oppositely-charged ions in opposite directions. This results in an exchange of ions between the inner stable Stern layer and the outer diffuse layer to give a metastable change in the ionic composition in the Stern layer, which may last for days. This effect is maximal where the forces are acting at right angles to the surface of the particle and results in localised areas having a different (and perhaps even reversed) surface potential. This explains the changes and reversals in ζ-potential observed by several workers (see Baker and Judd, 1996) when colloidal suspensions are magnetically conditioned [ζ-potential approximates to the difference in potential between the surface of the Stern layer and the bulk of the suspending medium (Shaw, 1992)]. It also explains the changes in the coagulation-rate of lyophobic colloids seen in many studies following magnetic treatment (see Baker and Judd, 1996), since their stability depends on the forces of repulsion between similarly charged particles. With a slight extension, it can also explain how magnetically treated particles become better crystal nuclei for scale-reduction. A heterogeneous and perhaps partially reversed distribution of charge over their surface resulting from conditioning should make the particles better able to attract both the anions and the cations needed for the crystallisation of salts.

A problem with Gamayunov's hypothesis, and any others which depend on Lorentz forces, is that it is not immediately obvious how water can also be conditioned when held *stationary* in a magnetic field, as reported by Higashitani *et al.* (1992) in their coagulation studies. However, this need not be a serious objection, since even in these circumstances, the colloidal particles are still in random Brownian motion, and over a prolonged period, some might be expected to acquire sufficient kinetic energy to "condition" themselves. This may explain why Higashitani *et al.* (1992) found that it took 10 min to fully condition water held static in a 0.4 T field, whereas a moving stream is normally conditioned in a fraction of a second.

Electrical water conditioners

The work of Higashitani *et al.* (1992), in which water was held static for differing periods in a magnetic field, suggests that there is an upper limit for the conditioning effect, but it is doubtful if most commercial flow-through magnetic conditioners achieve this since, according to Baker and Judd (1996), best results with scale-reduction are normally obtained in systems where the water is recirculated repeatedly through the conditioner. In theory, it is difficult to obtain high degrees of conditioning with simple commercial flow-through magnetic conditioners since the strengths of the magnets are limited for economic reasons (they are typically in the region of 0.01–0.5 T) and any attempt to increase the electrical forces generated by increasing the flow-rate is offset by the greater volume of water which then has to be conditioned.

Electrical water conditioners avoid this difficulty by applying an alternating or pulsed electromagnetic field via an "antenna" wound around the supply pipe. The result appears to be the same, but because the conditioning effect is generated by a moving electromagnetic field rather than by the motion of the water though a static field, it no longer depends on a rapid flow-rate. In fact, the level of conditioning is now *increased* at low flow-rates, which give a greater residence time in the conditioner, as noted by Lédion *et al.* (1980) who studied its effects on ζ-potential, and the highest levels of conditioning used in our own experiments were actually obtained by holding the water stationary within the device.

The wave-forms used in electrical conditioners vary, but they are frequently sharp pulses or audio-frequency square waves, which are rich in harmonics, often extending well into the radio-frequency spectrum. Fourier analysis shows that a square wave behaves as a sine wave of the same frequency plus all its harmonics. If proof be needed, place a radio set tuned between stations on the long or medium wave-band next to such a conditioner and you will almost certainly hear it! Sometimes, multiple frequencies may also be used in the belief that conditioning may be enhanced at particular frequencies which resonate with the particles to be "conditioned" (we will discuss this later). The "Water King" used in our experiments generated a square wave with a continually changing frequency in the audio range supplemented by a short burst of 100 kHz damped radio-frequency (RF) oscillations at the beginning of each half cycle. The RF component of the signal may be quite important, since the rapidly moving RF field should compensate for

the much lower field-strengths employed in these devices [about 2 μT for a "Water King" (data supplied by the manufacturer)] and is supported by the evidence of Chibowski *et al.* (1994) who showed that RF signals affect the ζ-potentials of colloidal calcium carbonate. This knowledge helped us design the water conditioner for our laboratory experiments. It was based on the "Water King", but to avoid possible inconsistencies in the results due to its variable audio-frequency, we took just the 100 kHz sinusoidal component and supplied it as a series of repeating pulses to the material to be conditioned. In our experiments, it worked at least as well as the "Water King".

Biological effects of conditioned water

Apart from its action on lime-scale, conditioned water also has many biological effects. Much of the early work on this was done in Russia with water which had been *magnetically* conditioned. Presman (1970) quotes investigations by Dardymov in 1965 who obtained significant stimulations of around 50% in the growth of sunflower, corn and soybean seedlings when watered with conditioned water. More recently, studies in Israel by Harari and Lin (1989) have confirmed the effect with muskmelon, where there were also changes in plant morphology and an increase in the yield and quality of the crop. Preliminary investigations in our own laboratory with a "Polar" magnetic conditioner revealed a 10% reduction in the time taken for dormant parsnip seeds to germinate when they were imbibed in conditioned tap water (Goldsworthy and Lagoa, unpublished). There are also reports of apparently beneficial effects on animals, for example, Lin and Yotvat (1988) report increased milk-yields from cows and more rapid growth-rates and generally improved health in farm animals drinking magnetically conditioned water. Since these effects require energy inputs many orders of magnitude greater than that supplied to the water in the conditioning process, it has to be acting by stimulating natural control mechanisms, rather than by supplying energy to the organism. Also, not all effects of conditioned water are beneficial, for example Patterson and Chestnutt (1994) found that conditioned water inhibited the rate of increase in carcass weight of lambs. In these experiments the water was conditioned *electrically* at a very slow flow-rate (no faster than the lambs could drink) and should therefore been highly conditioned (see Lédion *et al.*, 1980). Also, water which had been treated for 1–8 h in a *static magnetic field*, and should again have been highly conditioned (see Higashitani *et al.*, 1992), caused visible liver-damage in catfish (Garg *et al.*, 1995).

The rationale for our own studies was to see if these apparently conflicting results could be due to different levels of conditioning, and also to test our hypothesis that conditioned water produces its biological effects by increasing membrane permeability by a mechanism analogous to its effects on scale reduction. An increase in permeability was predicted on the grounds that biological membranes are stabilised by a monolayer of calcium ions which cross-link the phosphate moieties of the phospholipid bilayer. If the putative activated colloids from the conditioned water either removed some of these calcium ions or became semipermanently attached to them, a partial disruption of membrane structure might be expected with a consequent increase in permeability. We chose bakers yeast (*Saccharomyces cerevisiae*) as our model system because, being a microorganism, its surface area to volume ratio is extremely large, so any changes in membrane permeability should have a major metabolic effect and, because it is a eukaryote, any findings should also have relevance to higher organisms.

MATERIALS AND METHODS

Culture media were made with London tap water. Magnetic stirrers were not used at any stage, because they might also condition the water in the controls. The media were not autoclaved because the conditioning effect does not survive high temperatures. Contamination with other microorganisms was minimised by using a large inoculum and restricting the duration of most experiments to less than a day. The basic culture medium contained 5% glucose, 0.3% dried yeast extract, 0.3% malt extract and 0.5% peptone in nonconditioned tap water at pH 6.5. The inoculum was prepared by suspending 3 g of Allinson's active dried baking yeast (obtained from a local supermarket) in 300 mL of medium and allowing it to stand with occasional shaking for 1 h before use.

The electrical signal used to obtain controlled levels of conditioning was a 100 kHz sinusoid with a peak to peak amplitude of 6 V delivered as a series of pulses with a 1:1 mark:space ratio and a repetition rate of 1 kHz. The signal (which was constantly monitored with an oscilloscope) was generated by modulating a 100 kHz sinusoid with a 1 kHz square wave and applied from one terminal only of the generator to one end of the antenna. This consisted of ten turns of stranded copper wire wound around a plastic beaker just large enough to accommodate a 100 mL conical flask (an arrangement similar to that normally used with the "Water King" conditioner, with the beaker substituting for the water pipe). Conditioning was achieved by inserting the flask of medium into the beaker for the required length of time. Each flask contained 50 mL of medium prior to inoculation, duplicate flasks were set up for each treatment, closed with a cotton wool bung and incubated overnight at 30°C. The cultures were shaken and cell counts made with a haemocytometer at the beginning and end of the incubation, with between seven hundred and one thousand cells normally being counted for each treatment. The results were expressed as the percentage of extra cells produced.

In other experiments, the conditioned water was obtained from a tap with a "Water King" WK1 conditioner (Lifescience Products, Abingdon, Oxon. OX14 4YU) fitted to the supply pipe. To minimise possible errors due to the constantly changing frequency of the device, the water was run at about 3 L/min into an overflowing 10 L reservoir and samples ladled out as required. Nonconditioned water was obtained directly from the tap after the device was switched off and the water run for a

few minutes to clear any conditioned water from the plumbing.

RESULTS AND DISCUSSION

Conditioning generates a biologically-active agent

To see if the beneficial and damaging effects of conditioned water were due to different degrees of conditioning, yeast culture media were made up with tap water and exposed for different times to the pulsed 100 kHz conditioning signal. They were then inoculated with standard amounts of yeast and the increase in cell number determined after a standard overnight incubation. A typical result is shown in Fig. 1.

Conditioning caused a marked stimulation of cell multiplication, which peaked when the medium had been conditioned for around 15–30 s ($P < 0.001$), but this became a significant inhibition ($P < 0.05$) when the conditioning-time was increased to 1 min. This suggests that a biologically-active agent had been produced by the conditioning process which remained in the culture medium for long enough to affect cell multiplication. The peak at 15–30 s of conditioning suggests that there is an optimum concentration for this agent, above which it becomes inhibitory. This was confirmed in a similar experiment shown in Fig. 2, where the effect of diluting an inhibitory concentration can be seen. In this experiment, each culture solution was conditioned for what would normally be an inhibitory 2-min period, but was then diluted with different amounts of non-conditioned medium before use.

As expected, the 2-min conditioning period showed a marked inhibition compared with the unconditioned control, but diluting this medium by a factor of two with unconditioned medium converted this inhibition to a stimulation, with further

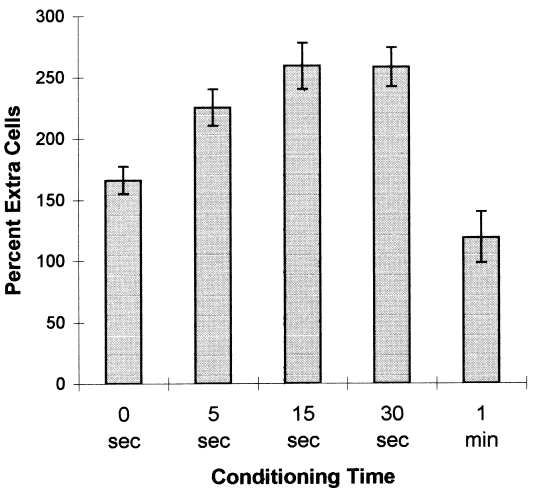

Fig. 1. A chart showing the optimum conditioning period for a culture medium to support yeast cell multiplication. It gives the means and standard errors for the percent increase in cell number after 22 h incubation.

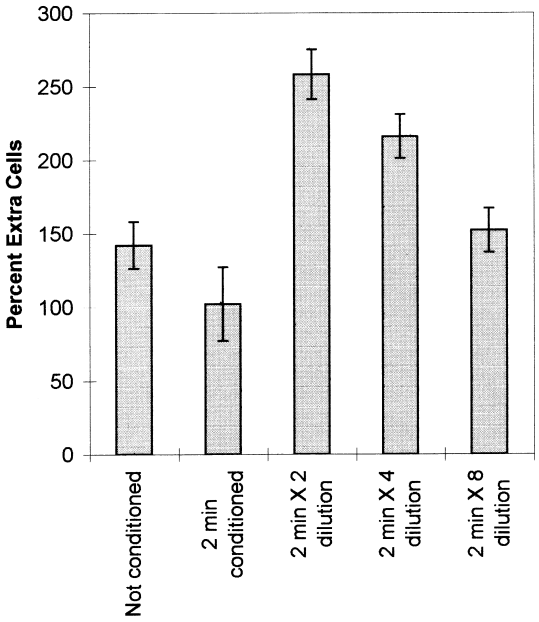

Fig. 2. Diluting a culture medium previously conditioned to an inhibitory level returned it to a stimulatory one. A $\times 2$ dilution series was made by diluting a medium previously conditioned for 2 min with unconditioned medium. The chart gives the means and standard errors for the percent increase in cell number after 22 h incubation.

dilution reducing the size of this stimulation. We can interpret this by saying that the long 2-min conditioning period produced a superoptimal concentration of the active agent which inhibited multiplication, but when this was diluted with unconditioned medium, it returned its concentration to nearer the optimum stimulatory level, and further dilution made it fall below this optimum. Both of these experiments have now been repeated four times. Although there was day-to-day variation in the absolute growth-rate of the cultures, perhaps due to variations in quality and chlorine content of the tap water, the pattern was always the same. As the conditioning period was increased, there was a stimulation of cell multiplication followed by an inhibition when it was increased still further, and this inhibition was always reversed by dilution with unconditioned medium.

A possible explanation is that the active agent in the conditioned medium is the same as the activated colloids which are believed to remove the calcium salts from lime-scale (Donaldson and Grimes, 1988). If they removed some of the monolayer of calcium ions, which coat and cross-link the phospholipid molecules of the cell membrane, or became attached to it and disrupted its structure, we might expect a weakening of the membrane and an increase in its permeability. This in turn could allow the entry of free calcium ions from the culture medium and stimulate growth. A growth stimulation might be expected because the cytosol normally contains very little calcium (about 1 μM), but is

extremely sensitive to any increase in its concentration. The entry of even minute additional amounts can initiate a "cascade" of enzyme activations which enormously amplifies the original "signal" and leads to changes in metabolism and gene-activation which can promote growth. Organisms normally use this process as part of a system of "second messengers" which link environmental information received by receptor molecules at the cell surface to the metabolic response (see Alberts *et al.* (1994) for a more detailed explanation). The entry of small amounts of additional calcium from weakly conditioned water could therefore cause quite dramatic stimulations of growth. On the other hand the larger concentrations of "agent" in strongly conditioned water could bring about a more lethal disruption of the membrane structure and inhibit cell multiplication, especially in microorganisms where virtually all of their external membranes are exposed to it. It seems likely therefore, that strongly conditioned water may possess a useful antimicrobial activity in its own right. This is consistent with the discovery (made accidentally, but now being commercially exploited) that recycling the water from fishponds through a conditioner can prevent the growth of the filamentous alga *Spirogyra* (blanket-weed), apparently without harming the fish (Lifescience Products — advertising literature). However, as we will see next, it may be even more useful when used in conjunction with conventional biocides.

The agent increases the effect of toxins

If our theory is correct, we might expect the increase in cell permeability brought about by conditioned water to increase the uptake of toxins and biocides too. When we measured the effect of conditioning a medium containing 0.4 mM copper sulphate (a commonly-used fungicide), the normally stimulatory 15-s treatment now *reduced* the yeast multiplication-rate by half, and a 10-minute treatment reduced it by three quarters (Fig. 3). This is consistent with the conditioning process increasing the penetration and hence the efficacy of the fungicide. This effect has possible commercial applications, since it could reduce the amounts of biocides needed to kill pathogens and other unwanted organisms.

To see if we could get the biocide-enhancing effect with the electrical signal from a commercially-produced conditioner, we repeated the experiment with the signal from a "Water King" WK1 and the results were virtually identical. However, such conditioners are intended to work with running water, so this was tested too. A "Water King" WK1 was fitted according to the manufacturer's instructions in the laboratory water-supply and the culture media made up using the conditioned water. The controls were made with water from the same source with the device switched off.

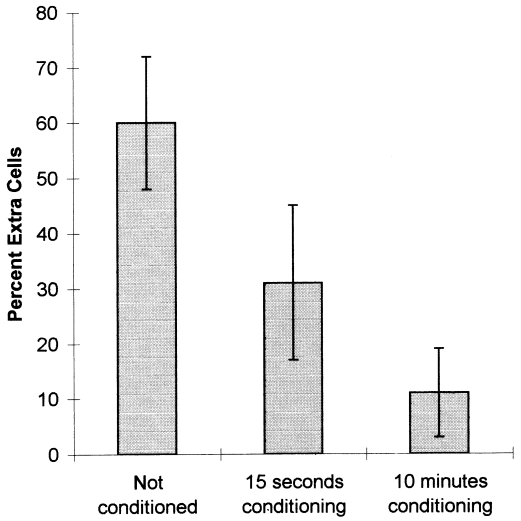

Fig. 3. Increasing the conditioning period increased the toxicity of 0.4 mM copper sulphate in a dose-dependent manner. The chart gives the means and standard errors for the percent increase in cell number after 22 h incubation in the presence of 0.4 mM copper sulphate.

The first experiment was to obtain a set of growth curves. The cultures had a restricted nutrient supply so that a complete growth curve could be obtained in 24 h. This was achieved by preparing a stock culture in unconditioned medium and diluting it by a factor of ten with either conditioned or unconditioned water. Figure 4 shows the curves for conditioned and unconditioned water in the presence and absence of 0.4 mM copper sulphate. In the absence of copper, the cells underwent about two doublings before entering the stationary phase, but multiplication was *faster* in the conditioned

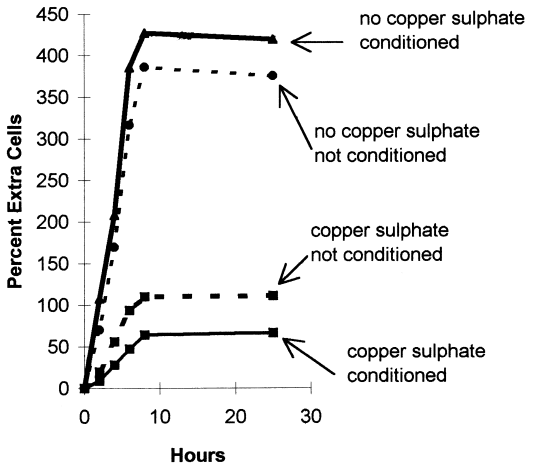

Fig. 4. Growth curves showing the effects of copper sulphate on the growth of yeast cultures after the medium was diluted ×10, with either conditioned or unconditioned tap water. Conditioned water on its own stimulated culture growth, but in the presence of copper 0.4 mM copper sulphate, conditioned water inhibited it. Each point is the mean for duplicate cultures which agreed closely.

medium. Multiplication in the presence of copper was slower and stopped after about one doubling, but here the effect of conditioning was reversed and it *reduced* the growth rate. When this experiment was repeated with a range of copper concentrations, the effect of conditioning was found to depend on the copper concentration. Figure 5 gives the cultures' growth after 24 h. In the absence of copper, conditioning was stimulatory, but as the copper concentration was increased, the stimulation due to conditioning first disappeared and then turned into an inhibition. Similar results were obtained when equimolar cobalt chloride was substituted for the copper (Fig. 6), suggesting that we may be looking at a general effect on cell permeability to toxins. Taken together, these findings show that, media made with water from the commercial conditioner operating under normal conditions also stimulates yeast multiplication and even increases their tolerance of low concentrations of heavy metals, perhaps due to extra calcium ingress stimulating metabolism to assist detoxification. However, when the heavy metal concentrations reach a critical threshold, this effect is reversed and conditioned water increases their toxicity, presumably because the degree to which calcium can stimulate metabolism and protect the cell is limited and becomes overwhelmed by the more rapid entry of the toxic ions when these are in high concentration.

The actual level of this threshold depends on the nutritive status of the cells. This was shown in a set of similar experiments, where the glucose concentration was reduced to 0.1%. A typical result is shown in Fig. 7. Culture-growth was now inhibited by conditioned water even in the absence of toxic ions, and the toxic effect of even low concentrations of copper was greatly enhanced. Possibly, the starving cells had less energy to repair damaged membranes or expel toxic ions, so that the effect of conditioned water was now always inhibitory. From these results, it appears that we can increase the efficacy of at least some biocides on microorganisms by formulating them in conditioned water, with the effect being greatest when their nutrient supply is restricted.

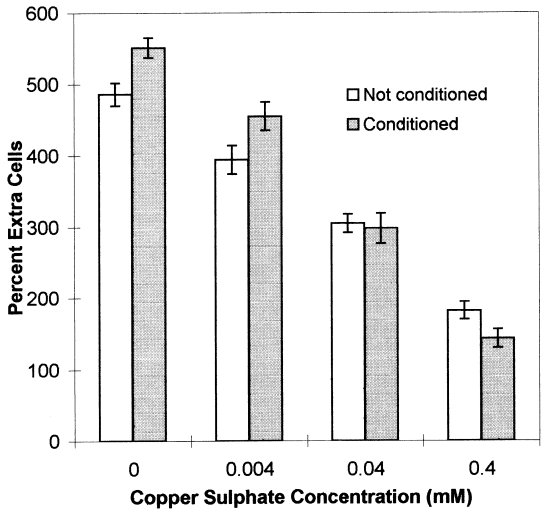

Fig. 5. Increasing the copper sulphate concentration converted the stimulatory effect of conditioned water into an inhibitory one. The chart shows the means and standard errors for the percent increase in cell number after 24 h incubation.

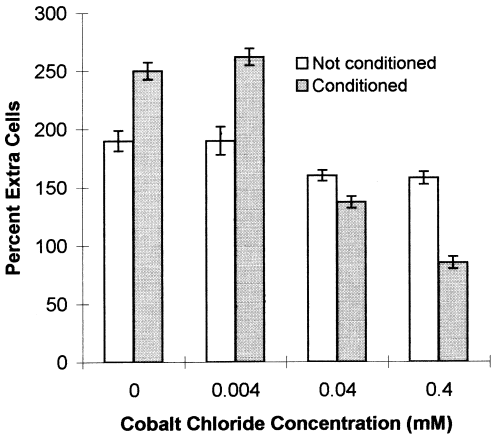

Fig. 6. Cobalt chloride behaved like copper sulphate. Increasing the cobalt chloride concentration converted the stimulatory effect of conditioned water into an inhibitory one. The chart shows the means and standard errors for the percent increase in cell number after 21 h incubation.

The implications for higher organisms

Can these findings explain the reported effects of conditioned water on higher organisms? In large

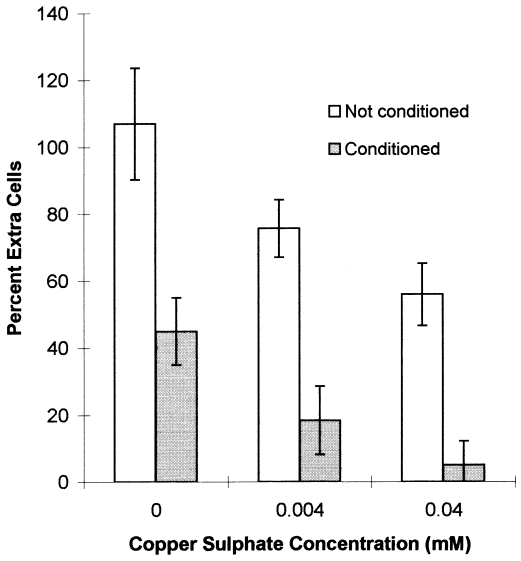

Fig. 7. When the yeast was starving in 0.1% glucose, conditioned water did not stimulate cell multiplication, but its enhancement of copper sulphate toxicity was more pronounced. The chart shows the means and standard errors for the percent increase in cell number after 21 h incubation.

organisms, the surface-area to volume ratio is much reduced and should lessen any increase in the concentration of cytosolic calcium, so the direct effects may be less marked. In plants, the large reported growth stimulations may in part be a secondary effect due to the conditioned water increasing the activity of the soil microorganisms which normally supply them with nutrients. This is supported by the observation by Harari and Lin (1989) of several-fold increases in soil nitrate following irrigation with conditioned water. However, we cannot rule out a direct effect on the plant itself; the surface area of the root/root-hair system is also extremely high and weakly conditioned water could also stimulate active nutrient uptake, as well as other metabolic processes by triggering the plant's own "calcium cascade".

In animals, the stimulatory effects could similarly be due to an activation of the gut microflora, or to an activation of the gut lining to increase food absorption. However, it is also possible that the putative colloidal conditioning agents can penetrate the gut lining by normal endocytosis to affect intracellular compartmentation and perhaps even enter the bloodstream. If so, the metabolic effects of conditioned water could be much more wide-spread in the body. This would be consistent with Russian work by Glebov *et al.* (1965) and Dardymov *et al.* (1966) quoted by Presman (1970) in which some of the internal organs of rodents fed with conditioned water underwent rapid size changes (possibly an osmotic effect due to changes in membrane permeability) and that their erythrocytes became less resistant to alkaline haemolysis (suggesting a weakening of their membranes). It could also account for the visible disruption of catfish liver-cell membranes seen by Garg *et al.* (1995), when the fish were exposed to conditioned water.

Relationship to direct effects of electromagnetic fields

There has been considerable research into the effects of weak electromagnetic fields on biological systems, ranging from their clinical use in bone-healing (McGinnis, 1989) to the activation of gene expression in tissue cultures (Liburdy *et al.*, 1993), and there are striking similarities between these effects and those of conditioned water. Like conditioned water, weak electromagnetic fields provoke diverse responses, often involving the stimulation of metabolism. Many workers now agree that the primary site of these stimulations is the cell membrane and that an early event is the ingress of calcium ions which then triggers the calcium cascade (Liburdy *et al.*, 1993). However, up to now, there has been no convincing explanation for how these extremely weak fields stimulate calcium uptake, nor is there any firm evidence that they react directly with any specific component of the cell membrane in order to do so.

From our results with yeast, it seems likely that at least part of the effects of electromagnetic fields on living organisms could be indirect and analogous to the conditioning of water. The electromagnetic fields, used to demonstrate biological effects, are often of the same type and magnitude as those used to condition water, and are therefore likely to disturb the electrical bilayers around the colloidal particles in living tissues in the same way. This could give them the power to abstract some of the structural calcium from the cell membranes to make them more permeable to external free calcium, and so stimulate the calcium cascade.

While this hypothesis may seem a little strange at first, it does explain some of the more curious effects of electromagnetic fields on living tissues. Firstly, it explains the "ion cyclotron resonance" effects which have been shown to occur in a whole range of organisms, from unicells upwards (Liboff *et al.*, 1990; Smith *et al.*, 1993). Cyclotron resonance occurs because ions tend to go into orbit around the lines of force of a steady magnetic field and will absorb energy and increase the size of their orbits if they also receive an oscillating electric or magnetic signal at their natural orbital frequency, which itself depends on the strength of the dc field. It has now been shown in several laboratories that many of the biological responses to weak alternating electromagnetic fields, have extrema at specific frequencies, the values of which depend on the strength of the prevailing dc magnetic field and correspond to the cyclotron resonance frequencies for biologically important ions such as calcium and potassium. This work has been well reviewed by Liboff *et al.* (1990) but the mechanism of how it affects biological systems is still unknown.

Liboff *et al.* (1990) also describe some particularly interesting experiments of their own, in which diatoms (which require calcium for motility) were able to move at suboptimal external calcium if also exposed to a combination of dc and alternating magnetic fields corresponding to the resonance conditions for potassium ions. However, under conditions promoting calcium resonance, the cells became unable to move, even with what would normally be adequate external calcium. It was as if potassium resonance stimulated calcium uptake, but calcium resonance inhibited it. This apparent antagonism between potassium and calcium resonance has also been demonstrated in other systems, including the growth of higher plants (Smith *et al.*, 1993).

Although these effects seem mysterious at first, it is possible to explain them in terms of surface chemistry. If the Gamayunov hypothesis is correct and the activation of colloidal particles by weak electromagnetic fields is due to ions being driven by Lorentz forces between the Stern and the diffuse ionic layers, it is likely that this will be most effective at their cyclotron resonance frequency where

the ions concerned can gather energy most efficiently.

Let us consider a colloidal particle in dynamic equilibrium with a solution containing calcium and potassium ions. If we apply an alternating electromagnetic field at the resonant frequency for potassium, potassium ions in the diffuse layer will absorb energy and increase the diameter of their orbits. Some of them will be driven into the Stern layer, which is itself adjacent to the particle surface, perhaps replacing some of the calcium ions already there. However, once in the much more densely-packed Stern layer the potassium ions will collide with its other components more frequently and be less able to resonate, so their ability to escape by a similar mechanism will be reduced. The net effect is a shift in the position of the equilibrium with some extra potassium ions now in the Stern Layer. Similarly, if the calcium ions are made to resonate, extra calcium will be present at equilibrium in the Stern layer. If these particles were then to contact a cell membrane, those which had been given the potassium frequency would be relatively deficient in calcium and able to withdraw calcium from the membrane. This in turn would weaken the membrane by reducing the cross-linking of phospholipids and increase its permeability to free calcium ions. External calcium could then enter the cell more rapidly along its diffusion gradient and stimulate the calcium cell-signalling cascade. Conversely, if the system had been treated with the calcium resonant frequency, the particles would be loaded with extra calcium. This could then be transmitted to the membrane to strengthen it, reduce calcium influx into the cell interior and inhibit the calcium cell-signalling cascade. In this way, exposure to the ion cyclotron resonance frequencies of either potassium of calcium would lead to the observed opposing effects on metabolism.

However, we do not have to postulate colloidal particles as intermediaries in this process. A similar mechanism could operate directly on the cell membrane. Ions could be driven at their resonant frequency from the surrounding medium into the much more densely packed layer of ions adsorbed on the membrane surface and so shift their concentrations at equilibrium. The potassium resonant frequency would tend to replace some of the divalent calcium ions with monovalent potassium, so that at equilibrium there would be less cross-linking of phospholipids, the membrane would be weakened, more free calcium allowed to leak into the cell, and the calcium signalling cascade stimulated. By the same token, exposure to the calcium resonant frequency would drive calcium onto the membrane so that at equilibrium it was strengthened, less free calcium allowed in, and the calcium cell-signalling pathway inhibited.

Whether the action of electromagnetic fields are directly on the membrane or via some colloidal intermediary (and it seems possible that they could both operate simultaneously), the effect on metabolism should be similar, a stimulation of the calcium cascade at the potassium frequency and an inhibition at the calcium frequency. This hypothesis explains many of the apparently antagonistic effects of the calcium and potassium resonant frequencies on biological systems. It also explains the curious nonmetabolic efflux of calcium, first noted by Bawin *et al.* (1975), when chick brain tissue was exposed to VHF radio waves modulated to act as a carrier for a 16 Hz ELF signal (which is the resonant frequency for potassium in the earth's magnetic field). This may now simply be interpreted as calcium leaving colloids or the cell surfaces as it becomes replaced by the resonating potassium to establish a new equilibrium.

The above is perhaps the simplest explanation for the biological effects of weak electromagnetic fields which explains most of the facts. It requires no special detection mechanism, cell structure, or organelle, and so may account for why such responses, peaking at specific ion cyclotron resonance frequencies seem virtually universal in living organisms, from unicells to higher animals and plants. Maybe the effects of weak electromagnetic fields on the surface properties of colloids and simple phospholipid membrane components are far more important to the proper functioning of biological systems than we have previously realised. If we are to apply them to greatest effect in medicine, biotechnology and agriculture, we need to learn much more about them.

CONCLUSIONS

1. The biological effects of conditioned water differ depending on the degree of conditioning. Strongly conditioned water inhibits the growth of yeast cultures, but weakly conditioned water stimulates it.
2. This is interpreted as being due to the conditioned water interacting with the structural calcium in the cell membrane to make it more permeable. Weak conditioning gives a slight increase in permeability and allows the ingress of small amounts of external calcium which activates the "calcium signalling cascade" and promotes growth. Strongly conditioned water causes more severe membrane damage which disrupts metabolism and inhibits growth.
3. Because of their larger surface area to volume ratio, microbes should be more sensitive to membrane permeability-changes than larger organisms. Consequently, conditioned water should be more toxic to microorganisms and may therefore have useful selective antimicrobial properties.
4. The apparent increase in membrane permeability caused by conditioned water extends to at least

some toxic materials. Dissolving biocides in conditioned water may therefore make them more effective.

5. There are parallels between the biological effects of conditioned water and those of weak electromagnetic fields applied directly to living organisms, both appear to modulate calcium entry into cells and they may share a common mechanism.
6. We propose that some of the hitherto unexplained biological effects of weak alternating electromagnetic fields are due to their disturbing the equilibrium between the ions which are firmly adsorbed to the cell membrane and colloid surfaces and those which are more free to diffuse. This affects membrane permeability and controls the ingress of free calcium ions, which in turn regulates metabolism via the calcium cell signalling cascade.
7. This effect is most clearly seen at the frequencies which correspond to the ion cyclotron resonance frequencies of specific ions. On theoretical grounds, electromagnetic treatment at the cyclotron resonance frequency for an ion should increase the proportion of that ion which is adsorbed on membrane surfaces. Treatment at the potassium frequency replaces some of the divalent calcium ions with monovalent potassium, which reduces cross-linking between phospholipids and weakens the membrane. This increases its permeability to free calcium, which then leaks in to stimulate the calcium cascade. Exposure to the calcium frequency increases calcium binding, strengthens the membrane and so inhibits the calcium cascade. This explains the often-observed antagonistic effects of the potassium and calcium frequencies on biological systems and also why exposure to the potassium frequency causes a measurable release of calcium from tissues.

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