11 Effects of Electrical and Electromagnetic Fields on Plants and Related Topics

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Anyone reading this book cannot fail to realize the importance of self-generated electric fields and currents in the energetics and control of metabolism in plants. We should therefore not be too surprised to find that externally applied fields also have effects. In this chapter, I will describe a few of the more significant findings from over a century of research and try to explain and sometimes reinterpret them in the light of more modern knowledge. The work is divided into three sections. Section 1 is on the non-polar effects of DC fields, where the effects are not related to the direction of the field. It ranges from responses to massive electric fields, such as those found in thunderstorms, to the effects of much weaker ones on the growth and differentiation of tissue cultures. Section 2 is on the polar effects of DC fields, where the direction of the response is related to the direction of the field and includes effects on polar growth and tropisms. Section 3 is on the effects of time-varying and alternating electromagnetic fields, where I will present evidence that a simple change in membrane stability can account for virtually all of the hitherto mysterious biological effects of weak electromagnetic radiation.

11.1 Non-polar effects of DC electric fields

11.1.1 High voltage natural fields and the rise and fall of electroculture

11.1.1.1 Phenomenology

Work on the effects of electrical fields on plants goes back several centuries, but the first person to carry out large scale experiments was Karl Lemström, who was a Professor of Physics at Helsinki. He had paid several visits to the Arctic, and was surprised how green and healthy the vegetation looked, despite the low light and temperature. He wondered whether this might be due to the weak electric currents carried through the atmosphere by air ions from the aurora borealis. His suspicions were confirmed when he looked at

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the annual growth rings of fir trees in the region, which showed a periodicity, with best growth during the peaks of sunspot cycles when the aurora would have been most active. To test his theory, he exposed a range of different crops in several European countries to high voltage gradients from wires suspended above them. The voltages were produced by an electrostatic generator and, from the length of the sparks it could produce, we can estimate that the gradients applied to the plants were approximately 10 kV/m. He found that the treated plants were greener, sturdier and often showed dramatic increases in yield, compared with the controls. Although the technique didn't always work, on average there was a yield stimulation of around 45% (Lemström 1904). This led to a flurry of activity by agricultural scientists hoping to exploit this effect, which had now been given the name *electroculture*. Amongst these were Blackman and his co-workers of Imperial College London, who used higher voltage gradients (20–40 kV/m) in both field and pot experiments. They found that it did not matter whether the overhead wires were positive or negative, but the amount of current flowing was important. A current of 10^{-11}–10^{-8} amps per plant normally stimulated growth but higher values were injurious (Blackman 1924; Blackman and Legg 1924). But the stimulations did not occur all the time. Out of 18 field trials with wheat, barley and oats performed by Blackman (1924), only 14 gave significant increases in dry weight, Murr (1963) only found a greening effect, and Briggs et al. (1926) found no significant response in a whole series of experiments in the USA. There were many claims and counter-claims about the beneficial effects of electroculture and the matter was highly controversial. However, most of the agriculturally oriented work was discontinued in the 1930s, largely because the cost, electrical hazards, and the uncertainty of getting positive results, made it uneconomic.

11.1.1.2 Ecological significance of natural electric fields

The conflicting results of these early workers now make sense. Plants seem to be using the very strong electrostatic fields associated with thunderstorms as a signal to let them make the best use of the rain (Goldsworthy 1996). If a plant in otherwise dry conditions is to use the rain to best advantage, it must respond quickly before the water drains away. But the synthesis of new proteins, chlorophyll etc. takes several hours, so it will be of selective advantage to start before the water trickles through the soil and can be sensed by the roots. The electric fields from thunderclouds are an excellent signal for this. Schonland (1928) measured voltage gradients of up to 16 kV/m under thunderclouds, which is the sort of gradient that was effective in the electroculture experiments. The first clue that plants may actually be *using* natural electric fields to stimulate growth came from Lemström (1904), who reported that electroculture was often *inhibitory* in dry weather, presumably because the anticipated rain never came and the plants' resources were being wasted. The

next clue came from Blackman et al. (1923) who found that exposing cereal seedlings to an electric field for just one hour was enough to stimulate growth, and the growth-rate continued to increase for at least four hours after the current was turned off. This suggests that the field had activated growth-promoting genes, which then remained active for some time. This phenomenon may explain the common perception that vegetation often looks unusually green after a thunderstorm. The requirement for subsequent rain may also explain the negative results for electroculture obtained by Briggs et al. (1926), since their work was conducted in one of the drier regions of the United States and, in order to avoid arcing, they switched off the current whenever rain was expected!

11.1.2 Low voltage fields and plant tissue cultures

Effects similar to high voltage electroculture can occur when weak DC currents are applied to plant tissue cultures. Currents of one or two microamps, of either polarity, applied between an electrode in tobacco callus and another in the culture medium gave several-fold increases in plantlet regeneration (Rathore and Goldsworthy 1985; Rathore et al. 1988). Even isolated protoplasts can be affected. Dijak et al. (1986) found that 20 mV applied for 4 h between a central silver anode and a circular silver cathode 25 mm apart in the culture medium caused a massive production of somatic embryos by *Medicago* protoplasts, whereas none appeared in the controls.

11.1.3 Mechanism of the non-polar effects of DC fields

The fact that stimulation of growth and regeneration by direct currents can occur in undifferentiated tissue cultures and isolated protoplasts suggests that the main effect is at the cellular level. In the light of modern knowledge, electrically induced calcium ingress into their cytosols seems the most likely cause. Most of the applied voltage will appear across the cell membranes because of the relatively low resistance of the cell contents. This will add to the membrane potential on one side of the cells and subtract from it on the other. Where it subtracts, it may open voltage-gated calcium channels and where it adds, there could be a non-specific increase in permeability due to transient pore formation in the hyperpolarized membrane (see Melikov et al. 2001). Either way, there will be an increase in membrane permeability to calcium ions. Because of the huge electrochemical gradient for calcium across the plasma membrane (about 4 orders of magnitude), even a very small increase in permeability to calcium will have a large impact on its cytosolic concentration. This can increase the rate of metabolism because calcium ions often form an integral part of the enzyme cascades that control many intracellular signaling processes. These cascades are enzyme-based amplifiers, where one enzyme molecule activates a large number of molecules of another

enzyme, which in turn activates a third enzyme and so on (Alberts et al. 2002). They normally allow very small stimuli such as a few hormone molecules to control often massive biological responses. By taking part in these cascades, calcium ions act as master gain controls that regulate many aspects of metabolism, and could affect chlorophyll synthesis, and the growth and regeneration in whole plants and tissue cultures.

11.2 Polar effects of DC electric fields

11.2.1 Direct current effects on single cells

Weak artificially applied electric currents can initiate the development of electrical polarity in single cells such as zygotes and so affect their direction of growth. This is probably because the asymmetric calcium ingress on the positive and negative sides of the cells results in metabolic gradients that determine which end of the cell grows first. A link between artificially applied electric currents and polar growth was first shown by Lund (1923) in zygotes of the seaweed *Fucus*, which becomes polar when it germinates. Although their direction of growth is normally controlled by the direction of light, Lund found that a weak electric current applied in the dark made them grow with their rhizoids (the first visible sign of germination) in line with the current. He speculated that the zygotes' polarities were normally controlled by electric currents generated by the cells themselves; but we had to wait until the 1960s for the work of Lionel Jaffe and his co-workers using *Fucus* and its close relative *Pelvetia* for these currents to be confirmed and measured. They used a vibrating probe to measure the current densities and radioactive tracers to determine their ionic composition (see Jaffe et al. 1974; Jaffe and Nuccitelli 1977). They found that following unilateral illumination, a weak electric current entered at a point that predicted the emergence of the rhizoid and left over the remainder of the cell surface. Similar currents predict the primary region of growth in many polarizing cells, including, moss spores, pollen grains and animal zygotes (Jaffe and Nuccitelli 1977). Artificially applied voltages seem to initiate polar growth, by triggering the development of the natural currents.

An interesting observation is that the direction of electrically-induced growth doesn't always correspond to the sign of the applied voltage. Peng and Jaffe (1976) showed that zygote growth usually begins towards the cathode, but it could also be towards the anode depending on the batch of cells used. This effect could also be voltage-dependent, since some batches of cells grew towards the cathode at low voltages but towards the anode at higher voltages. There is a similar voltage-dependence in the galvanotactic migration of animal cells (Mycielska and Djamgoz 2004), but the mechanism of this reversal is not yet understood. It does, however, indicate that the cells' voltage sensing

mechanisms may be more complex than once thought, and are programmable to give responses in either direction.

Novák and Bentrup (1973) discovered that the electrical effects on the germination of the *Fucus* zygote were voltage rather than current dependent when they found that they also occurred in pure electrostatic fields. This suggests that voltage-gated ion channels may be the sensors. The first ion to show polar uptake in the fucoid zygote is calcium (Jaffe et al. 1974), which suggests that voltage-gated calcium channels may be the main ones involved. It is argued that the localized calcium uptake stimulates metabolism around its point of entry and initiates rhizoid development. It also increases the translocation of other ions to give a much larger inflow of current, which is tightly focused at the point of calcium entry. The main function of this "amplified" trans-cellular current is probably to drive the electrophoresis of proteins with different charge densities to different regions along the electrical axis of the cell in the fluid mosaic of its membranes (Jaffe et al. 1974). These proteins could be enzymes or anchorage points for specific elements of the cytoskeleton, but the main effect is to establish a physiological polarity that controls the direction of growth and other polar metabolic functions. This mechanism is of fundamental importance to living cells since it provides a means for DNA, by determining the overall charge on proteins, not only to define their nature, but also to direct them precisely to different regions along a cell's axis using an electrical frame of reference. However, as far as the experimental scientist is concerned, the trans-cellular currents responsible for this are excellent indicators of cellular polarity that can be readily measured with a vibrating probe.

11.2.2 Direct current effects on multicellular structures

11.2.2.1 Effects on plant tissue cultures

The electrical control of cell polarity also occurs in the cells and tissues of higher plants, where it may help coordinate the polarities of neighboring cells. Although cellular polarity is normally quite stable, it does have to change occasionally, such as when a stem or a root initiates a branch or when there is a tropic curvature. Evidence for the mechanisms by which cellular polarities are regulated comes from vibrating probe studies on tobacco tissue cultures, where they seem to be controlled by both polar auxin transport and by electrical gradients.

Goldsworthy and Mina (1991) found evidence that polar auxin transport was important when they looked at the growth and electrical patterns of tobacco cells cultured with different auxins. When they were cultured in media containing the natural auxin indole-3-acetic acid (which can undergo polar transport) they tended to grow as filaments of elongated cells that were electrically polarized longitudinally; mostly in the same direction. However,

in a medium containing the synthetic auxin 2,4-dichlorophenoxy acetic acid (2,4-D) (which does not show polar transport), the cells were more commonly isodiametric and grew as random clusters with unstable and randomly oriented trans-cellular currents. This suggests that the polar transport of auxin is a prerequisite for clearly defined trans-cellular currents and orderly polar growth. This is consistent with recent work by Friml et al. (2003), with auxin transport mutants of *Arabidopsis*, from which they concluded that auxin gradients are important in establishing the apical–basal axis of the embryo. In particular, the polar efflux of auxin from donor cells appeared to determine the polarity and growth of its daughter cells so that they grew in orderly columns.

Higher plant cell polarities are also under electrical control. Mina and Goldsworthy (1991) applied a transverse positive electric current of either 3 or 100 $\mu A/cm^2$ originating from a point source to individual cells of tobacco cell filaments and mapped their own currents after the applied current was switched off. They found the cells had electrically re-polarized in line with the applied current, with their new negative ends next to the positive electrode. This effect would stabilize the polarities of the cells in a filament since the negative end of one cell is normally adjacent to the positive end of its neighbor. Closer examination revealed that the electrical patterns of the repolarized cell resembled those of a polarizing fucoid zygote. Current was entering at the point that had been nearest the electrode, but was leaving more uniformly over the rest of the cell; so could the mechanism be similar? Since calcium is important in controlling the polarization of the fucoid zygote, the tobacco experiment was repeated with no calcium in the external medium. Although the natural currents were not significantly smaller without calcium, the cells were unable to repolarize. The same happened even in the presence of calcium if cobalt ions were added. Since cobalt blocks calcium channels, it suggests that the cells responded to weak electric currents by opening voltage-gated calcium channels, which then reprogrammed their polarities in a way similar to that of germinating zygotes (Mina and Goldsworthy 1992).

The exact relationship between the control of polarity by auxin and electric currents is still unclear, but a simple explanation is that the day-to-day physiological polarities of cells are still controlled by the electrophoresis of membrane proteins driven by their trans-cellular currents as proposed by Jaffe et al. (1974), and it is this that makes them vulnerable to externally applied currents. The function of auxin is to focus the electrical patterns of the cells more precisely to accentuate cellular polarity. The mechanism by which this might occur is surprisingly simple. The point of current entry into the cell is already tightly focused at the apical end as described above for the fucoid zygote, but current egress (mostly via H^+ATPases) is more uniformly spread over the rest of the cell surface, to give only a crude electrical polarity. Polar auxin transport should sharpen this by generating a positive feedback loop that limits current efflux to a relatively small region near the basal end of the cell as follows. If, as might be expected from the proposals of Jaffe et al. (1974), the auxin efflux system is

targeted electrophoretically[1] away from the cell apex, it would still be relatively broadly distributed and give only a weakly polar auxin output towards the base. However, the released auxin should stimulate the activity of the local plasma membrane H^+ATPases (Taiz and Zeiger 2002), to give a corresponding pattern of proton efflux, also with maximum near the base. This will concentrate trans-cellular current-flow to this region and attract still more of the auxin efflux system. The process continues until most of the auxin efflux and proton efflux becomes focused in small area so as to amplify and stabilize cellular polarity. Cells then divide transversely to the direction of current and auxin flow, with the daughter cells sharing the same polarity to give the ordered polar filaments seen by Goldsworthy and Mina (1991). Therefore, both polar auxin transport and polar current-flow seem essential for the development of normal cell polarity in nature. Given the simplicity of the mechanism just proposed for auxin focusing trans-cellular currents and the fundamental importance of this for organized growth, we may be looking at one of the first functions for auxin ever to have evolved, perhaps preceding all others.

11.2.2.2 Tropic curvatures

The interaction between auxin and trans-cellular currents proposed above can also account for the transverse electrical potentials that occur in plant organs prior to tropic curvatures. For example, many workers have reported that the lower surfaces of organs (such as cereal coleoptiles) showing negative gravitropism become electrically positive when placed horizontally, and this is associated with an excess of auxin in the lower region. Also, adding auxin asymmetrically to the apex of the vertical organ gives a similar electrical effect and curvature.

We can now explain this by saying that the transverse auxin imbalance partially reorients the electrical polarities of the organ's cells by stimulating proton efflux from the parts of their plasma membranes exposed to the highest auxin concentration. The result is the development of the observed transverse electrical potentials and also the transverse pH gradients reported by Mulkey et al. (1981) in tropically stimulated organs. What it means is that the cells' normally longitudinal electrical polarities are now partially redirected sideways, which gives a tropic curvature as their direction of growth attempts to follow their new electrical polarities.

The reader will recognize the above as being a modification of the original Cholodny–Went hypothesis described in most textbooks (e.g. Taiz and Zeiger 2002). It still needs an apical transverse redistribution of auxin in response to the stimulus, and also a basipetal transmission of this imbalance, but the main

[1]The auxin efflux system is transported in vesicles via the cytoskeleton to its destination (see Muday et al. 2003 for a brief review) but the plasma membrane proteins that act as the targets for this transport are probably still put in position electrophoretically.

driving force for curvature is now a change in the direction of the polar growth of individual cells rather than a differential growth rate on either side of the organ. This overcomes one of the major objections to the original Cholodny–Went hypothesis; that being that the measured 2:1 ratio of auxin on either side of the organ is not enough to cause the observed difference in growth-rate. Because the relationship between auxin concentration and growth-rate is logarithmic, a difference in auxin concentration of orders of magnitude would be required if a growth-rate differential were to be the main driving force.

11.3 Effects of weak time-varying electromagnetic fields

11.3.1 Phenomenology

The effects of weak time-varying electromagnetic fields on living organisms are many and varied, and they have spawned a vast literature, largely in response to their alleged links to the promotion of cancer in animals (Wilson et al. 1990). Experiments have often proved difficult to reproduce in different laboratories for reasons to be described later, but there is little doubt that many of the effects are real.

Both the electrical and the magnetic components of the electromagnetic fields are effective. Biological effects have been reported with electric fields in the region of 10–10,000 mV/m (Adey 1990) and magnetic flux densities of the order of microtesla. It is difficult to be precise about the magnetic flux densities needed since the effect probably depends on their ability to induce electric currents in the tissue, and this depends on a number of other factors such as waveform. For example, pulses and square waves are more effective than sine-waves, at least partly because their rapid rise and fall times generate larger current spikes. It has been calculated that time-varying fields must induce tissue current densities greater than about 1 mA/m^2 if they are to produce biological effects (Tenforde 1990).

Effects of weak electromagnetic fields on growth and metabolism have been reported at all levels of evolution throughout the eukaryotes, with plants being no exception. They include changes in the motility of diatoms (McLeod et al. 1987), changes in the germination and seedling growth of radish (Smith et al. 1993), stimulation of root growth in maize (Muraji et al. 1998) and cress (Stenz et al. 1998) and cytological changes with faster resin production and senescence in mature pine trees (Selaga and Selaga 1996). Effects are most apparent at low frequencies (below a few thousand Hz) and much of the research work has concentrated on the extremely low frequency range, especially around 60 Hz, which is the frequency of domestic electricity supplies in the USA. Radio frequencies are mostly ineffective unless they are amplitude modulated at a low frequency, in which case they have a similar biological effect to the low frequency modulation envelope.

The fact that responses to electromagnetic field are so widespread and that they also occur in unicells suggests that they have a common mechanism based at the cellular level, but until now there has been no convincing explanation. Any explanation must account for each of the following generally accepted facts:

1. The observed biological effects of weak electromagnetic fields differ in different organisms and tissues, and their expression may also depend on their previous history.
2. The fields involved are usually too weak to cause significant heating.
3. The fields concerned usually contain both electrical and magnetic components but either can be effective on its own.
4. Pulses are often more effective than sine waves.
5. Weak fields may be more effective than strong ones and there may be one or more "amplitude windows" where they give maximal effects.
6. Only low frequencies work and some specific frequencies such as 16 Hz may be especially effective to give so called "frequency windows".
7. Radio frequencies can have biological effects, provided that they are amplitude modulated with a biologically active low frequency.

Despite the seeming complexity of the above phenomena, they can all be explained by a new and very simple hypothesis based on electromagnetically induced changes in the permeability of the phospholipid fraction of cell membranes.

11.3.2 Hypothesis

1. Weak time-varying electromagnetic fields are detected by living organisms because they generate eddy currents in and around their cells.
2. Low frequency eddy currents selectively remove calcium ions that normally stabilize cell membranes and replace them by less effective monovalent ions (mainly potassium), which increases their permeability.
3. This process is enhanced at the ion cyclotron resonant frequency for potassium because it increases the kinetic energy of potassium ions in the diffuse ion layer around the membrane and therefore their ability to replace calcium.
4. Voltage-gated calcium ion channels are involved indirectly by amplifying the effects initiated by the change in phospholipid permeability.
5. Radio waves that are amplitude modulated at the resonant frequencies for biologically active ions give responses because the asymmetrical motion of ions adjacent to the membrane demodulate the signal and promote their resonance.
6. The electromagnetically induced increase in membrane permeability weakens cellular compartmentation and lets in more free calcium ions into the cytosol to interfere with cell signaling.

   I will now discuss each item of the hypothesis in turn.

11.3.3 Why are eddy currents necessary?

A major problem in explaining the biological effects of weak electromagnetic fields is fact that they do not have enough energy to give chemical effects on individual molecules. The energy available to a molecule from biologically active signals may be many orders of magnitude below the thermal energy of that molecule (kT) and should therefore be negligible. However, in an electrically conducting medium such as a living cell or its aqueous surroundings, the electromagnetic forces can be summed by countless ions to generate eddy currents in synchrony with the incoming electromagnetic signal and so collect enough energy to give significant effects. A non-biological example of this is the antenna of a radio picking up weak signals from a distant transmitter. They may be orders of magnitude below the thermal energy of the atoms of the antenna but can still be detected by the currents they generate, which are then amplified by its transistors. Living cells can behave in a way analogous to transistors. Weak eddy currents flowing in and around them can selectively remove calcium ions bound to the phospholipid fraction of their membranes to make them more permeable (equivalent to the base of the transistor). This permits the entry of many more free calcium ions down a huge electrochemical gradient into the cytosol (equivalent to the collector current of the transistor), which reduces the membrane potential and interferes with calcium-based cell signaling.

11.3.4 How do low frequency eddy currents affect membrane permeability?

It is now widely accepted that biological membranes are stabilized by divalent cations (Baureus Koch et al. 2003). Evidence for this is that erythrocyte ghosts (red blood cells that have lost their contents) are protected from breaking up into vesicles by divalent cations (Steck et al. 1970; Lew et al. 1988). Also washed carrot discs leak intracellular potassium ions into distilled water but not into 1 mM calcium chloride solution (Goldsworthy, unpublished). The latter is probably due to the transient formation of pores in the phospholipid fraction of cell membranes, which gives a relatively non-specific increase in permeability. The generation and re-sealing of such pores has been demonstrated in artificial planar phospholipid membranes as spontaneous transient changes in their conductance (Melikov et al. 2001). Ha (2001) made a theoretical study of the role of divalent ions such as calcium in stabilizing membranes against pore formation. He concluded that their double positive charge is more effective at screening the natural repulsive forces between the negative phospholipids and, by being divalent, they can cross-link neighboring phospholipid molecules. Monovalent ions such as potassium are much less able to do either of these, so an electromagnetically induced loss of calcium from cell membranes and their replacement by

monovalent ions should increase their tendency to pore-formation and increase their permeability.

The first evidence that electromagnetic fields can remove membrane-bound calcium came from Bawin et al. (1975) and Bawin and Adey (1976). They exposed chick and cat brain slices to $^{45}Ca^{2+}$ and examined the effects of electromagnetic exposure on its subsequent release. They found that its release was stimulated when the tissue was exposed to VHF signals, amplitude modulated at what we now know to be the resonant frequency for potassium ions. This effect was insensitive to cyanide and they concluded that a purely physical process was releasing ions bound to the cell membranes. However, when the experiment was repeated with the low frequency component of the signal on its own, the effect was reversed. There was now a *minimum* for calcium release at the potassium frequency. This suggests that some of the dislodged calcium was now being absorbed into the tissue down its natural electrochemical gradient. The cause of this "mirror image" effect was unknown at the time, but can now be explained by the hypothesis. The explanation is that the tissue can extract the biologically active low frequency component from the signal (see section 3.7) but only at a low level. It may have been strong enough to release some of the membrane-bound calcium, but not enough to trigger the large-scale inward leakage of free calcium seen with the pure low frequency signal.

We can postulate two mechanisms by which weak electromagnetic signals remove membrane-bound calcium. One is amplitude-dependent and the other is frequency-dependent. Although they will interact with one another, it is convenient to consider them separately to begin with. In this section, I will concentrate on the amplitude dependent mechanism. The frequency dependent mechanism will be explained in section 3.5.

11.3.4.1 Amplitude dependent mechanism

Cell membranes are usually negatively charged and bind mineral cations reversibly (Ha 2001). Applying an alternating electric field tends to drive these ions off and on the membrane with each half-cycle. However, they will only dislodge if the combined effects of thermal agitation and the electrical forces trying to remove them exceed the forces binding them to the membrane. The threshold at which this occurs will depend on the charge/mass ratio of the ion concerned, the natural affinity of the membrane for it, and the availability of ions with a lower charge/mass ratio, with which it might exchange. In general, ions with high charge/mass ratios should be affected more and be dislodged at a lower voltage. The exact effect will depend on strength of the field. If it is below the threshold for any ion to dislodge, nothing much will happen. If the threshold voltage for all ions is exceeded, they will all have an equal tendency to dislodge but will return in the same proportions when the field reverses. Again, there will be no observable effect.

Between these extremes, there will be a range when the thresholds for only some ions will be exceeded and these will be preferentially dislodged. They may return when the field reverses, but if they have diffused too far, they may be replaced by other ions in proportion to their *local activities*. This will change the relative concentrations of the different ions bound to the membrane since specific ions with a high charge/mass ratio are being removed, but they are being replaced by a less specific mixture. The process will be repeated with every cycle to give a selective and progressive loss of ions with high charge/mass ratios.

Many ions may contribute to this effect by competing for sites on the membrane, but the most important for membrane stability are calcium and potassium. Calcium has a high affinity for the membrane and stabilizes it against temporary pore-formation (Ha 2001). Potassium has a lower affinity for the membrane but this is compensated by its high intracellular concentration. Potassium is easily the most abundant cation in living cells. A typical plant cell has a potassium concentration of 100 mM or more, which is about 5 orders of magnitude greater than cytosolic calcium. It is therefore a serious competitor with calcium for sites on the membrane. Since it has a charge/mass ratio only half that of calcium, it will also be harder to dislodge electrically and will be the most likely ion to replace any lost calcium.

Since the selective release of calcium described above can occur only over a narrow range of voltages, this gives us an "amplitude window" for changes in membrane permeability and their biological effects. Such windows have been reported by many workers (Bawin and Adey 1976; Blackman et al. 1982; Blackman 1990; Liboff et al. 1990), where biological effects were maximal within distinct but varied ranges of signal amplitude. Often, there were two or more windows. Multiple windows may be explained because eukaryotic cells have several membrane systems, each with differing surface properties, ionic environments and exposures to the electrical component of the applied field. All of these factors will affect the ease with which bound calcium might be replaced by other ions and therefore the optimum strength of electromagnetic signal needed. Each membrane system could therefore have its own window for maximum permeability and, since they often surround calcium-rich organelles, there could be several amplitude windows for biological activity, even in a single cell.

The proposed hypothesis also explains why the effects of weak electromagnetic fields only occur at low frequencies. This is because there must be time for the released calcium ions to diffuse well away from the membrane if they are to be replaced by monovalent ions before the field reverses. Also explained is the observation that pulses and square waves are more effective than sine waves of the same amplitude. This is because the rapid rise and fall times of the magnetic component of these fields give voltage spikes that catapult the calcium ions quickly away from the membrane, followed by a relatively long period for the lost ions to be replaced by less affected species such as potassium before the field reverses.

11.3.5 Why are some frequencies more effective than others?

The ability of different ions to replace dislodged calcium will depend on their relative chemical activities in the surrounding medium rather than their concentrations. These activities also include other components that may affect their ability to react, such as an increase in kinetic activity due to ion cyclotron resonance. The response will therefore hit an extreme value at the resonant frequency for the ion concerned.

11.3.5.1 What is ion cyclotron resonance?

Ion cyclotron resonance can occur when ions move through a steady magnetic field. Lorentz forces drive them into orbit around the lines of force of the steady field at a characteristic "resonant" frequency that depends on their charge/mass ratio and the strength of the steady field. If these ions are simultaneously exposed to either an electrical or a magnetic field that oscillates at this frequency, they absorb its energy and gradually increase the size of their orbits. This increases their kinetic energy, which increases their chemical activity and ability to react. The resonant frequency for any ion can be determined from the formula:

$$\text{Frequency (Hz)} = \frac{\text{Ion Charge (Coulombs)} \times \text{Steady Field Strength (Tesla)}}{\text{Ion Mass (Kg)} \times 2\pi}$$

11.3.5.2 What are the biological effects of resonance?

The two most important frequencies for biological effects are those for potassium and calcium. The resonant frequency for potassium in the Earth's magnetic field is around 16 Hz and that for calcium is about 32 Hz (the exact values depend on local field strength). The frequencies for these ions are important since they give extreme but opposite biological effects (Smith et al. 1993; Mehedintu and Berg 1997). Extreme responses at the potassium frequency have been reported so many times that their reality is beyond dispute (Tenforde 1990). Evidence that it is due ion cyclotron resonance was obtained by adjusting the steady magnetic field to different values, when the peak response occurred at the predicted new resonant frequencies (Smith et al. 1993). But until now, there has been no satisfactory explanation. An early proposal by Liboff (1985) that the helical movement of resonating ions lets them corkscrew their way through ion channels was criticized by several workers (Halle 1988; Tenforde 1990) because it needs an exact alignment of the channels with the magnetic field, their transit time in the channels is too short for even one orbit, and their high frequency of collision with other molecules in the solution and with the stationary sides of the channel may not permit their resonance anyway.

The present hypothesis avoids these difficulties. There is no time limit for resonance to build up, and the ions do not have to move in any particular direction. All they need do is to increase their kinetic energy and so enhance their chemical activity compared with competing ions. Collisions are also less problematical, since most of the ions near the negatively-charged cell membrane will be positively charged and will all tend to vibrate in synchrony with the electromagnetic field. This will occur regardless of whether they are actually resonating. They may also transfer some of this energy to adjacent uncharged molecules so that the whole region next to the membrane will have a component of its molecular motion that is synchronous with the applied field. Consequently, collisions of a resonating ion with any of these molecules will tend to reinforce rather than detract from its resonance.

Evidence that resonance can increase chemical activity, even in purely physical systems, comes from the work of Zhadin et al. (1998), who showed that the electrolysis of glutamate was enhanced by an electromagnetic field at its resonant frequency. The frequency corresponded to that of the unhydrated ion, which suggests that it is an effect at the electrode surface, since ions in the bulk solution are normally hydrated and would have a different frequency. I suggest that the most likely interpretation of these results is that the electromagnetic exposure of the glutamate ion to its resonant frequency increased its kinetic energy and chemical activity, which assisted its discharge at the electrode. It therefore seems probable that a similar electromagnetically induced increase in the chemical activity of potassium ions will enhance their ability to deposit on cell membranes to replace bound calcium and increase permeability.

Further evidence supporting the hypothesis comes from the observations that electromagnetic exposure near the resonant frequency for calcium gives opposite biological effects to the potassium frequency. Mehedintu and Berg (1997) found a significant stimulation of yeast multiplication at the potassium frequency but an inhibition at the calcium frequency. Smith et al. (1993) found that the potassium frequency stimulated the germination of radish seeds but the calcium frequency inhibited it. This is what we would expect, since the potassium frequency would enhance ambient potassium activity, help it replace membrane-bound calcium and *increase* permeability. The calcium frequency would increase the activity of ambient *calcium* ions so that they compete more effectively with potassium and *decrease* permeability. The two frequencies should therefore affect cell signaling and their metabolic consequences in opposite directions. This effect may prove useful in mitigating some of the claimed ill effects of electromagnetic exposure at 16 Hz and possibly other frequencies.

The hypothesis also explains some of the other more curious "frequency windows" phenomena. For example, Liboff et al. (1990) quote a maximum for diatom motility not only at the potassium resonant frequency but also at its even harmonics. However, the odd harmonics were inhibitory. Blackman (1990) quotes similar results for calcium release from brain tissue. We would

expect potassium resonance also to be supported by its harmonics and stimulate calcium release and diatom motility, but why were the odd harmonics inhibitory? This too can be explained by the hypothesis since the charge/mass ratio for the calcium ion is almost exactly double that for potassium. This means that the fundamental frequency for calcium corresponds to the first harmonic for potassium; thereafter every odd potassium harmonic corresponds to a calcium harmonic. Simultaneous calcium resonance where the harmonics share a frequency (the odd potassium harmonics) should therefore reverse the extra calcium release and consequent increase in permeability that might have occurred from potassium resonance. But not all frequency windows can be explained in terms of calcium and potassium harmonics. Blackman (1990) also reports some extrema for calcium release that didn't correspond to this relationship. We might be tempted to think that they could correspond to the resonant frequencies (or their harmonics) of other ions to allow them also to compete more effectively for the calcium sites on the membrane, but this needs further investigation.

11.3.6 How are ion channels involved?

Much research has been done on the possible roles of ion channels in bioelectromagnetic responses, but there is no convincing evidence that they are the *primary* receptors of the stimulus. By contrast, as we have just seen, there is considerable evidence that the phospholipid fraction of the cell membrane may be responsible. But if this is so, we must explain the observation by Barbier et al. (1996) that blocking calcium channels partially inhibited the electromagnetically-induced uptake of calcium in cultured cells, which suggests that ion channels must also play a role. This could be true, since even a non-specific increase in the permeability of the lipid fraction would partially short-circuit the membrane potential, reduce its voltage and open voltage-gated calcium channels. This would then amplify the effect, and make the whole response partially (but not completely) sensitive to the channel blocker. This interpretation is supported by Obo et al. (2002), who found no effect of electromagnetic treatment on calcium channel currents in animal cells when they were patch-clamped at a constant voltage. However, Baureus Koch et al. (2003) were able to show an increase in the passive leakage of $^{45}Ca^{2+}$ from inside-out spinach plasma membrane vesicles at the calcium frequency, presumably via voltage gated calcium channels, since these would be fully open in the absence of an actively maintained membrane potential. This is not inconsistent with our hypothesis since an electromagnetically-induced increase in calcium activity would also promote its *passive diffusion* through the ion channels. However, this still does not explain what happens under natural conditions, when these calcium channels would normally be held closed by the membrane potential. They also found no effect on calcium efflux at the potassium resonant frequency corresponding to that found by

Bawin et al. (1975), so it is probable that the two phenomena are not related and the hypothesis that it is the phospholipid fraction of the cell membrane that normally perceives the electromagnetic stimulus remains intact. However, we cannot deny that voltage gated calcium channels play a part in the biological responses to electromagnetic fields, but they seem only to amplify the response after the membrane potential has been reduced by the change in phospholipid permeability.

11.3.7 How do modulated radio waves give their effects?

Although much work has been done on the effects of radio waves on animals, relatively little has been done on their effects on plants. However, significant abnormalities have been detected in plants as diverse as pine trees (Selaga and Selaga 1996) and duckweed (Magone 1996) following exposure to pulsed radiation from radar installations, and there is no reason to believe that the mechanism by which they are perceived is significantly different from that in animals. It is widely accepted that continuous unmodulated radio waves are of too high a frequency to give biological effects but they do become effective when pulsed or amplitude modulated at a low frequency. It was once thought that non-linearities in the electrical properties of cell membranes rectify and demodulate the signal to regenerate the biologically-active low frequency. But these non-linearities disappear with carrier waves above a few MHz and the biological effects of modulated signals extend well beyond this. Also, even if the membranes could rectify these signals, it still does not explain how any extracted low-frequency component might work.

However, the present hypothesis explains how amplitude modulated radio signals, even at very high frequencies, can affect calcium release, membrane permeability and cell signaling; all without rectification. Let us draw an analogy with a child continuously bouncing a ball. The harder he hits it, the higher it bounces and the greater is its mean height. A coherent radio signal will do the same for the diffuse layer of unhydrated cations layer next to the negative surface of a membrane. Because the motion of the ions is limited in one direction by the membrane, the mean electrical centre of the layer will rise and fall in synchrony with the amplitude of the low-frequency envelope. These excursions are limited by the amplitude of the carrier wave and may be too small to give a direct biological response, particularly with high carrier frequencies. But if the modulating frequency corresponds to the resonant frequencies of any ambient ions, they will absorb energy from the slowly oscillating electrical centre of the cation layer and increase their chemical activity. This would explain the findings of Bawin et al. (1975) when they treated [potassium rich] brain slices with amplitude modulated VHF signals. The signals released calcium from the tissue, but only when the modulating frequency corresponded to the resonant frequency for potassium.

11.3.8 How does membrane permeability affect metabolism?

It has long been suspected that the multitude of different effects of weak electromagnetic radiation in different tissues is due to its promoting the entry of calcium into the cytosol, where it interferes with their characteristic cell signaling patterns. Liburdy et al. (1993) found evidence for this when they showed that the transcription of genes in cultured rat cells was enhanced by electromagnetic treatment and that this was associated with a calcium influx. They proposed that the extra calcium interfered with intracellular signaling cascades to affect gene transcription. Calcium ions often take part in these cascades, where they help to activate many aspects of metabolism. The huge variety of biological responses to electromagnetic fields can therefore be explained by the cells of different tissues or species having different cascades available for activation. These proposals also explain the poor reproducibility of many bioelectromagnetic experiments, since similar cells with different histories may have different cascades available. Also any factors that impair the cells ability to expel surplus calcium, such a starvation or stress, will affect the final response. Last but not least, a large increase in cell permeability to calcium may itself give stress responses, since cells are also programmed to use high levels of internal calcium as an indication of serious membrane damage. Responses to high calcium include the closure of gap junctions (Alberts et al. 2002) and possibly plasmodesmata, which could inhibit the transmission of an electrical signal through a tissue and affect our experiments. It may also be why electromagnetic exposure can also promote the transcription of stress-related genes such as that for the heat-shock protein hsp70 (Goodman et al. 1994). It therefore probable that an over-large increase in electromagnetic exposure could convert what might have been a stimulatory response into an inhibitory one.

Many attempts have been made to verify Liburdy's hypothesis by measuring cytosolic calcium with fluorescent probes such as fura-2. Although some significant effects of electromagnetic treatment have been reported, many of the results have been inconclusive. This is partly because it only measures transient free cytosolic calcium and not that which has been bound to organic molecules or homeostatically expelled from the cytosol. There are also technical problems, for example Ihrig et al. (1997) found that increasing the dose of UV to excite the probe gave an unexplained increase in the response to electromagnetic treatment. Perhaps the UV was damaging the ATP-driven calcium extrusion system to make electromagnetically induced calcium uptake more visible, which is consistent with Liburdy's proposals. Another extremely important finding by Ihrig et al. (1997) was that cells showing a significant increase in cytosolic calcium in response to electromagnetic treatment also showed a greater leakage of the fura-2. This suggests that the electromagnetically induced increase in membrane permeability *is not specific for calcium*. This is consistent with the present hypothesis and implies

that at least some of the metabolic effects of electromagnetic exposure may also be due to a more general breakdown of membrane containment and intracellular compartmentation.

11.3.9 Summary of the hypothesis

Time-varying electromagnetic fields induce eddy currents in and around living cells that remove some of the calcium ions that help to stabilize their membranes. These calcium ions are replaced by ions with a lower charge/mass ratio (mainly potassium) less able to stabilize the membrane. This increases the formation of transient pores to increase membrane permeability and affect metabolism.

Only low frequency signals are effective because a slow diffusion process must occur within each half-cycle for the membrane-bound calcium to be replaced by other ions. The *selective* removal of calcium from membranes can only occur at voltages close to the threshold for its release and results in "amplitude windows" for the biological response. Multiple amplitude windows occur because eukaryotic cells have several membrane systems, each with their own characteristics for calcium release.

Electromagnetic fields at specific frequencies are particularly effective and give rise to "frequency windows" for biological responses. These often correspond to the ion cyclotron resonance frequencies (and their harmonics) for biologically important ions; notably those for potassium and calcium. When an ion is exposed to its resonant frequency, it increases its kinetic energy and ability to compete for binding sites on cell membranes. Exposure at the potassium frequency (16 Hz) increases the kinetic energy of potassium ions, which enables it to replace calcium more easily in the cell membrane to *increase* permeability. Exposure at 32 Hz (the calcium frequency) increases the activity of calcium and makes it compete more effectively with potassium, to *reduce* membrane permeability. They therefore give opposite biological effects. The ability of the calcium frequency to reverse the effects of the potassium frequency (and possibly of those of other frequencies) is important, since it may provide a way to mitigate some of the claimed ill effects of exposure to weak time-varying electromagnetic fields.

Radio waves can also give biological effects, but only if they are pulsed or amplitude modulated at biologically active low frequencies. This is because the modulated wave makes the layer of cations next to the membrane swell and shrink in time with the low frequency envelope. The consequent rise and fall of its electrical centre feeds the resonance of ions tuned to the modulating frequency and allow them to accumulate enough energy, even from weak signals, to give biological effects.

The electromagnetically induced changes in membrane permeability affect metabolism principally by allowing calcium to enter the cytosol down a very large electrochemical gradient, where they give a variety of effects by

interfering with cell signaling. However, because the change in membrane permeability is not specific, there could also be other effects not necessarily attributable to calcium ingress.

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