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Research Highlights:

Photosynthesis, photorespiration, plant electrophysiology and the biological effects of electromagnetic fields.

The Evolution of Photosynthesis

I began working at Imperial College on photosynthesis, photorespiration and the way they evolved. Some of my ideas on their evolution were just shared with my students, but others were published. An interesting one is on "Why Trees are Green", which was first published in "Nature" and then as a feature article in the "New Scientist".

So why are trees green? If nature had done her job properly, they should be black to absorb all available light. I think they are green because the first known photosynthetic organisms were purple, like the present-day archaeobacterium *Halobacterium halobium*. They had no chlorophyll. Instead they had the purple pigment bacteriorhodopsin, which absorbs green light in the middle of the visible spectrum (it looks purple because we see a pigment as being the colours that it does not absorb).

However, they were unable to fix carbon dioxide and used light only as a source of energy. This primitive type of photosynthesis became less useful as the seas ran out of organic nutrients, most of it having been converted to CO₂. A good method of reducing CO₂ to organic compounds was essential if life was to survive. Nature probably had many tries at this, but the first really successful CO₂ reducing organisms were eubacteria living on the sea bottom. They used sulphur compounds in putrefying sediments as the source of electrons and light energy to transfer them to CO₂. But, because they had to live under a sea filled with purple archaeobacteria, most of the light they received was red and blue. Consequently, natural selection gave them a photosynthetic pigment that absorbed these colours strongly but reflected the less useful green, i.e. the green pigment chlorophyll.

Photosynthesis has come a long way since then. For example, it evolved to use water as a source of electrons instead of sulphur compounds. This meant that the green organisms were no longer confined to the sediments. They could now live in the surface waters or on land and had the whole visible spectrum for photosynthesis. They then evolved several accessory pigments that could absorb the newly available green wavelengths in the middle of the spectrum and pass their energy on to chlorophyll. Some plants are particularly good at this. Many seaweeds absorb most of the spectrum and look almost black. But most land

plants, perhaps because they have more light to play with, don't have such good accessory pigments, so trees still look green.

Photorespiration and the Evolution of the C4 Syndrome

The background to this topic is that most land plants (the C3 plants) waste massive amounts of energy because the enzyme used to fix CO₂ in the Calvin cycle also tries to fix oxygen. This destroys the CO₂ acceptor molecule and generates unwanted phosphoglycollate, which has to be metabolised to regenerate the lost acceptor. The process is called photorespiration it needs oxygen, releases CO₂, and uses about half the plant's photosynthetic energy. Photorespiration can be artificially inhibited by increasing the CO₂ concentration so that it can compete better with oxygen. This is done commercially by enriching the air in greenhouses with CO₂, when crop yield may be doubled. C4 plants do the same thing with a built-in enrichment system. This is based on their characteristic "kranz" leaf anatomy. Photosynthesis occurs in two layers of cells surrounding the vascular bundles. The outer layer (the mesophyll) fixes CO₂ using the enzyme PEP carboxylase to form 4-carbon organic acids. These are transported to the inner layer (the bundle sheath) where they are decarboxylated to regenerate CO₂, which is then refixed by normal photosynthesis. The system acts as a CO₂ pump, increasing the CO₂ concentration in the bundle sheath to a level where photorespiration is inhibited.

Since C4 plants often have high yields and make very good crops (e.g. maize and sugarcane) my first objective was to find a way to turn ordinary crop plants into C4 plants. To do this, I looked for a likely mechanism for the stepwise evolution of the C4 syndrome to see if it could be mimicked by artificial selection. It soon became apparent that the C4 pathway was complex and its mode of evolution totally unknown. The clue to how it could have arisen came from the kranz anatomy of C4 plants and the fact that they evolved in hot dry regions. They have evolved separately many times in different families. Their biochemistry is not always the same, but they always have "kranz" anatomy with the cells next to the vascular bundles fixing CO₂ from the decarboxylation of organic acids. I guessed that this may have evolved originally to recycle respiratory CO₂ from the roots when the stomata closed in dry conditions.

To check on this, one of my students (Mahdi Al-Mutawa) fed radioactive CO₂ to the roots of C4 plants in the dark and found that it was fixed into organic acids, apparently by PEP carboxylase. This in itself was not surprising, since plants normally fix CO₂ in the dark using PEP carboxylase as part of their respiratory pathway. It's part of a mechanism to make malic acid, which is the main substrate for plant mitochondria. The mitochondria use it to make their Krebs cycle acids and the amino acids derived from them. But what we found was that some of these acids were being transported to the shoot, where they seemed to be decarboxylated and the radioactive CO₂ refixed by photosynthesis. Most C3 plants are not very good at this (legumes are an exception, which may explain why they are widespread in the dry tropics). From this, we concluded that the an early stage in the evolution of the C4 syndrome was possibly the development of bundle sheath cells that were better adapted to decarboxylate organic acids coming from the roots and refix the CO₂.

If we accept this hypothesis, it is easy to see how the rest of the C4 pathway evolved. The selective force was dry conditions causing partially closed stomata. This favours plants generating a steep concentration gradient for CO₂ entering. In C3 plants, this is limited because photorespiration releases CO₂ and prevents the internal concentration falling to zero. C4 plants overcame this by inhibiting photorespiration. Since plants normally fix CO₂ into C4 organic acids in respiration,

all that was needed was to upgrade this capacity in the mesophyll cells and to transfer the products to the bundle sheath. They would then be decarboxylated by the system already used to deal with organic acids from the root. This released their CO₂, inhibited photorespiration and gave a steeper concentration gradient for CO₂ entering the plant. It could then manage with narrower stomatal apertures so less water was lost by transpiration. (typically, a C₄ plant loses only half as much as other plants per molecule of CO₂ fixed) and gave C₄ plants a tremendous advantage over most others in dry conditions.

Plant Electrophysiology

1. The Evolution of Action Potentials

Plants do not have nerves but can generate "action potentials" that look like nerve impulses but propagate through their ordinary cells. Sometimes they have a role in long distance communication, e.g. the folding of *Mimosa pudica* leaves when the plant is touched is controlled by action potentials propagating in the phloem. But this is not always the case. Some higher plant action potentials are confined to their cell of origin. Also microscopic unicells have them. This led me to think that they evolved originally for some other function, probably in a microscopic unicellular ancestor common to both animals and plants. But what did they do there and how did they later get their role in long distance communication?

I think their primary function was to switch off the cell's "membrane potential" when the membrane had been injured. The membrane potential is a voltage of several tens of millivolts generated by ion pumps across the cells external membrane. It is used, amongst other things, to supply energy for nutrient uptake. Its voltage is low, but because the membrane is so thin, the voltage gradient across the membrane is enormous (about ten million volts per metre!). The membrane therefore has to be an extremely good insulator. But if it were to be punctured, it would be unable to repair itself since the rapid flow of ions through the hole in this gradient would prevent it being sealed. Action potentials may have originally evolved in unicells to shut off the membrane potential for long enough for repair to occur. They are generated by ion channels in the cell membrane briefly opening in response to the drop in the voltage across the membrane that occurs when it is punctured. The response begins at the site of the injury, but spreads like a wave that short-circuits the whole of the cell's surface. This rapid spreading led to its being hi-jacked in evolution as a rapid means of communication in both plants and animals. Plants, such as *Mimosa* use their ordinary cells to transmit the signal and generally have a simple response. But animals developed special elongated cells to carry the signal. These were the first nerve cells, which eventually gave rise to our whole complex nervous system and control all our senses, movements and thoughts. [This hypothesis was first published in the "Journal of Theoretical Biology" and later as a feature article in the "New Scientist" under the title "The Cell Electric".]

2. DC Potentials and the Control of Cell Polarity

Animal and plant cells use steady DC electric currents to control their physiological polarity and direction of growth. A combination of ion pumps and channels generates a weak electric current flowing through the cell, with its point of entry normally determining the growing region. Physiological polarity is controlled partly by the electrophoretic distribution of differently charged membrane proteins along the cell's electrical axis and partly by the local ingress of calcium ions where the current enters, stimulating metabolism in the growing region.

We used a very sensitive device called a vibrating probe to measure current entering and leaving individual cells in plant tissue cultures. We wanted to know how neighbouring cells in a tissue maintained the same electrical polarity so that they could grow in the same direction. We found that individual cells generated

their own polar electric currents, but the direction of these currents could be changed by a brief application of a weak external current, after which the cell's new current was in the same direction as the one we had applied. This implies that the cells of a tissue may keep themselves aligned by sensing the currents generated by their neighbours and orienting their own currents to match. We also found that this reorientation of electrical polarity in an artificial current did not occur if calcium was missing from the culture medium or if the cell's calcium channels were blocked. This suggests that calcium entry via ion channels plays an essential role in the cells ability to detect and respond to weak electric currents. [See Mina and Goldsworthy, 1992].

3. Effects of Externally Applied Electric Fields on Growth.

There are many reports in the literature that plant growth is stimulated under high voltage lines. Work on this as a possible means of increasing agricultural yield began in the early 1900s and continued for several decades under the name "electroculture". It was later abandoned because the results were not always consistent and growth was often worse if the fields were applied under dry conditions. I wanted to know if this effect was real and, if so, why plants were so sensitive to electric fields

My research assistant (Alberto Lagoa) investigated this using plant tissue cultures. He found quite large stimulations of growth and they often became greener when weak electric currents were passed through them. Perhaps the cells were detecting the current by the same calcium-dependent mechanism that controls their polar growth and the calcium uptake was increasing their growth rate by acting as a second messenger. If so, the same thing could have been happening in the field experiments on electroculture, since similar currents carried by air ions would flow from the overhead wires to the crop. This ability to sense external currents may even have a selective advantage since strong electrical fields, similar to those used in electroculture, occur naturally under thunderclouds. These too should stimulate growth. It may even explain why your garden looks particularly green and lush after a thunderstorm. This may be an ecological advantage since the natural fields enable the plant to predict rain and activate its growth mechanisms in time to make the best use of it [See "Growing in Electric Fields", "New Scientist" Aug 23rd 1997].

4. Biological Effects of Physically Conditioned Water

Physically conditioned water is water that has been magnetically treated, either by passing it rapidly through a strong permanent magnetic field or exposing it to a much weaker pulsating one. Strictly speaking, it is not the water that is affected, but colloidal particles suspended in it as impurities. The electromagnetic treatment disturbs the shell of ions that normally surrounds these particles and (amongst other things) makes them more attractive to calcium ions

Conditioned water is used primarily to prevent and remove limescale in plumbing because of its calcium sequestering properties, but there are many reports that it also stimulates plant growth. Since the conditioning process is very cheap, there is a huge potential for increasing crop yield in hydroponics and even conventional agriculture at almost no cost, simply by irrigating with conditioned water. Quite a lot of people have already tried this, but the results have been inconsistent. Sometimes it worked, sometimes it didn't, no one knew why, and most people gave up.

However, we may be on the verge of a breakthrough. We have discovered that one of the factors affecting the response is the length of time for which the water is conditioned. We grew wheat seedlings in tap water that had been exposed to weak pulsating electromagnetic fields for varying lengths of time. In our set-up, we found that there were indeed stimulations of growth, with the best occurring with

water that had been conditioned for about 30 seconds. However, periods of conditioning in excess of a minute or more gave the opposite result and inhibited growth. Could this be the cause of all the inconsistent results? If so, we should be able to improve on things by subjecting the irrigation water to just the right level of conditioning. But what is the right level? It may be different for different water samples, different water conditioners and different crops. We needed a quick method of telling whether a given sample of conditioned water was likely to stimulate growth.

We tried various techniques, but in the end we discovered an electrical method that worked rather well. It is based on the principle that when plant roots take up nutrients, various ions flow in and out and change the voltage between the root and the surrounding medium. We discovered a simple way to measure this voltage and found that giving the roots conditioned water made it increase within a matter of minutes. Furthermore, the length of the conditioning period giving maximum voltage increase also corresponded to that giving maximum growth. This would not be surprising if growth correlates with nutrient uptake. More research is needed to discover how general this effect is, but we may have discovered a useful means to increase the yield of crops irrigated with conditioned water, just by getting the degree of conditioning right.

5. Mechanism of the Biological Effect of Conditioned Water

We set out to investigate this in yeast by adding either conditioned or non-conditioned water to the cultures and found that conditioned water increased the rate of cell division. Again, there was a maximum response when the water had been conditioned for about 30 seconds, with longer periods being inhibitory; i.e. it was just like the effect on wheat roots. What was happening? We had conditioned the water in a weak pulsating field of the order of microtesla, which is far too low to provide the extra energy needed for the growth effects. Instead it must be acting on a cellular control mechanism.

Perhaps there is a link between this and the ability of conditioned water to remove limescale. If conditioned water can remove calcium ions from limescale, could it not also remove some of the calcium ions that normally cross-link the negatively charged phospholipids in cell membranes? Phospholipids form the bulk of most cell membranes, which are only two molecules thick. The removal of the positively charged calcium ions that help bind them together would loosen the membrane structure and increase its permeability. If this resulted in extra free calcium leaking into the cell from outside (normally there is about a thousand times greater concentration of calcium on the outside than on the inside), it could stimulate metabolism and cell multiplication (cells normally regulate their rate of metabolism by controlling their internal calcium concentration). We checked on this by repeating the experiment in the presence of toxic heavy metal ions. This time the conditioned water inhibited cell multiplication, suggesting that there was indeed an increase in permeability, but this was now letting in more of the toxic ions.

6. Relationship to the Biological Effects of Electromagnetic Fields.

There are now a vast number of publications linking exposure to electromagnetic fields to various biological effects, at almost all levels of evolution, including man. In humans, they range from effects on brain function to the promotion of cancer and (amongst other things) have given cause for concern about the health effects of using mobile phones and various domestic appliances. However, there is as yet no proven explanation for the mechanism. But perhaps we now have one, based on our studies of conditioned water. I was struck by the similarity between our own results, with electromagnetically conditioned water, and those of many other workers who had applied similar electromagnetic fields directly to living organisms (including yeast). Perhaps we were looking at the same thing.

It would be quite reasonable to expect weak electromagnetic fields to also affect colloids in living cells so that they too could withdraw calcium from cell membranes, just like those in conditioned tap water. In this case, the internal

membranes should also be affected, resulting in the release of extra calcium from calcium stores inside the cell as well as from outside. However, they should both affect metabolism in a similar way and give similar effects.

This explains the very widespread but often inconsistent effects of weak electromagnetic fields. They are widespread because the control of metabolism by calcium appears to be universal in the animal and plant kingdoms. They are often inconsistent because calcium affects cells in many different ways. It tends to stimulate metabolism, but this can have a variety of effects depending on the nature of the cell, its physiological condition and what biochemical pathways and genes are available for activation. In addition, as we have discovered in yeast and wheat that it is possible to overdo the treatment, e.g. by conditioning the water for a too long a time. This may let in too much calcium, and there seems to be a stress response with growth being inhibited.

It is tempting to speculate from these results that intermittent exposure to weak time varying electromagnetic fields may not be totally harmful to living organisms (including human beings). By stimulating metabolism, they may even be beneficial. However, prolonged exposure may induce stress responses. A further risk is that the increase in metabolic rate may induce dormant but potentially cancerous cells in present some individuals to proliferate, which may account for the increase in the incidence of cancer in a small proportion of populations exposed to electromagnetic fields [see Goldsworthy, Whitney and Morris, 1999].

Selected Publications:

GOLDSWORTHY, A 1984 The cell electric. *New Scientist* **102** (1407), 14-15.

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GOLDSWORTHY, A 1996 Electrostimulation of cells by weak electric currents. In "*Electrical Manipulation of Cells*". Eds. Lynch, P., Davey, M.R. (Chapman and Hall, New York).

GOLDSWORTHY, A, WHITNEY, H and MORRIS, E 1999 Biological effects of physically conditioned water. *Water Research* **33**, 1618-1626.

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