The structural and functional effects of electromagnetic fields on the plasma membrane of *Vicia faba*, the broad bean.

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ABSTRACT

Vicia faba (broad bean) root-tip cells were exposed to electromagnetic fields at 50 and 60 Hz, square and sine waveforms and 0.1, 1, and 10 gauss. Levels of [^{3}H]-alanine uptake and ion efflux were measured at these parameters and compared to unexposed control seedlings. The ultrastructure of cortical cells from the zone of elongation exposed to a 1 gauss, 50 hertz, squarewave field was studied under the electron microscope.

In the first uptake trials alanine uptake via ATP dependant membrane carriers was stimulated by square waveform fields, but inhibited by 50 Hz fields. In the replicate trials alanine uptake was inhibited by both 50 and 60 hertz, square and sine waveform fields. The different response between trials was attributed to aging of the seeds used, owing to a six month chemical supply delay. This apparent aging of the seeds appeared to increase seedling susceptibility to modification by electromagnetic fields. The ion efflux trials saw no significant change in the pattern of ion efflux (as measured by conductivity) from exposed cells, although there was a significant decrease in hydrogen ion efflux at 0.1 and 1 gauss. A secondary inhibition effect on hydrogen ion efflux occurred with exposure to sine and square waveforms, but only in the presence of 0.1 and 1 gauss field amplitudes. The reduction in hydrogen efflux was most probably due to the inhibition of an active ATP dependent membrane carrier responsible for maintaining the transmembrane electrochemical gradient.

Under the electron microscope exposed cortex cells from the zone of elongation had significantly more pinocytotic vesicles than the controls. These vesicles were believed to be involved in bulk uptake of extracellular media, which may permit exposed cells to expand more rapidly than the controls.

Thus the functioning of three separate membrane transport systems were shown to be susceptible to functional modification, at least in the short term, by extremely low frequency electromagnetic fields. This introduces the potential for an enormous array of downstream effects to echo through-out the organism via signal transduction pathways.

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Chapter 1: INTRODUCTION

1.1 ELECTROMAGNETIC RESEARCH TODAY

Electromagnetic fields are a ubiquitous and unavoidable part of our industrialised world. Despite the conspicuous position of electromagnetic fields (EMF's) in the environment, there is still much to learn about how such a widespread phenomenon affects people and other organisms. Biological research into the effects of EMF's has in the past, and to some extent still is, subject to misgivings by some sections of the scientific community (Knave 1994, Philips, A. 1990b). This situation is due in no small part to extravagant claims made of the beneficial medical effects of extremely low frequency (ELF) EMF's made during the 1970's. Many of these claims have since been shown to be unfounded. Never-the-less, these studies have served to stimulate the interest of many serious scientific researchers.

Such research has resulted in the voice of caution being raised in the late 1970's, as increasing numbers of researchers drew correlations between levels of EMF exposure and elevated cancer risks especially in children (Bernhardt 1988, Bryant and Love 1989, Czerski 1988, Foster 1992, Frey 1993, Gadsdon and Emery 1976, Gledhill 1988, Litovitz *et al.* 1990, Repacholi 1988, Smith 1988, Stone 1992). Many of these reports have utilised highly emotive terms or have been sensationalised (Best 1990, Phillips, A. 1990a & 1990b, Smith 1988), resulting in a growing concern amongst the lay community over "the invisible threat in your own backyard". Upon closer examination, many of these reports have been found wanting, with some even out-rightly fraudulent, such as was recently seen in an article published in Science in 13 May 1994 (Bradley 1994). It was claimed that the application of low level EMF could drive chemical production of enantiomers toward one isomer or other, increasing the yield and reducing the cost of such production processes. The claim was withdrawn in the same journal on the 1 July (Clery and Bradley 1994) less than two months later, after numerous research teams failed to replicate the findings. Closer examination revealed that one of the original research team had "fixed" stock solutions used in the work. While such deliberate cheating is rare, problems with

deficiencies in scientific methodology or research techniques such as utilising inadequate controls (Myers 1985), failure to consider all possible sources of EMF exposure (Best 1990, Goodman, R. and Henderson 1991) or failure to fully describe the levels of exposure in their study subjects (Best 1990, Coleman *et al.* 1989) are all too common. In addition, some researchers have attempted to correlate the results of studies whose sites, subjects exposure levels and types of exposures had little or nothing in common (Paradisi *et al.* 1993). This comparing of studies as unalike as apples and oranges has unfortunately sometimes resulted in the publication of studies of limited scientific merit. Adding further confusion to an already inherently difficult and controversial field of research (Czerski 1988, Goodman, E. *et al.* 1986).

Currently the beneficial uses to which EMFs are being put are less widely advertised. These include accelerating chemical reactions and increasing the yields of chemical synthesis reactions (Bunting 1986), and a variety of healing properties from inducing the rejoining of stubborn bone fractures especially in the elderly (Bernhardt 1988, Czerski 1988, Fontanesi *et al.* 1986, Luben *et al.* 1982), to stimulating nerve regeneration, treating circulation disorders and soft tissue injuries (Binder *et al.* 1984). Balanced against these beneficial effects is the link between EMF's and several different ailments including cancer (Coleman *et al.* 1989, McDowall 1986, Taubes 1993), heart problems (Bernhardt 1988, Perry and Pearl 1988) and psychological disorders (Best 1990, Perry and Pearl 1988).

1.2 THE THESIS IN CONTEXT

Scientific research has recently demonstrated that ELF-EMFs can induce a bewildering array of reactions in living cells, both in *vitro* and in *vivo* (Frey 1993, Goodman, R. and Henderson 1991, Walleczek and Liburdy 1990). It is therefore important to understand how these fields impact upon living systems. As yet, however, little is known about how living organisms respond to ELF-EMF's (Bernhardt 1988, Goodman, R.and Henderson 1991, Male 1992, Sandweiss 1990). Also unknown is how the array of effects are induced within an organism, or which feature(s) of ELF-EMF induce the plethora of responses reported in the scientific literature.

The plasma membrane is a living cell's "doorway" to the world, across which nutrients are carried into the cell and through which the cell gains information from it's environment. Because of this interaction with the environment, the plasma membrane may be especially vulnerable to external stimuli. Many cellular functions can be modified by external stimuli, including EMF's (Frey 1993), and the plasma membrane might well be where such stimuli is perceived by the cell. Thus studying the effects of electromagnetism on the plasma membrane is especially relevant.

The relevance of this research is that it focuses on the effects of EMF exposure on the plasma membrane of *Vicia faba*, the broad bean. A plant system was chosen for four reasons. Firstly, because as far as could be determined by this author no results from previous studies into the effects of EMs exposures on the plasma membranes of plants have been done. Secondly, work can sometimes be performed using plant systems avoiding the ethical considerations involved in animal studies. Thirdly, work performed on plant systems can often be translated to animal systems, as there are many similarities. Finally, the economic importance of plants justifies the undertaking of such studies. As plants are the basis of nearly all the food-chains on earth, anything with the potential to disrupt their ability to gain nutrients from their environment should be considered important. *Vicia faba* was chosen because it has been widely used in scientific research, and as such, its requirements and tolerances as a biological system are well understood.

This thesis research includes both a structural and functional study of the plasma membrane. The functional aspect involved measuring the rate of amino acid transport across the plasma membrane into root-tip cells and investigating the levels of ions moving across the plasma membrane. In order to determine whether exposure to ELF-EMFs altered these two inter-related functions of nutrient transport across the plasma membrane. In both of these experiments, the roots of the plants were exposed to a variety of ELF-EMF's including three field intensities, two frequencies and two wave forms. The structural aspect of the research utilised an electron microscope to compare the ultrastructure of several membrane related organelles in control plants with those of plants

exposed to fields.

The careful use of controls and high replication numbers used in this work together with a wide variety of exposures and considerable care in maintaining uniformity of the environment, in the context of advances that have been made in membrane biology in recent years (Balnokin and Popva 1994, Brandt *et al.* 1992, Browning *et al.* 1992, Hansen 1990, Iseki *et al.* 1993, Lamfermeijer *et al.* 1990, Lemas and Fambrough 1993, Mata *et al.* 1993, Nagle and Scott 1994, Nordström *et al.*1994, Roos 1992, Soong *et al.* 1993, Williams *et al.* 1992, Xu, K. 1992) combine to make this study both topical and meaningful. It over-comes many of the problems such as inadequate controls seen in many previous studies. This research adds significantly to the growing body of knowledge on how ELF fields interact with biological membranes (Adey 1988, Blank 1992a, Rosen 1993, Paradisi *et al.* 1993, Osman and Cornell 1994, Luben 1991, Goodman,E. *et al.* 1986, García-Sancho *et al.* 1994, Farndale and Maroudas 1985, De Loecker *et al.* 1989 & 1990, Blank and Soo 1989).

1.3 THE THESIS AND IT'S AIMS

The primary aim of this research, was to determine whether the cells of *Vicia faba* respond to coherent alternating current (AC) electromagnetic fields, similar to those produced by domestic appliances, by modifying their behaviour at the level of the plasma membrane. Pursuit of this aim proceeded on two fronts, with research into the structural and functional effects of ELF-AC-EMFs on the plasma membrane.

(i) The functional study.

This involved an investigation of the transport of an amino acid and ions across the plasma membrane of *Vicia faba* root-tip cells, by membrane bound enzymes called ATPases.

ATPase enzymes are responsible for maintaining ion concentration and pH gradients across the plasma membranes and for transporting nutrients and ions into the cell. The movement of nutrients across biomembranes is usually coupled to the movement of ions, most commonly hydrogen ions. As such, measuring the

concentration of ions in the external media as well as the level of radio-labelled nutrient uptake, should more accurately reflect how EMF's affect transport across the plasma membrane than any single technique could.

Some studies (De Loecker1989 & 1990) have suggested that EMF's can induce a modification in the rate of amino acid transport in rat skin cells. While other studies had seen a modification in ion translocation across the plasma membrane in the presence of ELF-EMF's (Adey 1981, Coulton and Baker 1992, Czerski 1988, De Loecker *et al.* 1989 & 1990, Farndale and Maroudas 1985, García-Sancho *et al.* 1994, Liboff 1987, Walleczek and Liburdy 1990, Walleczek 1992), the work was, without exception, performed using animal cells.

The first aim of this the functional section of research was to determine whether or not the plasma membrane of *Vicia faba* root-tip cells responds to such ELFs. Certain ELF-EMFs may modify the rate at which alanine and/or ions, are transported across the plasma membrane. In order to determine this, the level of $[H^3]$-alanine uptake as well as the concentration of ions in the external media in which seedlings had been incubated was measured.

The concentration of ions in the external media was measured by both conductivity and pH. The ability of aqueous solutions to conduct electricity, or it's conductivity, is proportional to the concentration of ions dissolved in the solution. On the other hand the pH of an aqueous solution is the inverse of the log of the concentration of hydrogen ions in the solution. Any difference in the conductivity or pH of the media would be indicative of an alteration in the movement of ions across the plasma membrane. Any modification in extracellular concentrations could be due to either a change in the uptake of ions from the media and/or a modification in the rate of ion efflux from root cells.

The second aim of the membrane function section of was, if such a response is seen, then whether, and how, the response alters with the defining parameters of the EMF. To determine this, seedlings were incubated in the presence of twelve different electromagnetic fields, similar to those produced by domestic appliances.

(ii) The structural study.

The aim of this section of the research was to determine whether any physical abberrations were visible in the plasma membrane and associated organelles of root-tip cells exposed to EMF's. To this end the frequency of occurrence of various organelles was scored in the cortex cells of randomly selected root-tip sections from four seedlings exposed to an EMF and four control seedlings.

Until recently the microscopic structure of cells exposed to EMF's has remained largely unreported. Paradisi and associates (1993 and 1995) is one of the few exceptions to this rule. Their work, however was performed with protozoa.

Therefore this aspect of the research offers considerable potential towards determining a mechanism of interaction between EMF's and plant membrane systems.