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.

Chapter 2: LITERATURE REVIEW

Section 2.1: BIOELECTROMAGNETIC RESEARCH

2.10 INTRODUCTION.

(i) Bioelectromagnetic research.

Bioelectromagnetic research focuses on the effects of magnetic or electromagnetic fields (EMF) with living systems. The concept that organisms can sense external magnetic fields is not new. In fact it should be recognised that living organisms evolved in the presence the earths static magnetic field (Niwa *et al.* 1993). Much research has been performed which suggests that a variety of single cellular organisms and some animals can "sense" and respond behaviourally to fields of a similar intensity to the geomagnetic field. Such organisms include: bacteria (Foster 1992, Towe and Moench 1981), protozoans (Male 1993, Torres De Araujo *et al.* 1986), honey bees (Hsu and Chia-Wei 1994), hornets (Kisliuk and Islay 1977), land snails (Ossenkopp *et al.* 1990), sharks (Foster 1992), fish (Kirschvink *et al.* 1992, Quinn and Brannon 1982), birds (Able and Able 1995, Walcott 1974), humans (Kirschvink *et al.* 1992a). Most commonly such responses are navigational, with information from the geomagnetic field being used in migration (Able and Able 1995, Kirschvink *et al.* 1992, Quinn and Brannon 1982), homing (Walcott 1974), or orientation (Hsu and Chia-Wei 1994, Foster 1992, Kisliuk and Islay 1977, Towe and Moench 1981), see Appendix 1. Although in the case of land snails (Ossenkopp *et al.* 1990) and most developmental phases of hornets exposure to ELF-EMFs can be fatal. Thus it is widely acknowledged that some organisms do respond to the static geomagnetic field. An area which is more controversial is the effect of alternating electromagnetic fields of a similar intensity to the geomagnetic field (Anonymous 1994, Goodman, R.*et al.* 1993)

In recent years however public concern over exposures to the alternating current electromagnetic fields produced by electrical distribution equipment and domestic appliances has escalated. This concern has arisen in response to an

increasing number of published epidemiological studies which tenuously link exposure to such EMF with increased incidence of a variety of health disorders including cancers (Goodman, R. *et al.* 1993).

(ii) Non-ionising electromagnetic fields.

The primary emphasis of this review will be focused on low intensity, or non-ionising EMFs of the extremely low frequency (ELF) range. According to Reba Goodman *et al.* (1993) ELF-EMFs are those with frequencies below 300 Hz (see Appendix Two). This rather arbitrary setting covers the range of ELFs produced by domestic appliances (as shown in Appendix Three), as well as most of those produced by industrial equipment and power distribution systems.

Concerns about the effects of exposure to these types of fields on human health have been largely responsible for the rise of this field of research (Best 1990, Phillips, A. 1990a & 1990b). Therefore much of the work performed has been in the form of epidemiological studies of the effects of ELF-EMF exposures on various aspects of human health and welfare (Bernhardt 1988, Czerski 1988, Frey 1993, Gledhill 1988, Goodman *et al.* 1993, McDowall 1986, Smith 1988). As such a brief outline of this work will be given to set the background of non-ionising biomagnetic research.

2.11. THE HEALTH EFFECTS OF ELF ELECTROMAGNETIC FIELDS.

(i) Carcinogenicity.

Epidemiological studies have suggested that exposure to non-ionising EMFs elevates the rate of occurrence of various cancers amongst study subjects (Blanchard and Blackman 1994, Blank 1992a, Coleman *et al.* 1989, Foster 1992, Phillips,A. 1990a, Repacholi 1988, Speers *et al.* 1986, Stone 1992, Werthimer and Leeper 1987). Not all such studies have found correlations between EMF exposure levels and incidence of various cancers. For example a study carried out in Finland in 1993 by Verkasalo *et al.*, of 134000 children living near overhead powerlines found, "no statistically significant increases in cancers, leukaemia, or lymphomas. Although a statistically significant excess of nervous system tumours were found in boys with higher exposure levels". In summarising

this study the researchers concluded that, "a residential magnetic field (as generated by) transmission power lines does not contribute major to the public health problem regarding childhood cancers,". The researcher added a proviso to these findings in regard to the small number of cancers they observed making statistical analysis difficult.

As the numbers of published studies indicating a possible link between electromagnetic field exposure and cancer levels grew, a new set of researchers emerged. These people began to review and analyse the growing number of published epidemiological studies. In order to determine how strong the epidemiological evidence for the health effects of EMFs was Gary Taubes (1993), described the studies he reviewed as, "inconsistent and speculative". Kenneth Foster (1992) scrutinised several studies of the incidence of leukaemia, of which he states, "the increases in the odds ratios (a measure of relative risk) were near or below the level of statistical significance,". Not all reviewers were quite so sceptical. In 1993 Reba Goodman *et al.* described the epidemiological evidence as, "varying considerably; some (health) risks do seem traceable to EMF exposure". Thus the issue as to whether or not EMFs induce cancers is, as yet, far from being settled.

(ii) Infertility, pregnancy and infant health.

Another area of public concern about field exposures has arisen with the suggestion that exposure to EMFs could adversely affect fertility (Kanal *et al.* 1993 and Lundsberg *et al.* 1995), pregnancies (Best 1990, Werthimer and Leeper 1986) and Sudden Infant Death Syndrome, or cot death (Coghill 1990, Hanseen 1981). Not all studies have produced positive correlations between EMF exposure and negative reproductive effects (Kanal *et al.* 1993 and Lundsberg *et al.* 1995).

Thus the evidence implicating EMFs with infertility, abnormal pregnancy outcomes or SIDs is just as contradictory and sporadic as the carcinogenicity results. No consensus has been reached as yet by the scientific community about the putative effects of ELF-EMFs on human reproduction.

(iii) Other health effects.

Exposure to electromagnetic fields has also been linked to increased incidences of numerous other ailments including myocardial infarction, ischaemic heart disease, and depression, (Perry and Pearl 1988), chronic headaches, (Dowson *et al.* 1988), menstrual cycle modifications, clinical depression and abnormal levels of melatonin secretion (Best 1990). Przemyslaw Czerski (1988) suggested that EMFs might modify the threshold for cardiac stimulation. Reiter and Richardson (1992) indicate that circadian rhythms which are maintained by melatonin levels could be modified by exposure to ELF-EMFs which in turn could cause: fatigue and lethargy, mood alterations, endocrine malfunction, immunodeficiency, as well as increased risks of cancers. Bernhardt (1988) suggests a less direct route of potential harm via EMFs interfering with pace-makers. Finally, Smith (as cited in Phillips,A. 1990b) postulates that, "as many as one in one thousand people may be appreciably allergically affected by EM fields, amounting to 55,000 people functioning substantially below par in Britain alone,". A figure to stagger the imagination, but the statement should be recognised for what it is, the unsubstantiated opinion of one person, which is quite probably designed to shock.

Not all effects attributed to electromagnetic fields are negative. Some researchers have studied the putative healing properties on humans. One such study performed by Binder *et al.* (1984) which suggested that pulsed EMF (PEMF) induced healing of rotor cuff tendinitis.

(iv) Health effects synopsis.

The wide range of public health concerns have been raised around electromagnetic field exposures, but the "effects" are as yet unproven. Furthermore, considerable controversy still rages within the scientific community over the reliability of a large number of these studies; a situation which a large number of researchers, both within and outside the area, are all too ready to point out.

(v) Difficulties inherent in epidemiological studies.

The problems with interpreting the currently available body of research is

that such epidemiological studies have numerous inherent difficulties in methodology and unavoidable differences between study subjects and exposure parameters.

The most common criticism of epidemiological studies are the subject exposure parameters. This problem arises due to the difficulty involved in assessing the exposure levels of the human subjects (Best 1990, Israel 1994, Washburn *et al* 1994). This is due to the conflicting requirements of measuring instruments, and the complexity of the electromagnetic waves themselves and the diversity of potential sources of electromagnetic exposures. There is no consensus existing on where to collect EMF readings from, or how many readings should be taken (Delpizzo *et al.* 1991) and which measuring system(s) should be used to quantify exposures. Further complicating the process of assessing the ambient EMFs, the parameters of the fields that subjects are exposed to also needs to be related to the quantity of energy absorbed by the organism (Israel 1994).

Assessing EMF exposures is made more difficult because exposure systems and conditions differ between countries, areas within one country and even to some extent, between houses within an area (Siemiatycki 1993). Therefore no single formula for exposure assessment will function unilaterally. This dilemma makes for major complications in the evaluation of published studies (Siemiatycki 1993).

Another major challenge for epidemiological workers is the need to fully characterise environmental and behavioural factors likely to predispose subjects toward developing cancers or related disorders (Best 1990, Kanal 1993). Complicating factors such as: radon; smoking, both active and passive; alcohol consumption; age; diet; and an army of other potentially complicating factors. Failure to take such factors into consideration can invalidate the original intention of a study into EMF related disorders (Best *et al.* 1990).

(vi) Summary.

The epidemiological work involving human exposure to electromagnetic fields encompasses a large body of research, with much controversy, many

problems to be overcome and many questions yet to be answered. Nevertheless, it has been largely responsible for the rise of an allied field of research, into the effects of EMFs on living tissues: laboratory based *in vivo* and *in vitro* studies into the effects of electromagnetic fields.

2.12. THE LABORATORY STUDIES OF ELF ELECTROMAGNETIC FIELDS.

Under laboratory conditions, some of the dilemmas facing epidemiological researchers can be overcome. The exposure levels of the experimental tissues or organisms can be defined and closely controlled (Loscher and Mevissen 1994), to an extent that cannot be equalled in epidemiological studies (Rapley 1995). Experimental subjects can be contained within the EMFs, so that used in laboratory studies are substantially more uniform than those received by human subjects of the epidemiological studies (Rapley 1995). Additionally a range of environmental attributes can be regulated in the laboratory and a full description of subject behaviour (if animals are used), can be obtained.

However some of the experimental parameters used by laboratory based researchers will not accurately reflect the variable exposures received by the human subjects of epidemiological studies (Rapley 1995). As such the results of laboratory studies may be difficult to reconcile with those of the epidemiological studies.

2.13. LABORATORY SUPPORT FOR EPIDEMIOLOGICAL STUDIES.

Some studies by laboratory studies support the putative effects of EMFs on human health.

(i) The pineal gland and melatonin levels.

The hormone melatonin is produced by the pineal gland, and it is associated with an array of medical and psychological disorders (see Reiter and Richardson 1992 section 2.11-iv).

There have been a number of studies published which suggest that the synthesis of melatonin and its precursors by the pineal gland, is altered in the presence of EMFs (see Goodman, E. *et al.* 1995). Not all studies, however, have

seen any modification to melatonin levels in the presence of EMFs (Rudolph *et al.* 1988). Thus it is uncertain as to whether EMF exposure could induce changes in melatonin levels and the array of disorders associated with reduced melatonin levels (Best 1990 and Reiter and Richardson 1992).

One of the many functions of melatonin is in inhibiting a variety of cancers (Cos *et al.* 1991, Loscher and Mevissen 1994). Some research seems to suggest that this function of melatonin does appear to be impaired, or blocked by EMF exposures (Cos *et al.* 1991, Liburdy *et al.* 1993, Loscher and Mevissen 1994).

(ii) Embryonic developmental effects.

A large trial, replicated in several laboratories around the world, was carried out in 1989-1990 in order to determine whether PEMFs (of the types used by physiotherapists for healing induction) modified embryonic development (Berman *et al.* 1990). The results of this work varied between the different laboratories. That is, some saw an elevated incidence of deformities in exposed chick embryos, while other laboratories saw no difference between treatments and controls. An independent replication of this trial by Coulton and Baker (1991) also found no elevation in chicken embryo deformation in response to EMF exposures.

The variability in the results between experimental locals could have been due to several confounding factors including: differences in the local geomagnetic field, differences in exogenous EMFs produced by laboratory facilities (Coulton and Baker 1991, Goodman, E. *et al.* 1995), or even to subtle but telling differences in the strain of embryos used (Goodman, E. *et al.* 1995). These studies suggest that under very specific conditions EMF exposures might promote abnormal developmental processes. However as with the epidemiological studies there remains no clear consensus.

(iii) Healing induction.

Some research has provided support for the putative healing properties of PEMF exposures. Sakai *et al.* (1991) found that EMFs stimulated synthesis

glycoaminoglycan in bone and cartilage cells. A finding which the researchers suggested supports the clinical application of PEMFs to induce healing of bone and cartilage disorders.

Markov and Pilla (1994) demonstrated that 16 Hz EMF exposure could at different intensities either heavily inhibit or strongly stimulate the phosphorylation of light chain myosin in a cell free system derived from muscle tissue. The physiological importance of the phosphorylation of light chain myosin is that this step is a precursor step in the contraction of muscle fibres. The mechanism of this interaction is, as yet, unknown. But the researchers consider this work is the first step in determining the mechanism of the postulated curative effects of ELF-EMFs on muscle injuries.

In 1994 Vasil'eva *et al.*, presented a paper suggesting that clinical applications of ELF EMFs to 23 parasytolic (irregular heart-beat) children reduced levels of thromoxane in patients the blood plasma and activated Ca,Mg-ATPase in red blood cells. They observed a resultant improvement in the patients condition. The researchers suggested that further courses of "magnetotherapy may lower the risk of recurrent arrhythmia."

2.14, PLANTS ELECTROMAGNETIC FIELDS.

(i) The effects of intense static magnetic on plants.

The amount of literature on the bioeffects of fields on plants is considerably smaller than that covering the bioeffects of EMFs on humans. A large volume of literature linking exogenous fields to bioeffects on plants is concerned with interactions between static (DC) magnetic fields of very high magnitudes (Dunlop and Schmidt 1969, Mericle *et al.* 1964, Murphy 1942, Ssawostin 1930, Tolomei 1893), as compared to the geomagnetic field, or the non-ionising AC EMFs produced by electrical equipment (see Appendix Three).

Researchers have studied the effects of intense static magnetic fields on plants since last century (Tolomei 1893), with variable results. Some reports suggested growth responses in exposed plant material (Dunlop and Schmidt 1969, Mericle *et al.* 1964, Murphy 1942, Ssawostin 1930) others did not (Bayliss 1907, Favret *et al.* 1958, Mericle *et al.* 1964). Some other researchers

investigated the effects of intense static magnetic field exposure on protoplasmic streaming in plants, again with mixed results, some positive (Audus and Whish 1964, Ewart 1903, Ssawostin 1930), others negative (Audus and Whish 1964, Ssawostin 1930). But overall the results of such work was variable, and the results inconclusive (Audus and Whish 1964, Mericle *et al.* 1964).

(ii) Extremely low frequency fields and plants.

The earliest example of any priority being given to the study of low intensity magnetic/electromagnetic fields on plants (found by the author) was from the 1973 Workshop on Magnetic-Electric Fields and the Environment, held in Aspen, Colorado, (Franklin and Beal 1974). The conclusions of this workshop included a list of priorities for future research. The last item on this list was the need for, "better definition of field effects on living systems,". This recommendation was wide-ranging in its scope as it covered the full range of living plants, especially food plants. Suggestions of some areas involving plants requiring further consideration included: biorhythms, germination, mutation, and orientation.

At this same conference another workshop, The Workshop on Sources and Safe Levels of Electric and Magnetic fields (Kahn *et al.* 1974), also listed areas that were considered to require priority investigation. These concerned mostly high voltage fields, but also included electromagnetic fields such as would be produced by a proposed buried superconducting transmission line. The investigation of non-ionising ELF was listed as being of significantly lower priority. This attitude explains why the pre 1970's workers concentrated on intense fields rather than the non-ionising ELF-EMF. At the time, low frequency low intensity exposures were not as widely investigated as the larger fields, especially where plant interactions were concerned.

A study of the growth of trees under a naval communication's antenna in a forest in Northern Michigan (Reed *et al.* 1993), has received especially wide-spread attention since it's publication. The project results indicated that growth three species of forest trees was enhanced by exposure to 76 Hz electromagnetic radiation from a naval communications antenna. Two other species of trees did

modify their growth in response to the electromagnetic radiation.

However upon closer examination several problematic areas within this report become apparent. Plant ecologist Dr Jill Rapson Department of Ecology, of Massey University (New Zealand), suggests that one of the primary concerns arises from apparently inadequate pre-treatment validation, prior to the activation of the naval antennae, of the growth model used in the study with reference to the test and control forests . Only one year of pre-treatment data were collected; considering the average growth season in the region of 87 days and the weekly data collection interval, there can only be around 12 pre-treatment readings. Can 12 pre-treatment readings, from only one growth season, could provide an adequate validation for the growth model with reference to the forest under examination? This is a hard question to answer since the authors do not fully describe the parameters of growth model, how it was validified for the forests in question.

Another major problem pertains to the distance between the forests. 50 km is a considerably larger distance than would normally be considered advisable to have separating the test and control plots. This distance brings with it a new set of potentially confounding factors, such as differences in the soil and weather conditions. Dissimilarities exist in species proportions between the two forests, which further subvert the professed similarity between the test and control sites. On the subject of the two forests Dr Rapson states that the researchers "had no valid reason to expect similar growth between the two stands and although the growth model may have corrected for these differences, this process is not transparent in the published article". These problems strongly undermine the integrity of this paper as a serious piece of science.

The presentation of the paper is also concern, as in places it is difficult to follow what was done, for example; the statistical analysis of the data is another cause for concern. As one of the tables in the paper "±" ranges without describing whether they were: standard errors; standard deviations; or 95% confidence intervals (Henderson pers comm. 1995).

Despite these apparently serious shortfalls, discussions and citations of this study abound. One example of this is Constance Holden's 1995 paper,

where she cited unpublished findings by Thomas Burton, who compared aquatic algae living upstream of the naval antenna, with those within the emission field of the antenna. Burton's conclusions were, "our findings basically go along with the tree findings (by David Reed *et al.*) algal chlorophyll production was increased by electromagnetic exposure". Constance Holden's paper was balanced somewhat by her citing of John Stather, an expert in non-ionizing radiation at the U.K.'s National Radiation Protection Board. He describes the Michigan tree study as "intriguing" but in need of replication. Stather states that of the laboratory studies into the effects of EMF exposure that, "when properly controlled," most have turned out to be inconclusive.

In another discussion of the putative growth spurt of trees around the naval antenna Vincent Kiernan (1995) quotes one of the original researchers Glenn Mroz, as saying that "the study was not intended to determine the cause of the putative growth enhancement. But laboratory experiments (uncited) suggest that the electromagnetic fields might accelerate the transport of nutrients across the cell wall". This is a perplexing statement, as nutrients are not transported across the cell wall, but diffuse through it, with the neutrally charged, porous, cell wall offering almost no resistance to the diffusion of nutrients (Thomas pers comm. 1995). Mroz must be presumed to have been referring to the plasma membrane, since numerous laboratory studies suggest that some ELF-EMFs do modify the rate of transport of ions across the plasma membrane (Adey 1981, Coulton and Baker 1992, Czerski 1988, De Loecker et.al. 1989 & 1990, Fardale and Maroudas 1985, Liboff, A. *et al.* 1987, Walleczek 1992, Walleczek & Liburdy 1990). Therefore this is, most likely his point, which is potentially a valid one. All-in-all rather a lot has been read into this "intriguing" but flawed (perhaps fatally so) paper.

David Reed and associates cite three studies, which support the apparent "threshold effects" demonstrated in their paper. The studies were performed using plant systems, including; tomato yields, liverwort biomass and common garden cress, (*Lepidium sativium*) biomass. All three studies demonstrated enhanced growth at lower exposure levels, which fell away at higher exposures.

2.15. ELECTROMAGNETIC FIELD RESPONSES OBSERVED IN CELLS.

(i) Nuclear effects.

In order to determine whether, or not, EMFs interact with living organisms many researchers have seen the nucleus as a good place to look for EMF effects, as the nucleus is the control-room of the cell. In the past two decades many researchers have described modifications of cellular function at the level of the cell nucleus and the chromosome there-in. Effects seen on chromosome structure in cells exposed to ELF-EMFs have included: increased rate of mitotic indices in PEMFs (Dihel *et al.* 1985, Goodman, E. *et al.* 1986, Khalil and Qassem 1991), increased sister chromatid exchange at 50 Hz (Khalil and Qassem 1991), but at 60 Hz no increase in sister chromatid exchange was seen (Cohen *et al.* 1986), increase in chromosome breakage (Garćia-Sagredo and Montegude 1991) [although the fields used to induce the response were rather large, raising the question of heat damage, and chromosome puff enhancement in *Drosophila melanogaster* salivary gland tissue (Goodman, R. and Henderson 1988, Goodman, R. *et al.* 1992b).

With so many effects seen on chromosome structure it is not surprising to discover that modifications to the functioning of chromosomes exposed to ELF-EMFs have also been reported. Modified levels of transcription have been seen in a variety of organisms (Czerski 1988, Goodman, R. *et al.* 1983, 1987 & 1993, Goodman,R. and Henderson 1988, Litovitz *et al.* 1990, Phillips, J.L. and McChesney 1991), including human cells (Goodman, R. and Henderson 1991). In some cases the modified transcription levels have been isolated to very specific genes. These include: c-*fos*, c-*jun*, c-*myc* and the β sub-unit of protein kinase C (Phillips, J.L. *et al.* 1992), *c-myc* and histone H2B (Wei *et al.* 1990), the alpha subunit of RNA polymerase (Goodman, E. *et al.* 1993), the oncogenes *ras, src, raf* (Goodman, R. *et al.* 1992b), heat shock genes (Goodman, R. *et al.* 1992a & 1992b, Weisbrot *et al.* 1993), and growth related genes (Goodman, R. *et al.* 1992b). Another effect of EMFs at the nuclear level is that they appear to modify the responsiveness of cells to mitogens, compounds which stimulate cell division (Coghill 1990).

All this evidence of EMFs on DNA replication is described by Eugene

Goodman *et al.* (1995), as at best mixed; but in general, DNA does not appear to be significantly altered. Despite this, there is a growing consensus among investigators that transcription is altered by ELF-EMFs. Whether direct post-transcriptional effects occur has yet to be determined as there is no apparent disruption to routine physiological processes, such as growth and cell division. More usually a transient perturbation occurs followed by an adjustment to the normal homeostatic machinery in cells," (Goodman, E. *et al.* 1995). With a caveat on the putative effect of EMFs as promoters of cancers in the presence of a primary cancer promoters (Cain *et al.* 1993, Goodman, E. *et al.* 1995, Goodman, R. *et al.* 1993, Frey 1993, Loscher and Mevissen 1994, Paradisi *et al.* 1993), or by promoting the transcription of oncogenes (Goodman, R. *et al.* 1992b, Phillips, J.L. *et al.* 1992, Wei *et al.* 1990).

(ii) Biosynthesis.

Some studies have demonstrated that intracellular synthesis in exposed cells can be susceptible to modification by the presence of ELF-EMFs. Modifications to the rate of cellular synthesis of many products have been demonstrated including: protein production (Blank 1992a, Czerski 1988, De Loecker *et al.* 1989, Frey 1993, Goodman, R. and Henderson 1988), tubulin (Goodman, R. *et al.* 1992a), glycoaminoglycan (Sakai *et al.* 1991), thromoxane (Vasil´eva *et al.* 1994), and two apyrimidinic/apurinic endonucleases (Phillips, J.L. 1992), as well as a permanent significant reduction levels of a 5' nucleotidease in chick embryos (Moses and Martin 1993).

Without a doubt this list will continue to grow as research into the effects of EMFs on cells continues. What remains to be established is whether these effects are due to modifications in transcription, translation, regulation or enzyme functioning.

(iii) Electromagnetic fields and cellular membranes.

The cell membrane is a primary site of signal transduction for the control of cell growth, metabolism, and proliferation in response to chemical messengers such as ions, hormones, antigens and growth factors (Clery 1994). Because the

plasma membrane is seen as vital to normal cellular functioning much work has been performed on it. Thus interactions with ELF-EMFs at the level of cellular membranes are common in the scientific literature.

Effects on the movement of molecules across the plasma membrane into cells have included: increased active uptake of amino acids (De Loecker *et al.* 1989 & 1990), modified ligand binding to membranes (Czerski 1988, McLeod *et al.* 1987 & 1992), modified patterns of transmembrane ion movement (Czerski 1988), most notably enhanced level of Ca^{++} uptake (Liburdy 1992 & 1994, Lednev 1994) in the presence of EMFs modulated to ''cyclotron resonance frequencies'' (Fardale and Maroudas 1985, Liboff, A. *et al.* 1987, Walleczek and Liburdy 1990, McLeod *et al.* 1987, Sandweiss 1990, Walleczek 1992). However not all researchers have seen modifications in the level of Ca^{++} uptake in the presence of such EMFs (Coulton and Baker 1992). One report suggested EMFs were responsible for "activating" a membrane protein (ATPase) responsible for co-transporting calcium and magnesium into red blood cells (Vasil'eva *et al.* 1994). In 1988 Czerski suggested that ion channels in membranes might be opened by EMF exposure.

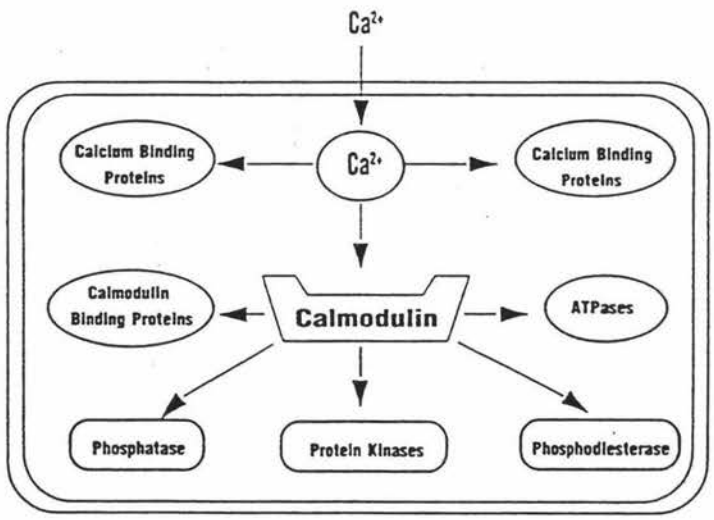
The cumulative effect of such studies is to suggest that the plasma membrane systems responsible for transporting molecules both into and out of living cells of a wide variety of different organisms might be sensitive to modification by ELF-EMFs. More generalised effects on the plasma membrane have included: the development of blister-like blebs (Paradisi *et al.* 1993), altered communication between bordering cells (Adey 1981, Cain *et al.* 1993), modified binding of hormones at the cell surface (Hiraki *et al.* 1987, Reiter and Richardson 1992) and slowed pre-synaptic membrane functioning, directly proportional to exposure duration (Rosen 1993 & 1993a).

Some researchers have reported modifications to various properties of the plasma membrane resulting from exposure to non-ionising ELF-EMFs. Membrane properties suggested as being subject to modification by EMFs include: the membrane potential (Durney *et al.* 1992), membrane conductivity and permittivity (Grandolfo *et al.* 1991), as well as the negative surface charging, and hydrophobicity (Goodman, E. *et al.* 1986), permanent reductions in levels of a

specific membrane related protein (Moses and Martin 1993) and altered transduction of extracellular signals across the membrane (Frey 1993).

The idea that the transduction of signals from the membrane to the cell is modified by EMF exposure seems to have gained considerable support amongst the research community (Brandt *et al.* 1992b, Best 1990, Cain *et al.* 1993, Czerski 1988, Dihel *et al.* 1985, Lednev 1994, Liburdy 1994, Litovitz *et al.* 1994, Luben 1982, 1991 & 1994). The putative signal transduction effects of EMF exposure could be of considerable biological significance, as the binding of ligands (especially Ca^{2+}), hormones (parathyroid hormone and melatonin) and other messengers to the membrane results in a variety of intracellular responses (Clery 1994, De Loecker 1990, Luben 1991 & 1994, Markov and Pilla 1994), see Fig, 2.1.

Fig 2.1, INTRACELLULAR CA^{2+}-CALMODULIN SIGNALLING.



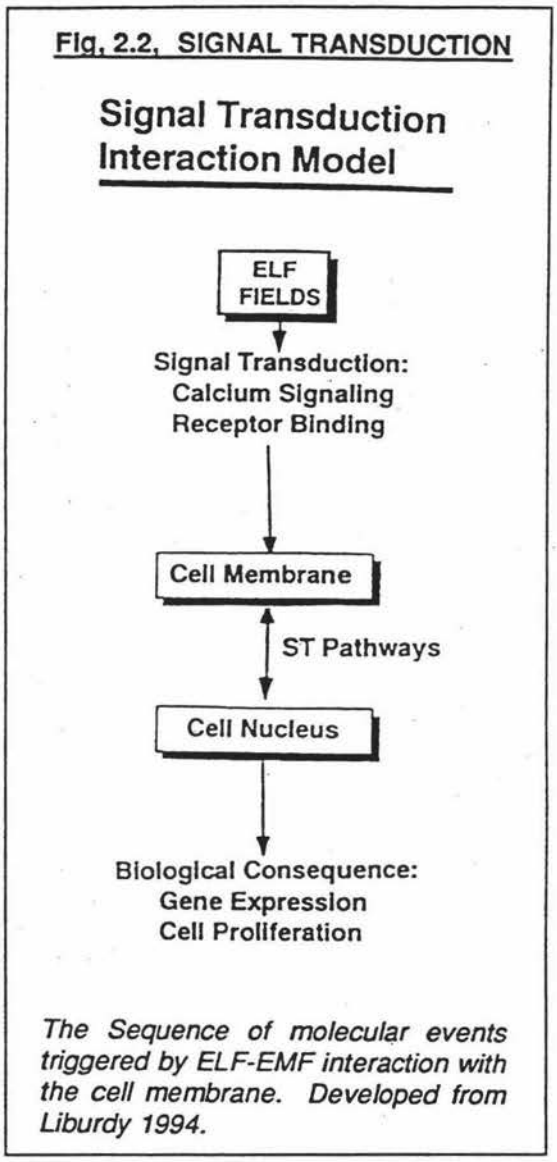
This diagram (from Markov and Pilla 1994) demonstrates some of the range of signalling activities that the ubiquitous Ca^{2+}-binding protein calmodulin is involved in within the cell.

Modified signal transduction responses could include secondary messenger activation and gene activation leading to altered macromelecular transcription and transduction (Clery 1994, Markov and Pilla 1994), see Fig. 2.2.

Not surprisingly then, many EMF investigators suggest cellular responses

to a variety of ELF EMF fields are induced via a membrane dependant mechanism, rather than a nuclear mechanism (Brandt *et al.* 1992b, Best 1990, Cain *et al.* 1993, Czerski 1988, Dihel *et al.* 1985, Goodman, E. *et al.* 1986, Liburdy 1994, Litovitz *et al.* 1994, Paradisi *et al.* 1993, Sakai *et al.* 1991, Tenforde 1992, Walleczek and Liburdy 1990, Walleczek 1992). In fact most theories addressing the mechanism of interaction between biological systems and EMFs suggest that the plasma membrane is the primary site of action (Goodman, E. *et al.* 1995). But if such a mechanism does occur, then it cannot be the sole site of interaction between EMFs and cells, as has been demonstrated by several experimenters using cell free systems (Goodman, E. *et al.* 1993).

Fig. 2.2. SIGNAL TRANSDUCTION



The Sequence of molecular events triggered by ELF-EMF interaction with the cell membrane. Developed from Liburdy 1994.

2.16. DISCUSSION OF LABORATORY STUDIES.

(i) Windows of effect.

The relation between the reaction induced by an EMF stimulus and the applied stimulus is neither simple, nor linear. In many instances there is a threshold level below which no response is observed, followed by a response that rapidly plateaus. Once the plateau of effect is achieved no further increase in response will be elicited by increasing field intensity or duration (Goodman, E. *et al.* 1995). In other instances there are frequency or intensity windows, within which responses can be seen to be very specific and differ between experimental systems. Outside these 'windows of effect' there is usually no response, or if

a response is observed then it is in the opposite direction (Goodman, E. *et al.* 1995).

These windowed responses can be demonstrated by a brief look at the published laboratory research studies into the effects of EMFs on cellular transcription. Much work has been done in this area and responses are usually elicited within specific windows. Thus the researchers often need to expose cells to a wide array of fields in order to see an array of responsive loci.

Not all chromosome transcription loci that are susceptible to modification by EMF exposure respond to the same field was demonstrated by Reba Goodman *et al.* (1992a & 1992b). She showed in three different *Drosophila* chromosomes that some genes will modify their transcription in response to a variety of EMFs, while other genes will respond to only one very specific field. Therefore in order to elicit a response from the genes requiring very specific stimulation the researcher(s) must hit upon the precise window of effect.

Frequency windows were demonstrated by Wei *et al.* (1990), who found that the genes they studied responded to 60 Hz fields by doubling the rate of transcription. But in the presence of 45 Hz fields, the response was quadruple that of the control.

In contrast to these responses to very specific windows Eugene Goodman *et al.* (1993) demonstrated using a cell free transcription system isolated from *Escherichia coli*, that similar fields of considerably different magnitudes can induce a similar response. But the trials that were exposed to weaker fields took longer to respond, eventually achieving a level of transcription similar to that in cells exposed to stronger fields.

Generally, however, in order to be sure of detecting a response to EMF stimulation in sensitive material bioeffects, researchers may to need apply a variety of different field conditions. Otherwise a lack of response could simply indicate that the correct combination of field conditions needed to induce an observable response was not meet.

(ii) Reproducibility.

Many published studies reporting bioresponses to EMF exposure have

proven difficult, or impossible, to replicate by independent researchers (Goodman, E. 1995). A study of modified embryonic development in the presence of non-ionising PEMFs performed by Berman *et al.* (1990) is a good example of the difficulties encountered in such studies. Berman and associates took considerable care to ensure uniformity in experimental procedures and fields applied to the developing chicken eggs. But still the results between the different laboratories varied considerably, and an independent replication of the experiment also came up negative (Coulton and Baker 1991).

Eugene Goodman *et al.* (1995), suggested that, "these studies strongly indicate that eggs from a different egg-laying flock, although obtained from the same supplier, will differ in their responsiveness to EMF exposure. Eggs from a given flock may either show susceptibility to a field in all experiments or no effect at all. If correct, this might in part explain why the carefully replicated ''Henhouse'' experiment (Berman *et al.* 1990) still showed differences between laboratories. In addition it also reinforced the previously voiced caution that subtle and as yet unidentified factors can impact on the final outcome of EMF experiments."

(iii) Confounding factors in laboratory studies.

With such examples to hand it is becoming increasingly apparent that considerable care must be expended by bioeffects researchers to fully describe the physical, chemical, physiological, and field parameters under which experiments are performed.

The factors that *must* be considered (according to Goodman, E. *et al.* 1995) include:

1. Differences however subtle in the serum batches used to incubate samples. Small differences can profoundly alter the growth and responsiveness of cells.
2. Utilisation of experimental strains which differ even in apparently trivial aspects. Cell density, the number of cells, and growth conditions prior to experimentation can potentially impact on the final result.
3. Local fields, both the static geomagnetic field and the ambient EMFs produced by assorted laboratory equipment and facilities need to be measured carefully

and recorded accurately in order for independent replications to be successful.

4. Serious consideration should be undertaken prior to a decision to utilise transformed cells (cancer cell lines). Question have been raised as to whether utilising abnormal cells to elucidate mechanisms of EMF interactions is a viable option.

5. Because of problems associated with exact replication of prior experiments, anybody attempting to replicate work should spend time in the original investigators laboratory, in order to understand all of the subtleties of the experiment prior to attempting a replication in their own laboratory.

It is to be hoped that careful consideration of these guide-lines by future investigators will eliminate most of the replication problems which currently plague this field of research.

2.17. THE CURRENT POSITION OF ELF-EMF BIOEFFECTS STUDIES.

A huge body of evidence, some of it circumstantial, has accumulated in the last two decades indicating that under some, often very specific, circumstances ELF-EMFs may interact with living systems.

(i) On going criticisms.

The biggest hurdle to gaining acceptance for the concept of non-ionising EMFs being able to affect the day to day functioning of living cells, and in turn entire organisms comes from theoretical considerations of the fields.

One major area of concern is that non-ionising electromagnetic/magnetic fields are low energy, ie. weak signals. So it is considered by many that the random thermal and electrical noise inside a living cell is loud enough to swamp any signal from these exogenous fields (Goodman, R. *et al.* 1993, Litovitz *et al.* 1994). Some reports suggest that the local thermal and electrical noise inside a cell may be 100 to 1000 times louder than the signal generated by low energy EMFs fields (Litovitz *et al.* 1994).

Furthermore non-ionising ELF fields are considered to be too weak to act on cellular mechanisms through known physical mechanism such as dielectrical breakdown or particle displacement (Goodman, R. *et al.* 1993).

How can this apparent paradox be reconciled? Work performed by Litovitz *et al.* (1994), may provide a clue. By using a previously developed system known to respond to ELF-EMFs the researchers demonstrated that the experimental cells responded to a coherent (rhythmical) EMF field. But when an incoherent 'magnetic noise' field was overlaid over the coherent field, the response disappeared. From this the investigators suggest that it is the temporal coherence of the artificial fields that enables them to be 'heard' over and above the intrinsically incoherent or random local thermal-electrical noise inside living cells (Litovitz *et al.* 1994).

Another possible answer to the enigma as to how the fields are heard has recently been posed by investigators working in an affiliated field of research studying magnetic field reception. The scientists were attempting to isolate the biologically precipitated ferromagnetic mineral, magnetite (Fe_3O_4) from various tissues (Kobayashi *et al.* 1995), but were encountering problems with contamination of their samples by environmental magnetite particles. This contamination Kobayashi *et al.* (1995) suggested might provide at least a partial answer as to how biological samples have appeared to be able to recognise non-ionising EMFs. The researchers found that under laboratory conditions contaminating magnetite particles (usually in the sub-100 nm size range), were present not only in dust in the air, but also adsorbed onto the surfaces of laboratory equipment, present within the glass and plastics, and even in reagent-grade laboratory chemicals and water.

Contamination of experimental systems by these particles might provide at least a potential answer as to how *in vitro* biological samples have appeared to be able to recognise non-ionising EMFs Kobayashi *et al.* (1995). Kobayashi and associates quoted previous work which had demonstrated that magnetite particles are readily taken up by human white blood cells, and it is quite likely that a wide range of other cells may also ingest the particles. To make matters worse many of the techniques used during cell-culture protocols concentrate these contaminants into the final rinsate, forcing the particles into close proximity with sample cells, in numbers far higher than would occur under natural conditions. Such particles ingested into, or adsorbed onto the surface of cells may provide

a simple mechanism to account for links between non-ionising EMF exposures and *in vitro* biological effects. Ferromagnetic particles interact strongly with magnetic fields and once adsorbed onto or ingested into cells in sufficient numbers the particles could potentially transfer mechanical energy gained from the external fields on to cellular structures, thereby overcoming the signal to noise ratio problems envisaged by physicists.

Kobayashi *et al.* opened the final paragraph of their communiqué with this statement, "We are not aware that the authors of any of the published studies on *in vitro* EMF effects have either controlled for, or attempted to reduce the levels of, ferromagnetic contamination." Kobayashi *et al.* softened this statement by adding that the particles are difficult to detect and quantify, but that their presence should not be ignored.

This communiqué offers a new challenge to bioeffects researchers contemplating *in vitro* studies, as only the utilisation of sophisticated "clean room" laboratory techniques can prevent contamination by these particles (Joe Kirschvink [co-author of the Kobayashi *et al.* communiqué], bionet.emf-bio March 1995). In their communiqué to the editor of Science (Kobayashi *et al.*) the researchers merely reported their finding and the possible implications it had on *in vitro* studies of EMF bioeffects. On the internet Joe Kirschvink went further quoting his co-author Michael Nesson as saying, "We're waving a red flag about what may be causing any supposed biological effects from these electromagnetic fields ... It is important, because our studies question the usefulness of many or most of the published laboratory research on the biological impact of EMFs."

Sorting out how much impact ferromagnetic particle effects have had on published work, or if indeed they have had any impact at all is going to take many years of careful work. It should be recognised, however, that any effect induced by the presence of ferromagnetic contaminants, would be restricted to *in vitro* laboratory studies. The "contamination" has no relevance to *in vivo* and human epidemiological studies and the results of these types of studies stand unchallenged by this very recent finding (Kobayashi *et al.*).

Thus this research is not undermined by contamination by ferromagnetic particle problems, as the research utilised intact *Vicia faba* seedlings.

(ii) In conclusion.

The position of bioelectromagnetic research today was very well summarised by Michael Nesson (in Joe Kirschvink internet March 1995) who said, "The jury is still very much out", with no concordance even between researchers or reviewing experts. Some acknowledge the sheer weight of experimental evidence as being undeniable (Frey 1993, Goodman, R. *et al.* 1993, Goodman, E. *et al.* 1995). Others do not (Knave 1994), with the main obstacle to acceptance of the bioeffects of non-ionising ELF-EMF being the lack of a clear mechanism of interaction on which researchers can agree upon (Anonymous 1994, Linder 1994, Loscher and Mevissen 1994, Wood 1993, Tenforde 1992). Many models have being put forward such as: cyclotron resonance (Durney et al 1992, Male 1992), conformational perturbation of enzymes (Browning 1992), electroconformational coupling (De Loecker 1989 & 1990, Weaver and Astumian 1992) but none have quietened the critics.

It should, however, be acknowledged that even if EMF exposures do induce health related disorders, the cases are rare and seldom fatal. Thus in the interim a policy of prudent avoidance of unnecessary exposures as suggested by The Consumer Institute (Anonymous 1994) would seem the most judicious policy.

Section 2.2:

MEMBRANE TRANSPORT OF NUTRIENTS

2.21. TRANSMEMBRANE TRANSPORT MECHANISMS IN PLANTS.

The movement of molecules across the semi (or selectively) permeable plasma membrane into cells can be divided into two major processes (Koryta 1991), by the cost in energy consumed to the cell. The first processes involves the passive, diffusion based, movement of molecules across the plasma membrane entailing no energy expenditure by the cell. The second uptake process is by active, energy consuming, translocation of molecules across the membrane by a transport system associated with enzymatic membrane proteins

called ATPase complexes, or ATPases (Iseki *et al.* 1993).

(i) Passive transport mechanisms.

The passive, or non energy-consuming movement of molecules across the plasma membrane can, in turn, be subdivided into two mechanisms. The first mechanism involves the diffusion of substrates directly across the lipid bilayer of the membrane, either by physio-chemical interaction between the molecule and the membrane or by simple diffusion of non-polar molecules (Iseki *et al.* 1993, Ohki and Spangler 1992). A diverse array of exogenous molecules gain entry into living cells by diffusion (Iseki *et al.* 1993). The second passive uptake mechanism involves the interaction of molecules with pores or channels in the membrane (Iseki *et al.* 1993, Koryta 1991, Läuger 1985, Lien and Rognes 1977, Watson and Fowden 1975, White and Tester 1994), which are specialised to facilitate the diffusion of specific molecules into the cell.

(ii) Active transport mechanisms.

The active (energy consuming), carrier based transport system can also be subdivided into two major groups. The first group of active carriers are called uniporters. Uniporters are ATP dependent carriers, which are relatively specific for the transportation of one type of substrate (Higgins 1992). Substrates known to be transported by uniporters include amino acids, sugars, inorganic ions, polysaccharides, peptides and even proteins. Some uniporters transport substrates into cells, while others export them, but none move substrates both ways. All the uniporters that have been studied have considerable structural similarity, even those isolated from distant phyla (Higgins 1992).

The second group of active (energy consuming) carrier based transport of molecules into cells is by ATP dependent carriers. These couple the transport of two, often quite different, molecules across membranes. Coupled active transport can, in turn, be subdivided into two subgroups (Koryta 1991), based upon the direction of movement of the solutes and on how the energy to transport the substrates is derived.

In the first group of active coupled ATP dependent carriers, the two

interrelated solute molecules are transported across the membrane in the same direction, with both chemicals moving against their electrochemical gradients. The carrier is called a symporter and the uptake process is described as primary active uptake (Koryta 1991). In primary active uptake the energy required to transport both molecules comes from ATP.

In the second group of coupled, ATP dependent carriers the two solute molecules are transported across the plasma membrane in opposite directions. One into and one out of the cell, with both molecules moving against their electrochemical potentials. In this case the carrier is called an antiporter and the uptake process is described as secondary active transport. In secondary active transport (Koryta 1991), the energy used to fuel the transportation of only one of the two solutes against its electrochemical potential is supplied by ATP. The energy needed to transport the second solute is stored in the electrochemical potential of the first solute, to be released as the molecule(s), usually ions, diffuses back across the membrane down its electrochemical gradient. This is the transport process often used to transport monosaccarides and amino acids into plant cells (Cooper *et al.* 1991, Koryta 1991).

It should be understood that the ratio between the two molecules transported in secondary active transport processes is not necessarily 1:1, as Koryta (1991) showed in a very detailed modelling of the functioning of an ATPase carrier responsible for translocating Na^+ and K^+ ions (described as a Na^+,K^+-ATPase). This ATPase carrier translocates three sodium ions out of the cell and two potassium ions into the cell.

Under some conditions, the transportation of the two solutes by antiporters can be uncoupled so that only the ion exporting phase of the transport mechanism is in action (Balnokin and Popova 1994, Cooper *et al.* 1991, Nørby 1987, Reinhold and Kaplan 1984), maintaining ion and the pH gradients across the plasma membrane.

(iii) Differentiating between active and passive transport.

The active and passive modes of substrate uptake into cells can readily be differentiated by the addition of the metabolic inhibitor DNP to the experimental media. DNP stops cellular production of ATP. With the supply of ATP cut, the energy supply to the active transport system runs out and the active mode of transport quickly stops (De Loecker *et al* 1989 & 1990, Lien and Rognes 1977). Lien and Rognes for example showed the presence of DNP considerably reduced the uptake of amino acids into barley leaf tissue, demonstrating that the majority of amino acid uptake occurs via an energy-dependent transport system.

2.22. AMINO ACID UPTAKE INTO PLANT CELLS.

In plants, sucrose is the most commonly translocated organic compound, although a range of organic compounds are translocated via the phloem, including amino acids (Peterson *et al.* 1977). Furthermore, amino acid transport within a plant is not entirely restricted to the phloem (Van Bel 1990). There is an exchange of solutes between the xylem and the phloem, so the movement of amino acids within a plant can be quite complex, with some amino acids likely to become available to leaves via xylem as well as to the roots via the phloem.

Amino acids do enter plant cells by the passive uptake process (Kinraide 1981, Lien and Rognes 1977, Reinhold *et al.* 1970). Reinhold *et al.* suggests that passive uptake of amino acids may represent a major form of amino acid uptake when extracellular concentrations of amino acids are high. Plants may require ATPase carriers to scavenge amino acids efficiently when extracellular concentrations are low.

(i) Active amino acid uptake.

ATPase carriers responsible for transporting amino acids into plant cells have been observed and reported upon since the 1950s, with researchers demonstrating active uptake of various amino acids into a variety of plant tissues (Birt and Hird 1956, Lea and Norris 1976, Lien and Rognes 1977, Trond and Nissen 1978, Watson and Fowden 1975). This work continued through the 1980s and 1990s (Borstlap and Schuumans 1988, Despeghel and Delrot 1983,

Dietz *et al.* 1991, Jung and Lüttge 1980, Kinraide 1981, Reinhold and Kaplan 1984, Thume and Dietz 1991, Xia and Saglio 1990, Zheng-Chang and Bush 1990 & 1991), with growing understanding of the kinetics and specificity of amino acid carrier systems in plant tissues.

(ii) Coupling amino acid transportation with that of ions.

The active, ATP dependent, membrane carriers responsible for the uptake of animo acids into plant tissues have been demonstrated to co-transport ions (Despeghel and Delrot 1983, Reinhold *et al.* 1970, Reinhold and Kaplan 1984). Thus the uptake of amino acids into plant cells is, at least in some instances, coupled to the movement of ions across the plasma membrane. The ion coupled to amino acid transport in plant tissues most usually involves Na^+ (Reinhold and Kaplan 1984).

(iii) ATPase carrier amino acid specificity.

The ATPase carriers responsible for conveying amino acids into plant cells, group amino acids for transportation purposes rather than recognising and transporting them individually (Williams *et al.* 1992). The groupings of amino acids recognised by ATPase carriers systems, are (usually) acidic, neutral and basic amino acids. Which group, or groups, of amino acids are transported by any given ATPase carrier varies. Individual ATPase carriers are usually specific for the transport of amino acids from only one or two of these groupings (Cooper *et al.* 1991, Dietz *et al.* 1990, Jung and Lüttge 1980, Kinraide 1981, Lien and Rognes 1977, Watson and Fowden 1975, Yingst 1988) but carriers have differential specificities. Jung (1980), on the other hand, found only one generalised amino acid transporter in *Lemna gibba* which transported all of the amino acids.

Thus no generalised rules exist by which amino acids are transported by a given carrier, with ATPase carriers from different plants, different tissues within a plant (Brandt *et al.* 1992, Lemas and Fambrough 1993, Williams *et al.* 1992, Xu, A *et al.* 1992), or from tissues of different developmental phases within a plant (Lanfermeijer *et al.* 1990, Reinhold *et al.1970*), all displaying quite different

specificities.

(iv) Kinetics of amino acid uptake.

The rate at which active amino acid transport systems translocate amino acids into plant cells has been shown to vary over time (Borstlap and Schuurmans 1988, Lien and Rognes 1977, Trond and Nissen 1978, Zheng-Chang and Bush 1991). One of the factors seen to modify the rate at which amino acids are transported into plant cells is the concentration of the amino acids themselves. Lien and Rognes (1977) demonstrated that at least 2-3 modes of amino acid uptake operate in a H^+-ATPase carrier in barley leaf tissue. At low solute concentrations uptake is by a slow linear mode (most likely a combination of diffusion and slow active transport according to Lien and Rognes). Immediately after the introduction of an enriched level of amino acids, uptake became rapid and linear (possibly due to the activation of a metabolically driven active uptake system Lien and Rognes suggest). After 10-12 minutes of rapid amino uptake, the rate of uptake fell back to a slower, although still elevated, linear uptake rate that persisted for at least 5 hours. Lien and Rognes work suggests the existence of multiple uptake modes for amino acids in plants. This suggestion is supported by numerous competitive inhibition and kinetic studies of amino acid uptake in plants (Dietz *et al.* 1991, Furman 1986, Jung and Lüttage 1980, Kinraide 1981, Koryta 1991, Reinhold *et al.* 1970, Reinhold and Kaplan 1984, Trond and Nissen 1977, Watson and Fowden 1975). These researchers have demonstrated that uptake of amino acids is via a range of carriers (and facilitated diffusion channels) with differing substrate (in this case amino acid) affinities, many of which have overlapping specificities.

(v) The structure of ATPase carriers.

ATPase carriers have three domains, a cytoplasmic domain, a membrane spanning domain and an extracellular domain. Of these the cytoplasmic domain is the largest and contains the site of ATP hydrolysis (Mata *et al.* 1993). There is considerable debate occurring over how many functional subunits make up the ATPase carrier (Chow and Forte 1992, Lemas and Fambrough 1993, Nørby

1987). Ligand binding studies suggest the protein to be a monomer, while *in situ* studies using physical methods suggest that the enzyme is a dimer or oligomer (Nørby 1987), although the debate is far from settled. After reviewing the relevant literature Nørby concluded that ATPase carrier functioning is due to tight structural associations between two or more functional units which behave as a single unit.

(vi) Functioning of ATPase carriers.

There are many models of ATPase carrier function (Cooper *et al.* 1991, Iseki 1993, Koryta 1991, Reinhold and Kaplan 1984, Repke and Schön 1992). Often the models differ only in quite minor details, but sometimes the differences are substantial. Most models agree that the ATPase carriers undergo conformational changes as they translocate substrates across membranes and that the energy for these conformational changes is supplied by ATP. Beyond this, however, the models begin to diverge, with little agreement on how many conformational changes occur and whether, or how extensively, the tertiary structure of the protein is affected by the conformational changes (Esmann *et al.* 1992, Karplus *et al.* 1981, Koryta 1991, Mata *et al.* 1993, Repke and Schön 1992, Robinson and Pratap 1993). Many researchers accept that any conformational changes occurring must be restricted to small localised regions within the enzyme (Karplus *et al.* 1981, Robinson and Pratap 1993), with "small flexions at key hinge regions" potentially inducing the dramatic alterations in relationships between the protein domains of the enzyme (Karplus *et al.*). Another area of disagreement is on how the substrates are physically translocated across the membrane (Koryta 1991, Mata *et al.*1993, Robinson and Pratap 1993).

Thus while much has been learned about the behaviour of active energy dependent carriers in cellular membrane there is a notable lack of consensus about how these effects are achieved.

(vii) Control of nutrient uptake by ATPase carriers.

It is clear that many different modes of amino acid uptake occur, which fits

in well with a model of the substrate concentration control of ATPase carrier transportation presented by Hansen (1990). In this model Hansen does not consider plant amino acid transporters specifically, but rather ATPase carriers as a generalised group. Hansen suggested that "The control behaviour of transporters may be compared with the management of city buses: At low passenger concentrations, the active buses can take more passengers if the number of people increases (by filling normally empty places). This is the direct effect of the substrate effect ... However, if the buses are saturated (all places occupied), the number of transported passengers per bus stays constant, and additional buses have to be activated in order to transport more *substrate* ... In that case somebody (the sensor) has to appraise the crowds and phone the agency, which activates more buses. The difference of the control models between biological transporters is that the adjustment to the load really works in the living cell." The model was supported by detailed mathematical modelling, which suggested that with active coupled ATPase carriers it would seem likely that two sensors would be required, one for binding to the substrate (the activator loop), and one for binding to the substrate antagonist (of the inhibitor loop). When Hansen presented his paper there was little experimental evidence for the existence of two separate sensors.

Solute concentration is not the only factor which controls active carrier transport. Zheng-Chang and Bush (1990 & 1991) found that the rate of amino acid transport into sugar beet leaves was dependant on extracellular pH rather than solute concentration. This seems to suggest that the control of the rate of active transport of amino acids into plant tissues is more complex than a feed-back loop operating alone.

2.23. THE MOVEMENT OF IONS ACROSS PLANT CELL MEMBRANES.

(i) Types of ion transport.

The movement of ions into plant cells occurs via many of the same pathways as amino acids, such as: passive diffusion (Iseki *et al.* 1993, Ohki and Spangler 1992), facilitated diffusion via pores or channels in the membrane (Iseki

et al. 1993, White and Tester 1994), active energy dependant uniporters (De Jaegere *et al.* 1993, Higgins 1992), and antiporters (Anner *et al.* 1984, Aronson 1985, Sanko 1990, Vasilets and Schwarz 1993).

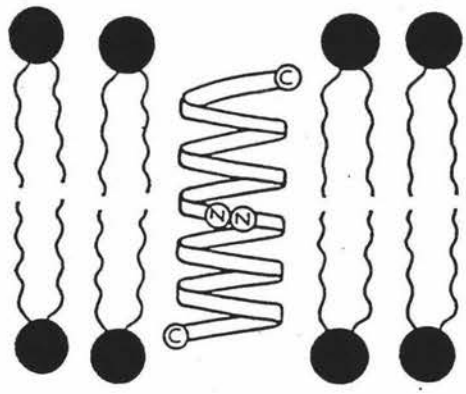
(ii) Transmembrane movement of ions via antiporters.

Antiporters responsible for transporting ions across cell membranes (Anner *et al.* 1984, Aronson 1985, Kirtley and Inesi 1992, Sanko 1990, Vasilets and Schwarz 1993) maintain separate concentration gradients for both ions, with one ion accumulated inside the cell and the other (usually H^+) actively pumped out of the cell. In some instances the export function of such carriers can be decoupled from the uptake function (Racker *et al.* 1992), so that the electrical and concentration gradients across the membrane are maintained.

(iii) Facilitated diffusion of ions across membranes.

Facilitated diffusion across biomembranes is better understood with ions than with amino acids. A variety of cellular membranes contain special pores, or ion channels, which facilitate the diffusion of ions across the membrane (Furman *et al.* 1986, Koryta 1991, Läuger 1985, McLeod *et al.* 1992). Koryta (1991) describes these ion channels as being composed of two helices attached by ethanol-amine end groups to the hydrophobic layers of the membrane (see Fig 2.3). The helices thus form a membrane spanning channel through which ions may diffuse.

Fig. 2.3, The structure of ion channels.



*The structure of ion channels proposed by Ivanov in Koryta 1991. **N** - the hydrophobic ethanol amine ends of of the helix, situated inside the lipid phase of the membrane. **C** - the hydrophylic carbon end groups.*

Koryta suggests that the interior of ionic channels is ordinarily partitioned by hydrogen bridges between the amino acid subunits of the channel, so that

ions can only move through the channel under the power of the electrochemical gradient. However, in response to an appropriate transmembrane electric potential (which is developed and maintained by the ATPase carriers in the membrane), the conformation of the channel can change (Läuger 1985, Koryta 1991, McLeod *et al.*1992) thereby disrupting the hydrogen bridges and facilitating the free diffusion of ions through the channel. Which ions are able to pass through the channel is dependent on the radius of the channel (Koryta), as hydrogen ions which are small pass through such channels readily, while larger ions such as lithium move through channels slowly. Läuger 1985 demonstrated that under some unusual conditions the rate of ion transport through such channels may be limited by the rate of conformational transitions of the channel.

(iv) Passive diffusion of ions across biomembranes.

Work by Iseki *et al.* (1993) using rat intestinal brush-border cells, has resulted in a model for the passive diffusion of organic cations across membrane being developed, which may have parallels in plant systems. The researchers demonstrated that movement of cations across biomembranes was linked to the transmembrane electrochemical gradient, with uptake stimulated when the exterior is acidic as compared to the interior. Furthermore, uptake was also seen to be proportional to the level of phosphatidylserine in the membrane.

From these observations Iseki *et al.* developed a model for ion transport which involves positively charged ions interacting electrostatically with the negatively charged molecule of phosphatidylserine (or other phospholipids) in the membrane. Once the cation is bound to the membrane it is able to diffuse up the pH gradient into the cell. The investigators conclude that the passive diffusion of cations into cells may not be described as free, since it depends upon both the limited availability of anionic phospholipids in the membrane and by the pH gradient across the membrane.

(v) Control of ion diffusion into cells.

In conclusion both passive diffusive and facilitated diffusion via ion channels may be subject to at least partial regulation by the transmembrane

electrical and chemical gradients set up by the active energy consuming membrane carriers.

2.24, EFFLUX OF SOLUTES FROM PLANT CELLS.

(i) General efflux.

Net, or visible, efflux of solutes (amino acids or ions) from plant cells could be due to several mechanisms (Reinhold and Kaplan 1984), such as: (1) it could constitute the "leak" of a "pump and leak" system. Such a system could involve simple diffusion, or be via a carrier-mediated leak pathway; (2) It might be a proton symport in the outward direction; (3) it could be the result of outward movement via a symport functioning 'in reverse' under some rather specific circumstances. Thus, observed or net, efflux is the end result of simultaneous influx and efflux processes. Previous studies of unidirectional fluxes of solutes using double labelling (Guy 1981) have shown that such apparent effluxes can actually be entirely due to a reduction in influx and not due to raised efflux at all.

(ii) Efflux of amino acids.

Efflux of amino acids from plant cells has been observed (Reinhold and Kaplan 1984), but the extent of such "leakage" is extremely low (Berry *et al.* 1981). One of the factors limiting such efflux is that a "pool" of amino acids is actively sequestered inside the vacuole. Once within the vacuole, amino acids are unable to "leak" back across the tonoplast into the cytosol (Reinhold and Kaplan 1984). But once the concentration of amino acids within a cell reaches a set plateau, efflux from the cell appears to be commensurate with influx (Guy *et al.* 1980).

2.25, FACTORS WHICH MODIFY UPTAKE ACROSS MEMBRANES.

Energy dependant membrane carriers are very sensitive to the conditions under which experiments are performed. A huge range of factors can critically affect the out-come of experiments. Thus an overview of these factors is

required so an understanding of the degree to which such trial conditions must be regulated and the environment standardised.

(i) Modification of ATPase function by environmental stimuli.

Researchers have demonstrated a variety of environmental factors involved in modifying the solute transporting behaviour of ATPase enzymes. These factors include: temperature (Lien and Rognes 1977), light levels (Lien and Rognes 1977), electric fields (Blank 1992a, Tsong and Astumian 1986 & 1988, Tsong *et al.* 1989, Tsong and Zhou 1992), and electromagnetic fields (De Loecker,W. *et al.* 1989 & 1990). Therefore all of these factors must be closely regulated, in order for meaningful results to be obtained.

(ii) Modification of ATPase function by the incubation media.

Not surprisingly, the incubation media used during uptake trials is also critical. The features of the extracellular media which modify carrier function include: the media pH (Bush 1991, Cooper *et al.* 1991, De Michelis *et al.* 1991, Despeghel and Delrot 1983, Hansen 1990, Kotyk *et al.* 1991, Lien and Rognes 1977, Williams *et al.* 1992, Zheng-Chang and Bush 1990 & 1991), the water potential of the media (Williams *et al.* 1992), ion concentrations (Brandt *et al.* 1992, Cooper *et al.* 1991, Lien and Rognes 1977, Yingst 1988), especially Ca^{++} (Brandt *et al.* 1992, Cooper *et al.* 1991, Lien and Rognes 1977, Yingst 1988); and competing solutes (Dietz *et al.* 1990, Jung and Lüttge 1980, Kinraide 1981, Reinhold and Kaplan 1984, Zheng-Chang and Bush 1990 & 1991).

(iii) Modification of ATPase function by various xenobiotic chemicals.

A variety of xenobiotic compounds have also been shown to regulate the function of ATPase carriers including: a variety of ions (Cooper *et al.* 1991, Nørby 1987, Thume and Dietz 1991, Yingst 1988) which is to be expected since the electrochemical gradient of one of the enzymes substrates provides the energy required to transport the second; the fungal toxin fusicoccin stimulates proton extrusion by antiporters in higher plants, which in turn stimulates uptake of the coupled compound (Colombo *et al.* 1978, De Michelis *et al.* 1991, Despeghel and

Delrot 1983) where once again the mode of effect is clearly on the proton motive force; vanadate (Williams *et al.* 1992), which increases membrane permeability to some ions; moderate concentrations of denaturating chemicals (Browning *et al.* 1992), nucleotides (Nørby 1987) which mimic the binding of the γ-phosphate of ATP; compounds which uncouple the cell's energy cycle (Cooper *et al.* 1991, Lien and Rognes 1977, Reinhold and Kaplan 1984, Trond and Nissen 1978); a monoclonal antibody to the cytoplasmic domain (Chow and Forte 1992). Many other chemicals can also effect the function of ATPase carriers (Brandt *et al.* 1992, Mata *et al.* 1993, Williams *et al.* 1992, Xu, A. *et al.* 1992), including heavy water, erythrosine B, diethylstilestrol (Kotyk *et al.* 1991).

(iv) ATPase control at the tissue level.

Different tissues within an organism may have different ATPase isoenzymes (Brandt *et al.* 1992, Lemas and Fambrough 1993, Williams *et al.* 1992 and Xu, A. *et al.* 1992). Often these isoenzymes have very different uptake kinetics and substrate affinities (Brandt 1992, Despeghel 1983, Williams 1992). ATPase isoenzymes within a tissue can also vary with the developmental phase of the tissue (Lanfermeijer *et al.* 1990, Reinhold *et al.* 1970).

ATPase carriers are also regulated at the tissue level by plant hormones such as auxin (Colombo *et al.* 1978).

(v) Control of ATPase functioning by biotic chemicals.

Many compounds within the cell have a role in modulating the function of membrane bound carriers. Some compounds regulate ATPase carriers directly, such as ATP concentrations (De Michelis *et al.* 1978, Nørby 1987, Thume and Dietz 1991, Xia 1990, Zheng-Chang and Bush 1991) where the nature of the effect is readily apparent. Although the mode of action may be subtle, as was shown by Dietz *et al.* 1990, who supplied target cells with an analogue of ATP that ATPases were unable to hydrolyse and saw enhanced amino acid uptake. This suggests that the cell may have been "fooled into believing it has a larger pool of ATP available than was actually present".

Associations between calcium ions and regulatory enzymes are very

important controllers of ATPases with researchers demonstrating that such conjoined compounds regulate a variety of membrane carriers (Brandt *et al.* 1992, Cooper *et al.* 1991, Lien and Rognes 1977, Yingst 1988), of which Ca^{++}-calmodulin is the most widely reported (Brandt *et al.* 1992, Cooper *et al.* 1991, Yingst 1988). Douglas Yingst published a good review of this phemonena in 1988 of which this is a brief synopsis.

Calnaktin: modifies the function of some ATPases in the presence of Ca. The nature of the interaction is not understood, but appears to involve sensitising the carrier to calcium levels within the cell. Other indirect modes of interaction for calnaktin have been suggested, such as interaction with a pathway being regulated by calcium levels, or involvement with a hormone-calcium loop. But these have yet to be confirmed.

Calmodulin: Some researchers maintain that calmodulin acts on the carriers via synergistic interaction with other controllers and Ca_i rather than by a direct interaction. Under differing conditions calmodulin may inhibit or stimulate carrier functioning.

Protein kinase C: the effect of this protein is the least understood of the three, while it appears to stimulate ion pumping; so do phorbol esters. Phorbol esters in turn stimulate protein kinase C levels as part of a feed-back regulatory loop. So it is not known whether the protein kinase C, or any one of several associated compounds related to the Ca/hormone binding pathway such as diacylglycerol are responsible for the observed effect. Yingst suggests that the effect on the ATPase could be attributed to a more general alteration in membrane lipids, rather than a specific stimulation of the pump by protein kinase C. This opinion is not shared by Nordström *et al.* (1994) who suggest that protein kinase C is responsible for maintaining the cytosolic pH of the cell by regulating the H^+ activities of antiporters.

2.26. ELECTROMAGNETIC FIELDS AND MEMBRANE TRANSPORT.

(i) Via ion channels.

It has been suggested (McLeod *et al.* 1992) that a combination of alternating and static EMFs of specific amplitudes corresponding to the

theoretical conditions needed for cyclotron resonance of unhydrated calcium ions enhanced the uptake of calcium ions. McLeod *et al.* suggested the fields act by gating the conformational changes of the ion channels, so controlling the transit of ions through the channels.

(ii) Via ATPase carriers.

Some research suggestive of an effect of EMFs on ATPase carriers have been published (Clery 1993, Del Giudice *et al.* 1988, Goodman, E. *et al.* 1995, Vasil´eva *et al.* 1994). The most convincing of which was De Loecker *et al.* 1989 and 1990, who determined that the movement of two different amino acids into rat epithelial cells was enhanced by specific EMF exposures. However, this effect was only apparent when the normal concentration gradients of Na^+ and K^+ across the plasma membrane of the target cells were undisturbed, demonstrating that the uptake of these two amino acids was via a system of antiporters. De Loecker *et al.* suggested that the modification of ATPase functioning was induced by the carriers, deriving energy for conformational changes directly from the electromagnetic fields by a process termed electroconformational coupling.

(iii) EMFs and target molecule conformational changes.

In both the above examples the researchers concluded that the EMFs stimulated biological response by inducing a conformational change in target structures. That such conformational changes do occur, at least in ATPase carriers, is gaining acceptance (Esmann *et al.* 1992, Mata *et al.* 1993, Repke and Schön 1992, Robinson and Pratap 1993). But there is little consensus as to how many changes occur, or how extensively they affect the structure of the molecule.

Electroconformational coupling model as an explanation for EMF interactions is reasonable only if the required conformational changes involved are minor, such as the "small flexions at key hinge regions" of ATPases as suggested by Karplus *et al.* 1981, or modifications to the hydrogen bonds of ion channels as proposed by Koryta 1991. Otherwise the type of low energy EMFs seen to induce biological responses would appear to be too weak to physically displace particles (Goodman, R. *et al.* 1993), a significant force would be required

to induce the major three dimensional changes in the structure of biological molecules researchers that Robinson and Pratap 1993 suggest occur during routine functioning of ATPase carriers.

Therefore such models of EMF interactions are speculative pending better understanding of the detailed functioning of the target molecules and the control systems governing them. The situation has been very clearly stated by the eminent EMF researcher Allan Frey (bionet.emf-bio, January 1995) "The problem in the EMF experiments is also due, in part, to the need for basic biology to advance further. And that is happening now at an accelerating and phenomenal pace." Thus determining how an exposed biological system responds to EMFs may be valuable in determining how the interaction was induced.

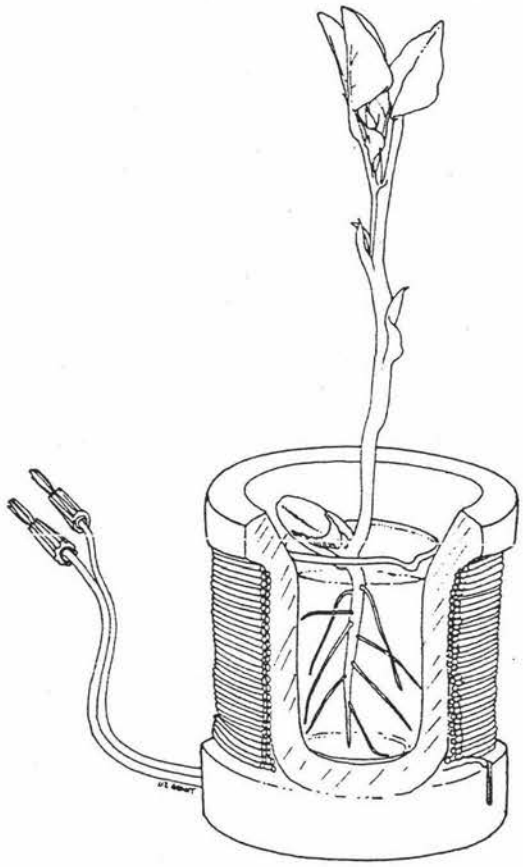
Chapter 3:
MATERIALS AND METHODS.

Section 3.1: MEMBRANE FUNCTION STUDIES

3.10, SEEDLING PREPARATION.

Vicia faba, variety "Coles Early Dwarf," was used for all experimental work. Broad beans were grown in a 2:1 medium vermiculite to coarse river sand for 7 days in a controlled environment growth chamber with a 24 hour photo-period at 21°C +/- 1°C. The seedlings were then gently removed from the media and all traces were washed off the roots with distilled water, taking care to avoid damaging any of the root initials.

Fig 3.1, THE INCUBATION APPARATUS



During alanine uptake trials four seedlings were incubated inside the coil, while only one was incubated at a time during the ion efflux trials.

Seedlings were then screened for uniform development. Any poorly germinated seedlings were discarded, as were those with pronounced lateral roots which could be subject to damage during transplanting. Twelve to sixteen seedlings from each batch, with few or no lateral root initials and a well grown epicotyl, were selected. The primary root was then excised approximately 4 cm below the seed. The plants were then placed on polystyrene floats with their roots immersed in 2 litres of Watson's (*et al.* 1975) liquid

incubation media (see appendix four) and returned to the growth chamber for a further four to five days, until seedlings were 11 to 12 days old. Alanine uptake experiments were performed with either 11 or 12 day old seedlings, dependent on availability, as at this age the seedlings were in similar developmental stage.

Prior to initiating experimental work, beans were once more screened for uniformity of development. Only plants with healthy well developed lateral roots, and a healthy shoot were used for experiments.

3.11, THE ROOT-TIP SAMPLES.

(i) Root-tip fresh weight.

To determine the average weight of the root-tips used, 120 root-tips, 15 mm long, were collected from both eleven and twelve-day old seedlings. These samples were weighed immediately after excision, as biological tissue samples dehydrate very rapidly once detached from the plant.

(ii) Root-tip dry weight.

The eleven-day old root-tip samples were dried at 98° C for two days and re-weighed.

3.12, [^{3}H]-ALANINE UPTAKE TRIALS.

(i) Radioactive pulse.

The procedure used to measure the level of [^{3}H]-Alanine movement across the plasma membrane into the root-tip cells was the same in all trials. Prior to starting uptake trials four seedlings were transferred into approximately 400 ml of fresh liquid media for 30 minutes and allowed to acclimatise. Seedlings were then gently transferred into a 25 ml beaker (taking care to avoid damaging any of the roots) containing 20 ml liquid media, adjusted to pH 6.5, containing 37 kBq cm^{-3} (1 μCi/ml, see appendix five for a description of these measurement systems) [^{3}H]-Alanine (Amersham, New Zealand). The beaker was immediately placed inside the magnetic coil (see FIG 3.1), in a small cabinet inside the growth room, for a 40 minute radioactive pulse period. (The specifications of the magnetic coil can be found in appendix six). After the pulse the seedlings were plunged into

a 200 ml becker of ice cold distilled water to stop uptake and moved to a radioactive lab for the next stage. Once there (less than 1 minute in transit) the seedlings were removed one at a time and six root-tips, randomly selected, of 1.5 cm were excised from each plant.

(ii) Control trials.

During control trials, the plants received no exposure to electromagnetic fields other than the local AC and DC EMFs. The beaker containing the plants was placed inside the coil, without it being turned on, to ensure that there was only one difference, the electromagnetic field, between the control and the test trials. The were no ambient AC EMFs of greater than 1mG in the cabinet where the trials were performed.

(iii) Chemical inhibition of uptake trials.

These trials were performed under identical conditions to the controls. But with the chemical inhibitors of ATPase enzymes, ouabain or dinitrophenol added to the media at a concentration of 10^{-3}M prior to the start of the pulse. The incubation proceeded as with the controls.

(iv) Electromagnetic exposure trials.

Plants in these trials were treated identically to the controls, with the exception that during the radioactive pulse period, test plants were exposed to continuous alternating electromagnetic fields with the following characteristics:

field intensities of: 0.1, 1 or 10 Gauss;

field frequencies of: 50 or 60 Hz;

sine or square waveform;

(see appendix five for a description of these measurements). The electrical component of all fields was considered to be negligible due to the low frequencies used with respect to the coil's physical dimensions.

(v) Apoplastic wash.

The six root-tips from each plant (total of twenty four per trial) were placed in a flask containing 100 ml distilled water and shaken gently for a five minute rinse. The root-tip samples were rinsed a thus three times to wash the excess [^{3}H]-Alanine from the root apoplast.

(vi) Scintillation counting.

Each root-tip was transferred to a clean scintillation vial containing 350 µl of deionised water. Once the root-tip samples were sealed in scintillation vials, the samples could be held for up to two hours, allowing a further trial to proceed in tandem with the first. At approximately 4.30 pm, 1 ml of NCS-I (tissue solubiliser form Amersham) was added to each vial. This step was performed under minimal light levels to avoid problems with chemiluminescence. The vials were then shaken slowly at 47.5°C (+/- 2.5°C) in a Gallenkamp orbital incubator for incubation overnight. At 9 am the temperature of the incubator was turned down to 20°C to cool the vials while inside the incubator. Once the vials reached room temperature, approximately 30 minutes later, 13 ml of OCS (xylene based organic scintillant, from Amersham) was added to each vial, again under minimal light conditions. Samples were then read on a scintillation counter as soon as possible, within a few hours, to reduce the impact of chemiluminescence on the DPM readings.

Experimental trials were performed in two phases. Early trials to determine experimental parameters were read on a Beckman LS3801 Scintillation Counter. This group included the uptake time course discussed in Chapter 4. The amino acid uptake trials, both tests and controls, were read on a Wallac 1409 Liquid Scintillation Counter. The Wallac scintillation counter has an internal programme designed for the reading of xylene based cocktails, which was used for reading all the alanine uptake trials. This programme compensates for false readings (or chemiluminescence) form the cocktail, thus providing a more accurate count.

(vii) Data collection and analysis.

Samples were read for one minute on the scintillation counter and the level

of radioactive alanine in the sample recorded as disintegrations per minute (DPM, see Appendix five for a explanation of this measure). The results for all 24 samples, from four different plants, were pooled to determine the mean uptake and standard errors for each trial. These results were then analysed by the SAS General Linear Models Procedure to determine the significance of these results. Results were further analysed using the Duncan's multiple range test for independent variables to determine the effect of the different field parameters (field intensity, frequency and wavoform) on [^{3}H]-alanine movement across the plasma membrane.

(viii) Replications.

The control trial was replicated three times, giving a total of 96 readings from twelve plants over four trials. This provided a solid statistical basis upon which to evaluate the effect of the various electromagnetic fields on the movement of [^{3}H]-Alanine across plasma membranes of the root-tip cells.

3.13. ION EFFLUX TRIALS.

(i) Experimental procedure for efflux trials.

The broad beans used in those trials were grown in the same manner as those used for amino acid uptake trials. Each seedling was rinsed three times with deionised water, and gently shaken dry. A single seedling was then placed in a 25 ml beaker, taking care to avoid damaging any of the roots. The beaker containing 20 ml of deionised water was placed inside the coil as shown in Fig 3.1. Test plants were exposed to the same range of electromagnetic fields as was used in the alanine uptake experiments previously described. After 15 minutes, the plant was discarded and the liquid was transferred to a clean opaque plastic scintillation vials. Blanks contained only deionised water, with separate blanks used for the pH and the conductivity readings. Each experiment was replicated four times with 11 day old beans, and five times using 12 day old bean seedlings giving a total of ten replicates for each trial. Samples were stored in a refrigerator at 4°C until all samples were collected.

(ii) Conductivity measurement.

Conductivity of the deionised water (as microsiemens, see Appendix five for a description of this measurement system), in which the seedlings were incubated was measured, inside a 20°C controlled temperature chamber using a Radiometer Copenhagen CDM-83 conductivity meter. Samples were read on the auto range setting using a CDC-304 probe calibrated with NaCl.

After these tests the samples of incubation media were retained at 4°C for later reading on a pH meter.

(iii) pH measurement.

The same samples were then measured for pH using a Solstat pH/mV meter EMP-1000, calibrated first to pH 7 then to pH 4. The meter was recalibrated in this way after each set of ten replicates. To produce the greatest possible uniformity in readings, the pH of the samples was recorded exactly two minutes after the probe was immersed in the sample to reduce any instability.

Section 3.2: MEMBRANE STRUCTURE STUDIES.

3.20. SAMPLE PREPARATION.

(i) Seedling preparation.

Seedlings were grown as described in section 3.1.1, then carefully transported, in fresh incubation media, to the Keith Williamson electron microscope unit. The four control seedlings were fixed immediately on arrival. The test seedlings were incubated for 40 minutes inside a continuous alternating electromagnetic field of: 1 gauss, 50 Hz, with a square waveform. Root samples were then excised 15 mm back from the root-tip.

(ii) Fixation.

0.5 mm sections were excised from the cut end of root samples and transferred to the primary fixative comprising: 3% gluteraldehyde, 2% formaldehyde in 0.1M PO_4 buffer, pH 7.2 at room temperature for 2 hours.

Samples were then given three washes in phosphate buffer at room temperature.

(iii) Dehydration series.

Samples were dehydrated in a graded acetone series of: 25%, 50%, 75%, 95%, and twice at 100%

(iv) Embedding and curing.

Continuously stirred samples were infiltrated in a 50/50 mix of acetone and Polarbed 812 epoxy resin overnight at room temperature.

(v) Sectioning.

Once cured, the resin blocks were then closely trimmed around samples. One micrometre sections were cut from the blocks and heat mounted on glass microscope slides. The sections were then stained with 0.05% Toluidine Blue in 0.1M phosphate buffer and viewed under a light microscope to locate areas of interest, such as cells of the cortex.

Blocks were then retrimmed for electron microscope sections. Sections approximately 90 nm thick were cut though the entire width of the root using a Reichert Ultra E diamond knife ultramicrotome.

(vi) Section mounting.

Sections from the four test and control plants were mounted on copper microscopy grids.

(vii) Staining.

Mounted sections were then double stained using saturated uranyl acetate in 50% ethanol, followed by a simplified lead citrate staining for electron microscopy as developed by Venable and Cogshall 1965.

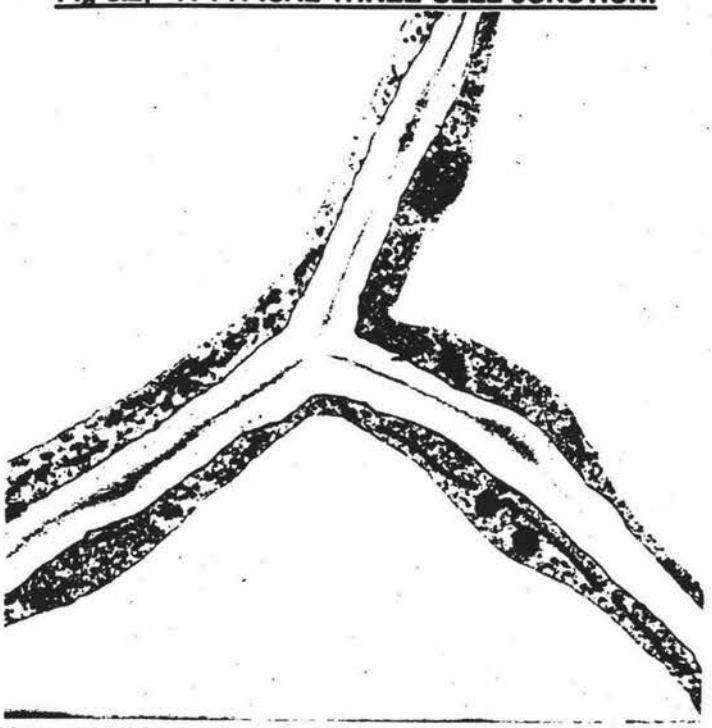
3.21, SAMPLE COLLECTION AND PROCESSING.

(i) Microscopy and collecting the micrographs.

Sections were viewed with a Phillips ZOIC transmission electron microscope at

a magnification of 11,200x. A section of the root cortex was randomly selected and the field of view centred on the nearest junction of three cells, see Fig. 3.2, as an example. If the cells were plasmolysed the site was rejected and another was randomly selected. Care was taken to ensure that the same cell did not feature on more than one micrograph, but as the micrographs were collected from more than one mounted section of each specimen plant, some overlap may have inadvertently occurred. A micrograph of the section was then taken. This process was repeated until ten micrographs had been collected for each of the test and control plants.

Fig 3.2, A TYPICAL THREE CELL JUNCTION.



Mag x11,200. A thin strip of cytoplasm can be seen around the margin of these three immature cortical cells. Most of the space inside the cells was occupied by the vacuole.

(ii) Scoring the micrographs.

Cells in these micrographs were scored for seven structures, five of which are (often) functionally associated with the plasma membrane. These included: golgi stacks; intracellular vesicles; clusters of intracellular vesicles; extracelluar vesicles; clusters of extracelluar vesicles and plasma membrane derived, or pinocytotic vesicles. These were the test organelles. Two structures were included in the count to act as an internal control in order to determine whether test and control plants used for the trials were essentially the same. These were the endoplasmic reticulum and mitochondria. A full description of all seven

structures, as defined during this research, can be found in Appendix seven. These definitions have been rigidly applied to all structures counted during this research. Any structure which did not clearly fit into a class was eliminated from the count.

Chapter 4: RESULTS

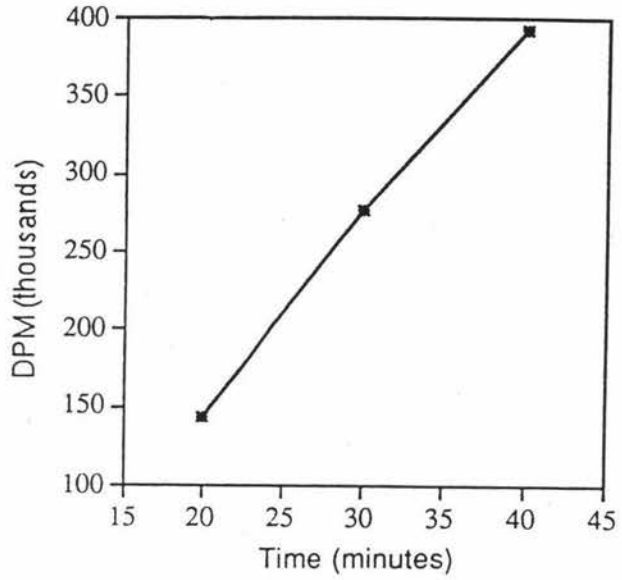
Section 4.1: MEMBRANE FUNCTION STUDIES.

4.10, [^{3}H]-ALANINE UPTAKE TRIALS.

(i) Pulse duration.

A Beckman Scintillation Counter was used to determine the relationship between the duration of incubation and the level of alanine within root-tip cells. Alanine uptake was found to be directly proportional to the duration of the trial (see Fig 4.1). In view of these results, a 40 minute incubation period was selected for all subsequent uptake trials. A Wallac Scintillation Counter was used for all subsequent experiments.

Fig 4.1, [^{3}H]-ALANINE UPTAKE OVER TIME



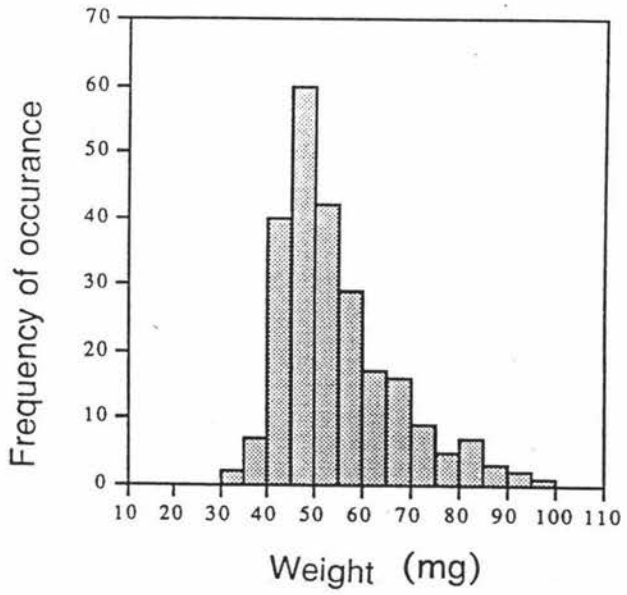
Plants were incubated for 20, 30 & 40 minutes to determine what effect this had on the DPM.

(ii) Root-tip samples.

The weights of the 240 root-tips formed a skewed bell curve (see Fig 4.2), with the majority of the samples weighing between 40 and 60 mgs. From the drying of the 11 day old samples it was found that the root-tip samples had dehydrated by an average of 93%.

To determine the

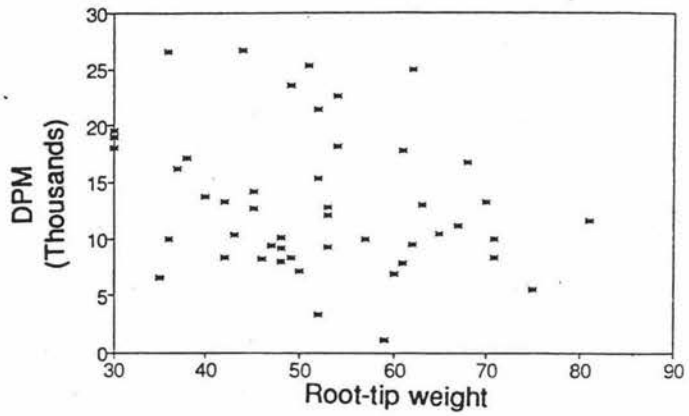
Fig 4.2, SAMPLE WEIGHT DISTRIBUTION



This graph was calculated from the wieghts of 120 root-tip samples from both eleven and twelve day old seedlings.

To determine the relationship between sample weight and [^{3}H]-alanine uptake, the root-tips from a control trial were weighed after the apoplastic wash, but prior to the addition of NCS-tissue solubiliser. The samples were subsequential treated in the same manner as other trials. The results can be seen in Fig 4.3. There was no correlation between the weight of the root-tip and the level of alanine taken up, from which it was determined that the weight of the root-tips do not directly affect movement of alanine into *Vicia faba* root-tip cells.

Fig 4.3, ROOT-TIP WEIGHT AND UPTAKE.

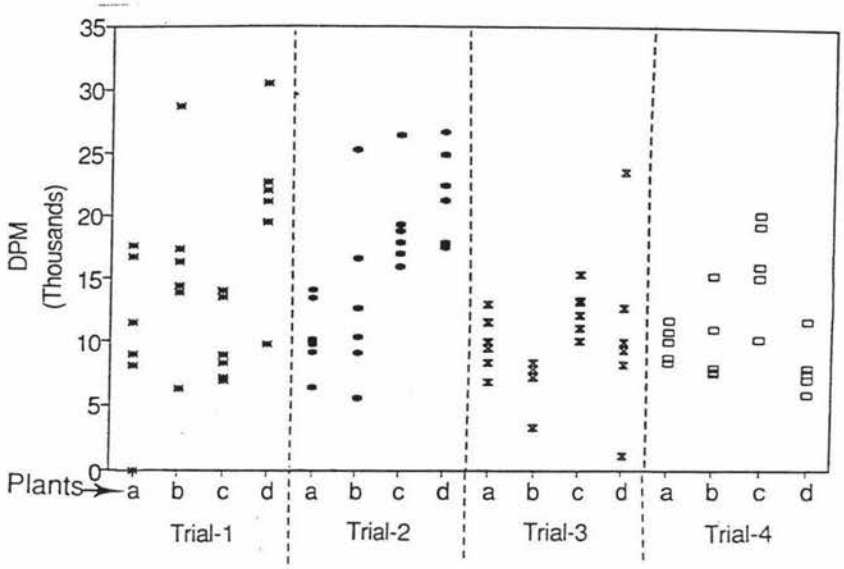


The weight of the root-tip sample is plotted against it's DPM, to determine whether root-tip's weight affects the level of [^{3}H]-alanine movement into the root-tip cells.

(iii) Control trials.

In order to provide a solid statistical foundation, the control trial was replicated three times, giving a total of 94 samples, with an average DPM of 13159. This level of replication was deemed necessary as the DPM values of samples within, and between plants, varied quite widely as is shown in Fig 4.4.

Fig 4.4, VARIABILITY OF UPTAKE IN CONTROL TRIALS.

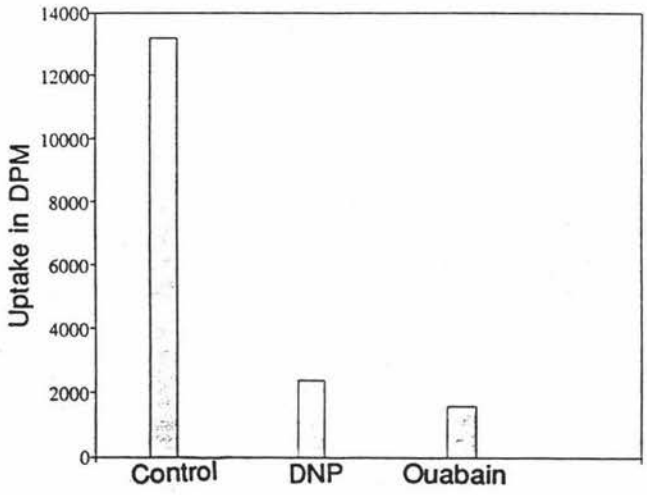


This graph shows the variability between the DPM values of control samples. All four control trials are shown, with the six samples from each plant aligned in a vertical column above the letter denoting the plant.

(iv) Chemical inhibition of uptake.

Movement of alanine into root-tip cells was strongly inhibited by both DNP and ouabain at a concentration of 10^{-3} M (see Fig 4.5), both of which are known to inhibit active ATPase based membrane transport of nutrients.

Fig 4.5, CHEMICAL INHIBITON OF UPTAKE.
(each bar represents one trial with 24 samples)



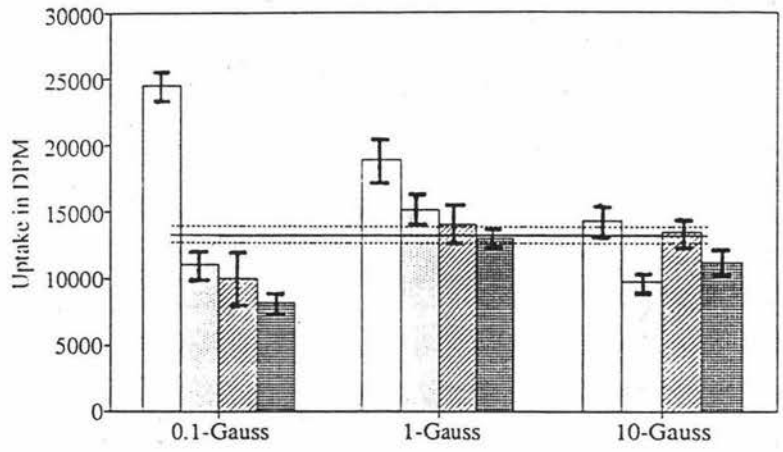
DNP and ouabain were added at a concentration of 10^{-3}M.

(v) Electromagnetic field exposure trials.

In the next series of experiments plants were exposed to 12 different electromagnetic field protocols. Prior to beginning the trials, the temperature in the incubation beaker was monitored over a 40 minute period for all 12 fields. None of the fields induced a temperature elevation in the beaker.

The results of the alanine uptake trials are shown in Fig 4.6. Alanine uptake increased in two of the test trials to an extent that the standard error bars of the tests did not overlap with those of the controls. In contrast, five of the EMF exposure trials had a DPM count lower than the controls by a level greater than the standard errors of the trial and the controls combined.

Fig 4.6, THE EFFECTS OF EMF'S ON ALANINE UPTAKE



- Sq/50 Hz, -Sq/60 Hz, - Si/50 Hz, - Si/60 Hz, Control, ... Upper & lower standard errors of the control, Sq = square waveform, Si = sinewaveform. In this graph each bar represents the average DPM of one EMF exposed trial, with standard error bars. In each trial six root-tips were collected from each of four plants. The average control value is shown as a horizontal line, with upper and lower standard errors drawn as dotted lines.

One of the initial aims of this project was to determine which of the various parameters, if any, of applied electromagnetic fields were involved in modifying the functioning of biological enzymes, *in situ*. Overlapping of the standard error bars led us to adopt more sophisticated statistical analyses than simple graphic interpretation.

(vi) Statistical analysis of the exposure trials.

Dunnett's two tailed t-tests were used to analyse the four control and twelve test data sets. The results of this analysis are shown in Table 4.1.

These tests demonstrated that of the three electromagnetic field parameters (magnitude, frequency and waveform), only waveform and hertz were involved in modifying alanine transport. These statistics were valid to a confidence level of 99%.

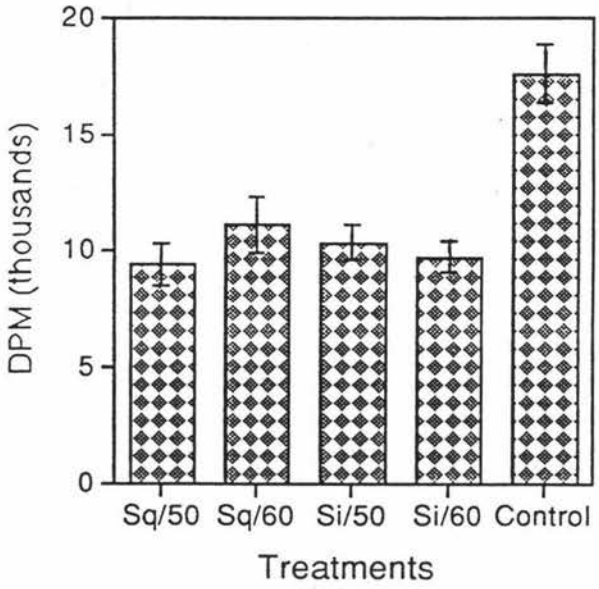
Table 4.1, ANALYSIS OF EMF PARAMETERS.

FIELD PARAMETER	LEVEL	DIFFERENT FROM CONTROL ?
Gauss	0.1 Gauss	No
	1 Gauss	No
	10 Gauss	No
Hertz	50 Hz	Yes
	60 Hz	No
Waveform	Squarewave	Yes
	Sinewaves	No

Dunnetts two tailed t tests for variables, was used to determine which of the EMF parameters modified the level of alanine transported into root-tip cells. As compared to the mean value and the level of variability set by the controls. With a confidence level of 99%

Only the 50 Hz field frequency appeared to modify the level of alanine uptake. Trials exposed to 60 Hz fields did not differ from the control. Of the two waveforms tested only trials with a square waveform differed from the controls.

Fig 4.7, REPLICATE 1 GAUSS EMF TRIALS



Replicates of one gauss field trials using NCS-II, a different compound from that used in the intial trials. Thus direct comparisons can not be made with the original set of trials.

4.11, REPLICATE TRIALS.

(i) Methodology differences.

A replicate set of trials were performed using one gauss fields only, in order to confirm the above listed findings. Owing to a change in company supply the tissue

solubiliser NCS-I, which was used to break down cellular membranes prior to scintillation counting in the original trials was replaced with NCS-II. The active ingredient of both products was quaternary ammonium hydroxide. In NCS-I the compound was present at a concentration of 0.6 Mol, in NCS-II this was reduced to 0.5 Mol. This introduced an unavoidable methodological difference between the two sets of trials. As this compound was suspected of involvement in the chemiluminescent problems encountered during this research. Therefore the results of the replicate trials cannot be directly compared to the original trials. Accordingly a new control trial was run for these trials.

(ii) Replicate EMF data.

The results can be seen in Fig 4.7. The four test trials all displayed a level of [^{3}H]-alanine uptake lower than the controls. All four test trials had a DPM value markedly lower than that of the control.

(iii) Statistical analysis of replicate EMF data.

The results of the replicate trials were analysed using Dunnett's two tailed t tests, see Table 4.2. These replicates differed from the original trials in that the results of both of the frequency and waveform treatments differed from the controls. These statistics were valid to a confidence level of 99%.

Table 4.2, ANALYSIS OF EMF REPLICATES.

FIELD PARAMETER	LEVEL	DIFFERENT FROM CONTROL ?
Hertz	50 Hz	Yes
	60 Hz	Yes
Waveform	Squarewave	Yes
	Sinewaves	Yes

Dunnetts two tailed t tests for variables, was used to determine which of the EMF parameters modified the level of alanine transported into root-tip cells. As compared to the mean value and the level of variability set by the controls. With a confidence level of 99%

4.12. ION EFFLUX TRIALS.

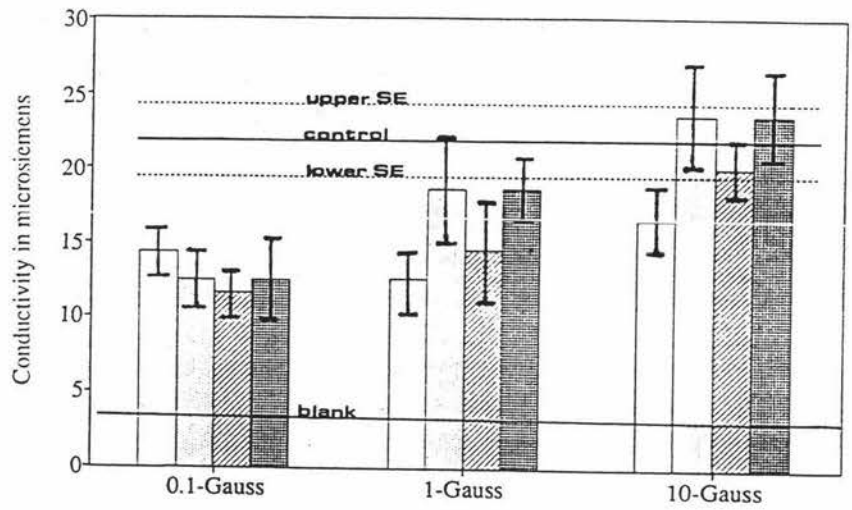
In this series of experiments plants were exposed to the same twelve electromagnetic fields used in previous trials.

(i) Conductivity trials.

The results are shown in Fig 4.8. None of the EMF's tested induced an increase in the level of ions in the incubation media. Seven of the fields (all four 0.1 gauss fields, 1 gauss/squarewave/50 Hz, 1 gauss/ sinewave/50 Hz, 10 gauss/squarewave/50 Hz) appeared to inhibit the *net* outward movement of ions from root cells. The remaining five fields did not induce any clear response at the level of ion movement into or out of roots in the incubated seedlings.

Fig 4.8, THE EFFECT OF EMF'S ON ION EFFLUX

(The conductivity of a liquid increases in direct proportion to the concentration in solution. This solution was deionised water in which seedlings had been incubated)



Sq/50 Hz, Sq/60 Hz, Si/50 Hz, Si/60 Hz,

SE = standard errors of the controls, Sq = square waveform, Si = sinewaveform. Each bar represents the average conductivity of ten samples, five using 11 day old seedlings and five with 12 day old seedlings. With standard error bars. The blank was fresh deionised water.

The graphic representation of the conductivity results were suggestive of an EMF modulating transmembrane ion movements. However analysis of this data with Dunnett's two tailed t tests did not find any significance in these results at the 95% confidence level, see Table 4.3.

Table 4.3, MEDIA CONDUCTIVITY ANALYSES.

FIELD PARAMETER	LEVEL	DIFFERENT FROM CONTROL ?
Gauss	0.1 Gauss	No
	1 Gauss	No
	10 Gauss	No
Hertz	50 Hz	No
	60 Hz	No
Waveform	Squarewave	No
	Sinewaves	No

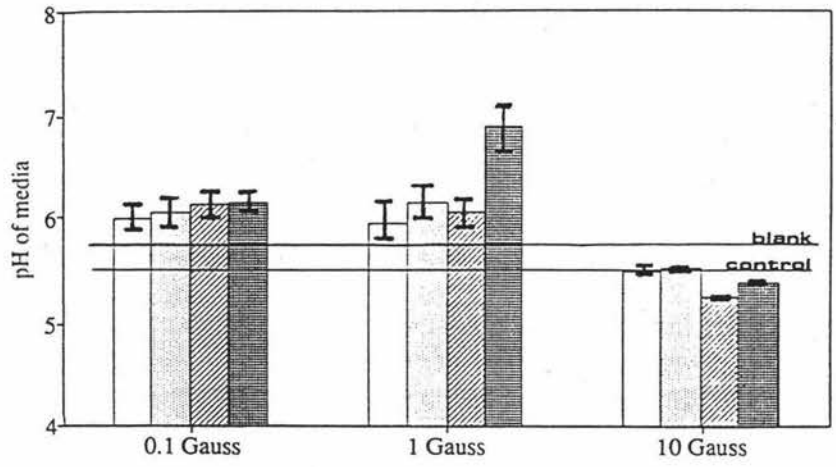
Dunnetts two tailed t tests for variables, was used to determine which of the EMF parameters modified the level of alanine transported into root-tip cells. As compared to the mean value and the level of variability set by the controls. With a confidence level of 95%

(ii) pH trials.

The results of the pH readings of the sample incubation medias can be seen in Fig. 4.9.

Fig 4.9, THE pH OF SEEDLING MEDIA & EMF'S

(pH is a measure of hydrogen ions, so any alteration in the pH of the incubation media indicates that there was a change in the movement hydrogen ions in root cells)



Sq/50 Hz, Sq/60 Hz, Si/50 Hz, Si/60 Hz,
Sq = square waveform, Si = sinewaveform. Each bar represents the average value of ten samples, five using 11 day old seedlings and five with 12 day old seedlings. With standard error bars. The blank was fresh deionised water. No standard error bars are given for the blanks or controls as the values were too small to indicate on a graph of this scale.

In all but two of the ten gauss field trials, the pH was higher than in the blanks and controls, indicating that there were fewer H^+ ions present. Only in the two 10 gauss sine waveform fields did the concentration of H^+ ions in the external media rise, as was seen in the trial illustrated in Fig 4.9.

Statistical analysis of this data by Dunnett's two tailed t tests showed significance of the gauss effect at the 95% confidence level, see Table 4.4. While if the effects of wave and gauss were paired for analysis then it was found that both waveforms did modify uptake, but only when paired with 0.1 and 1 gauss field intensities, see Table 4.5.

Table 4.4, MEDIA pH ANALYSES.
The first of the analyses of the pH data. With single field variables being compared to the controls.

FIELD PARAMETER	LEVEL	DIFFERENT FROM CONTROL ?
Gauss	0.1 Gauss	Yes
	1 Gauss	Yes
	10 Gauss	No
Hertz	50 Hz	No
	60 Hz	No
Waveform	Squarewave	No
	Sinewave	No

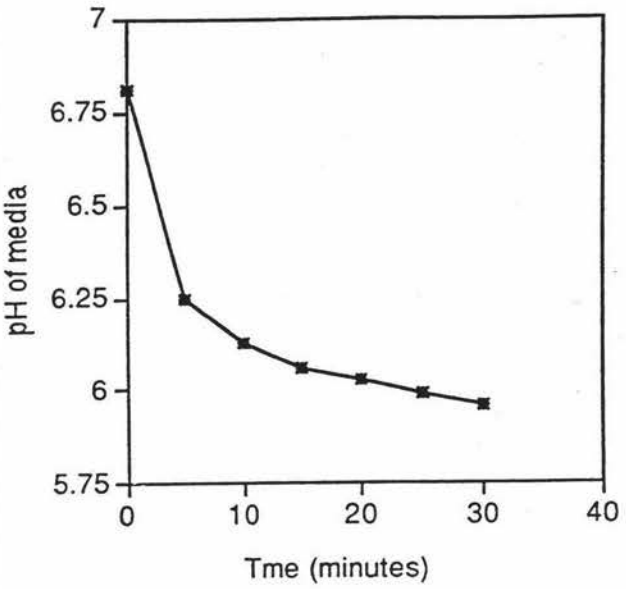
Table 4.5, PAIRED ANALYSES OF MEDIA pH.
In the second set of the analyses of the pH data. The waveform and gauss variables were paired for comparison with controls.

LINKED GAUSS-WAVE ANALYSES		
Squarewave	0.1 Gauss	Yes
	1 Gauss	Yes
	10 Gauss	No
Sinewave	0.1 Gauss	Yes
	1 Gauss	Yes
	10 Gauss	No

Waveform is put before gauss on this table for clarity of presentation. It should not be taken as meaning that waveform is more important than gauss.

Another trial involving media pH was performed at the very beginning of this experimental work, in which the three seedlings were incubated in 20 ml of the same incubation media as the seedlings were grown in for 30 minutes. This was done in order to determine whether the seedlings would modify the pH of their external media. The results of this trial can be seen Fig 4.10. This graph shows that over time the three *Vicia faba* seedlings acidified the extracelluar nutrient media.

Fig 4.10, pH DRIFT OF MEDIA OVER TIME



Three seedlings were incubated in 20 ml of Watson's nutrient medium. The pH of the medium was read at 5 minute intervals for 30 minutes.

Yet media acidification did not occur when the incubation media used was deionised water, see Fig 4.9. The reason for this is not apparent.

4.20. SCORING FOR STRUCTURES/ORGANELLES.

The ten micrographs each showing a randomly selected junction between three cells collected for each of the four test and four control plants were scored for the presence or absence of the seven organelles in three partial cells (see Table 4.6)

Table 4.6. DISTRIBUTION OF ORGANELLE IN ROOT-TIP CELLS

ORGANELLE	CONTROLS			EMF-EXPOSED		
	Total Count in the Control	Mean Count per Plant	Freq	Total Count in the Tests	Mean Count per Plant	Freq
Endoplasmic reticulum	117	29.5	97.5%	116	29	96.7%
Extracelluar vesicles	56	14	46.7%	56	14	46.7%
Intracellular vesicles	37	9.25	30.8%	36	9	30%
Golgi stacks	17	8.5	14.2%	23	5.75	19.2%
Mitochondria	62	15.5	51.7%	62	15.5	51.7%
Pinocytotic vesicles	33	8.25	27.5%	51	12.75	42.5%
Vesicle clusters extracellular	11	2.75	2.75%	9	2.25	2.25%

The presence of organelles in a total of one hundred and twenty cells were counted for the EMF exposed and the control trials.

(i) The internal controls.

The two internal control structures/organelles, the endoplasmic reticulum and the mitochondria occurred in frequencies which were identical, or very nearly so, in the cells of the test and control plants. Thereby demonstrating the similarity between the test and control plants.

(ii) The test organelles/structures.

The intra and extracellular vesicles and the clusters of extracellular vesicles were also present in nearly identical frequencies in the test and the control cells examined.

Golgi activity appeared to be enhanced in the test plants with 5% more golgi stacks in the test plants cells, while there was a 15% elevation in pinocytotic vesicles activity in the tests cells.

(iii) Statistical analysis of organelle distribution.

The counts for the seven different types of organelles present in the 120 test and 120 control cells were then analysed by a t-test to determine the statistical significance of the frequency of occurrence of the organelles, (see Table 4.7). The power of this statistical analysis was significantly reduced by the necessity of pooling the data for each plant prior to the analysis, due to the extremely low counts of the organelle on each micrograph (there could be 0-3 examples of each organelle in each micrograph). This analysis confirmed that most of the organelles had similar frequencies in the test and control treatments. Only the pinocytotic vesicles had exhibited a significant difference from the controls. It is generally accepted that if the probability of the results occurring by pure chance alone is 5% or less, then they are regarded as being

Table 4.7, ORGANELLES FREQUENCIES

ORGANELLE	PROBABILITY	SIGNIFICANT
Endoplasmic reticulum	77.96%	No
Extracelluar vesicles	Same as control	No
Intracellullar vesicles	87.699%	No
Golgi stacks	45.07%	No
Mitochondria	Same as control	No
Pinocytotic vesicles	5.60%	Probably
Vesicle clusters extracelluar	66.38%	No

Sections of three cells were scored for organelle presence in each micrograph. Such small numbers are difficult to analyse by conventional methods so organelle numbers where totalled for each of the four test and four control plants. These numbers which were then analysed with a T-test.

"statistically significant". The 5.6% probability seen in the pinocytotic vesicle frequency is tantalisingly close to this value. It is most likely that this probability value would have been lower if the data could have been analysed without pooling the values for each plant.

Chapter 5: DISCUSSION

Section 5.1: MEMBRANE FUNCTION TRIALS.

5.10, THE BACKGROUND FOR THIS SECTION OF RESEARCH.

(i) Experimental aims.

These trials were intended to determine whether or not external EMFs could alter the movement of amino acid alanine and ions across the plasma membranes into and out of *Vicia faba* root cells. Prior to this investigation no published works were available on the effects of ELF-EMFs on membrane function in plants. Thus, investigations utilising animal systems provide the background for this research.

(ii) Relevant published work.

Work performed by De Loecker *et al.* 1989 & 1990 suggested that exogenous ELF-EMFs affected the movement of amino acids across the plasma membrane into rat epithelial cells. However this finding was somewhat undermined by a failure of the researchers to fully describe the EMFs utilised and which fields induced alterations in the rate of amino acid transport into cells. Despite these problems, the paper was seen as being worthy of follow up.

The other published work of particular relevance to this research was performed by Garciá-Sancho *et al.* in 1994 involving the movement of ions into human cell lines in the presence of ELF-EMFs. Garciá-Sancho *et al.* exposed a variety of human cell types to a range of different EMFs. The result was a 12-32% elevation in the level of uptake of ^{42}K into two classes of cancer cell lines only within a very specific field window of 14.5-15.5 Hz and between 0.1 and 2 gauss.

(iii) Parallels with this project.

While both De Loecker *et al.* and Garciá-Sancho *et al.* have worked with animal systems strong parallels exist between these works and the current research project. In that the ELF-EMFs applied to the experimental tissues are very similar, also despite the basic differences between plant and animal cells

(plant cells are surrounded by cell walls and animal cells are not) the mechanisms for translocating compound across biomembranes are often very similar (Higgins 1992, Nørby 1987, Rahaminoff 1989, Xu, K. *et al.* 1992). Thus it is reasonable to extend this field of research which is currently deeply rooted in animal based systems outward to encompass plant systems.

5.11, THE EXPERIMENTAL METHODS UTILISED.

(i) Amino acid uptake methods.

Methodology developed by Watson and Fowden 1975 to measure the movement of amino acids into maize roots was selected as being suitable for the nutrient transport section of this work, especially as Despeghel and Delrot 1983 had already determined that *Vicia faba* (leaf cells) cells actively take up alanine. As Watson and Fowden's methodology utilised a simple, straightforward and non-invasive procedure to measure the level of amino acids in sample tissue.

During this research it was found that the level of [^{3}H]-alanine uptake, as measured by DPM, varied significantly (see Fig. 4.4) both within and between plants and trials. This variability problem was overcome by the use of a large number of replicate samples in order to provide a statistically solid mean value for each treatment.

(ii) Transmembrane ion migration methods.

It should be recognised that the comparative level of ions in the media after an incubation, as opposed to the level present prior to incubation is a result of both uptake and efflux processes, so that a variety of different transport channels could potentially be acting on the transmembrane distribution of ions across the plasma membrane (Reinhold and Kaplan 1984). This has been demonstrated by studies utilising double labelling techniques, which have shown that such fluxes apparent effluxes of solutes to be entirely due to a reduction in influx rather than raised efflux (Guy 1981).

The media samples collected during the ion efflux trials were read on both a conductivity and a pH metre. The reason for this two pronged approach was that the conductivity reading would give an overview of the distribution of ions

across the plasma membrane at the end of the 15 minute incubation. Any large scale alteration in the distribution of ions would indicate that the plasma membrane had been damaged by the treatment, becoming leaky, while reading the pH of the media would detect subtle changes in the concentration of hydrogen ions in the extracellular media.

In the conductivity trials, the conductivity of the controls was far higher than the blanks, indicating a net efflux of ions effluxed from root-tip cells into the extracellular media had occurred (see Fig 4.8). However the difference was small, from which it was concluded that the experimental seedlings suffered no mechanical damage to their roots during the transfer from the liquid growth medium to the incubation beaker.

The control reading on the pH metre, as seen in Fig. 4.10, was also different from the blanks, indicating that the concentration of hydrogen ions in the extracellular media was different. This difference in the concentration of hydrogen ions was less than that seen in the earlier trial shown in Fig. 4.9. The difference was most likely due there was only one seedling in the beaker and that the incubation time was less, only 15 verses 30 minutes.

(iii) Statistical analysis of the translocation data.

Dunnetts two tailed t tests were chosen for its ability to analyse for main effects, even in the presence of interactions between the effects. Dunnetts allows for such interactions by adjusting each effect (in this case the main effectors would be the various parameters of the EMFs applied) for all other effects. While it is acknowledged that most statistical methods texts advise not to test for main effects if there is interaction present (Varela-Alvares 1995), in this situation it would not have been sound to assume that there was no interaction between the EMF parameters. Dunnetts analyses the data for significant variance both above and below the mean of the control data. For these reasons Dunnetts two tailed t tests were the most viable option for analysing this data.

5.12. STANDARDISING THE EXPERIMENTAL CONDITIONS.

As has been discussed in the literature review, much of the prior work on

investigating electromagnetic field phenomena has drawn criticism for being inadequately defined and standardised. This has made independent replication of much of the work difficult, if not impossible. Considerable care was taken during the course of this research to describe experimental parameters so as to avoid these difficulties.

(i) Incubation conditions.

The incubation conditions during the course of this research were carefully controlled to ensure uniformity of experimental conditions trials. Light, temperature, the pH and ion concentrations of the media, as well as the time of the day during which trials were performed were kept uniform throughout the course of this work.

The uptake trials were performed inside a wooden cabinet in a controlled climate chamber. As such, the only electrical equipment closer than three metres from the incubation site was the climate control equipment
(lights and fans at least 60 cm, and growth cabinet control panel ≈3 m from the incubation site), external corridor lighting (≈4 m), and the electromagnetic field production equipment (≈30 cm). Thus potentially confounding external electromagnetic field sources were minimised, and those that were present would have been uniformly present for all trials.

The incubation duration was set at 40 minutes, as this allowed time for differences in the rate of alanine uptake to accumulate inside the root-tips. At the same time this avoided an overlong exposure time which can potentially mask transient alterations in the biological systems induced by EMFs (Phillips,J.L. 1992 and Rosen 1993). Thus, setting the exposure duration for less than an hour minimises the opportunity for sample tissues to "acclimatise" to the EMFs.

(ii) Experimental tissues used.

Broad bean seedlings of eleven or twelve day-old seedlings were used during these uptake trials dependant on availability. The developmental phase of seedlings at this age were very similar. Twelve-day old seedlings were slightly taller with slightly longer roots, but the diameter of the roots were similar. For the

efflux trials, an equal split of eleven-and twelve-day old seedlings were used.

The distribution of sample weights displayed a skewed bell curve, with a distinct clustering between 40 and 60 mg in Fig. 4.2. Thus, the rate at which alanine diffuses into the root apoplast for uptake should be similar in eleven-and twelve-day old seedlings. This assumption was justified by the results of to be the case Fig. 4.3, where it saw shown that the rate at which the tritium labelled alanine entered root-tip cells was independent of the weight of the individual root-tip sample.

(iii) Scintillation counting.

Early experimental radio-labelling work, to set experimental parameters, was read on a Beckman LS3801 Scintillation Counter. The later experimental trials were read on a Wallac 1409 Liquid Scintillation Counter. The change was made to the Wallac counter as its internal programme for reading samples in a xylene based scintillation cocktail overcame the chemiluminescence problems encountered when the Beckman Scintillation Counter was used.

5.13, THE [^{3}H]-ALANINE UPTAKE TRIALS.

(i) Amino acid uptake mechanism (s).

The purpose of the chemical inhibition trials (see Fig. 4.5) was to determine the mechanism(s) by which the alanine was entering the *Vicia faba* root-tip cells. These trials demonstrated that the alanine uptake observed during this work was largely due to active energy-dependant ATPase carriers. A smaller portion of the uptake, just under 20% of the total uptake, represented the movement of alanine into root-tip cells by a diffusion based mechanism.

(ii) Alanine uptake in electromagnetic fields.

The original 12 EMF exposure trials (see Fig.4.6) demonstrated that exposure to such fields can significantly modify the ability of *Vicia faba* root-tip cells to accumulate alanine. This effect occurred only within rather specific frequency and waveform windows (see Table 4.1). Of the three field intensities

tested during this section of research, none appeared to be involved in modifying the level of alanine uptake in the subject tissue. However, both the field frequency and waveform significantly modified alanine uptake. On the frequency front, 50 Hz fields induced a decrease in the level of alanine uptake while 60 Hz field exposures did not modify alanine uptake. On the waveform front, squarewave fields increased alanine uptake, but sinewave fields did not modify alanine uptake. Both of these field frequency and waveform effect on alanine uptake had statistical probabilities of $P \leq 0.01$ (less than or equal to one in hundred).

The replicate trials performed using only 1 gauss fields (see Fig. 4.7), showed that responses to the EMFs were less specific in their field requirements than the original trials. Reduced levels of alanine uptake were seen in response to both field frequencies and both waveforms tested (see Table 4.2). These effects had statistical probabilities of $P \leq 0.01$. The reason or reasons for this differential response between the original and replicate trials is not immediately apparent.

The six month delay between the original experiments and the replication trials, resulting from an unavoidable delay in chemical supply may explain the differential response. As Goodman, E. *et al.* 1995 pointed out, subtle differences in experimental tissues, including the storage of material prior to experimental work, can potentially impact on the final result. If this aging of the experimental material is the reason for the difference, then it would seem to suggest that seedlings grown from "old" seeds were more sensitised to EMF stimuli than those from "fresh" seeds. But aging alone does not explain why only inhibition was seen in the replicate trials, when both inhibition and stimulation were seen in the original trials. This differential response might be worthy of further investigation to elucidate the reason for this differential response.

Another possible explanation for the difference may be the smaller number of samples in the replicate trials. The original trials yielded 285 test samples from 12 trials, were compared to 92 control samples from four trials, while in the replicates 96 test samples, from four trials, were compared to only 24 controls from a single trial. This is unlikely to be the reason for the differential response,

as the results of both sets of data had statistical probabilities of $P \leq 0.01$. The age of the biological samples was the only obvious difference between the two series of trials.

5.14, THE ION EFFLUX TRIALS.

By the use of conductivity and pH measurements of the incubation media any net alteration in the concentration of ions in the extracellular media could be detected. Any modification in the movement of ions across the plasma membrane induced by exposure to electromagnetic fields should be detectable.

(i) The conductivity trials.

This technique did not detect any modification in the level of ions in the extracellular media (at the 95% confidence level) after samples were exposed to EMFs (see Table 4.3). This result may indicate that the technique was not sufficiently sensitive to detect the changes that occurred, as a broad specificity probe was utilised to measure the conductivity of the media. It is more likely however, that this result indicates the lack of a large scale change in the pattern of ion movements across the plasma membrane in the presence of ELF-EMFs. In turn this indicates that no physical damage was caused to the membrane by exposure to the array of electromagnetic fields used during this research.

(ii) The pH trials.

The results of these trials (see Table 4.4 and 4.5) indicated that the level of H^+ ions in the extracellular media was significantly lower (statistical probability $P \leq 0.05$) in the ELF-EMF exposed test samples than the controls. Not all of the EMF parameters contributed equally to this effect. Table 4.4, of the statistical analysis of the effects of the various parameters of the applied EMFs indicates that the intensity of the EMF was the most important parameter involved in eliciting an affect on the movement of H^+ ions across the plasma membrane. There was a secondary effect on alanine uptake by the field's waveform, but this secondary effect only occurred through an interaction with the field intensity (see Table 4.5), once again this effect was statistically significant to a level of $P \leq 0.05$.

Therefore the inhibitory effect of the field intensity, at 0.1 and 1 gauss, on the efflux of H^+ ions from root-tip cells was compounded by both square and sinewaves.

This modification in the movement of H^+ ions may be due to the modification of any one of several different transport mechanisms, including reduced diffusive or active efflux or reduced diffusive or active uptake of ions (Reinhold and Kaplan 1984). If an active transporter of H^+ ions was involved in producing this redistribution then it would have to be either an antiporter, or a symporter functioning in the export mode only. Since the deionised water media contained no compounds for coupled transport with the H^+ ions. Further evidence that the ion efflux effect is a separate effect from the alanine uptake comes from Reinhold and Kaplan 1984, who suggested that the active uptake of amino acids in plants is most usually coupled to Na^+ transport.

Therefore it would seem most reasonable to assume that the EMF effect on ion transport was due to reduced efflux, rather than increased uptake, as the deionised water medium would not have contained significant levels of H^+ ions.

5.15, MEMBRANE TRANSPORT AND EMFS EXPOSURES.

(i) Uptake channels.

The combined results of the alanine uptake and the ion efflux work indicate that transport of compounds across the plasma membrane of root-tip cells in *Vicia faba* is susceptible, at least in the short term, to modification by EMFs. Supporting the findings of De Loecker *et al.* 1989 & 1990, and Garciá-Sancho *et al.* 1994.

Clearly more than one transport channel was involved, since both uptake of nutrients and efflux of ions from root-tip cells were susceptible to modification by ELF-EMFs (Despeghel and Delrot 1983, Reinhold *et al.* 1970, Reinhold and Kaplan 1984).

(ii) Windows of effect.

The transport channels affected appear to be susceptible to differing "windows" based on different EMF parameters. Such windows of effect have

been widely reported in the scientific literature (Goodman, E. *et al.* 1993 & 1995, Goodman, R. *et al.* 1992a & 1992b, Wei *et al.* 1990).

Alanine uptake into *Vicia faba* root-tips cells was inhibited by the 50 Hz fields and stimulated by the squarewave fields. This response fits in with Eugene Goodman *et al.* (1995) who stated that responses of biological systems to EMFs are usually within specific frequency or intensity windows, which are often very specific to the experimental system. Outside these 'windows of effect' there is usually no response, or if a response is observed then it is in the opposite direction.

The efflux of H^+ ion was modified by EMF exposures with the effect primarily dependent on the intensity of the field, with secondary affect by waveform, but only through an interaction with field intensity. The only response on ion efflux was an inhibitory effect. No reverse effect as seen in alanine uptake was apparent in the ion efflux trials. Such an effect may conceivably have been seen if a wider range of fields were utilised.

The interaction shown by statistical analysis to occur between the field intensity and waveform in the H^+ ion efflux trials justified the selection of Dunnetts two tailed t tests to analyse this data. If a more traditional type of statistical test had been applied, this effect would not have been detected.

Thus all aspects of the applied EMFs can potentially be involved in the induction of modified membrane functioning in *Vicia faba* dependent on what membrane system is under investigation. There was no suggestion that the membranes are damaged in the process.

(iii) Sites of EMF interactions.

What remains unclear from this work is, with what membrane local or component the EMFs are interfacing. Further work would be required in order to determine whether the EMFs interacted with: the compound or ion being transported (as is proposed by Male's 1992 cyclotron resonance model); with energy dependant membrane carrier or carriers (as is proposed by the electroconformational coupling model presented by De Loecker *et al.* 1989 & 1990, Tsong and Astumian 1986, Tsong *et al.* 1989); with the membrane itself

(Weaver and Astumian 1992), with some form of membrane traffic controller based on a feed-back loop, of the type proposed by Hansen 1990; or indeed whether the effect was between the EMF and a generalised stress mechanism (Czerski 1988, Paradisi *et al.* 1993, Phillips,J.L. *et al.* 1992), possibly nuclear based, with the nucleus in turn regulating the function of various components of the membrane.

Further work with a more precise and controlled biological system would be required to determine the answer to these questions. But in the light of the differing, but very specific, windows of effect it would seem plausible to consider that the EMFs interact with more than one functional unit within the target cells. In turn this would appear to make it unlikely that the target was a general stress mechanism, since such a mechanism would be expected to show susceptibility to a range of stimuli, as has been demonstrated with heat shock genes (Blank 1992a, Goodman, R. *et al.* 1992a & 1992b, Wei *et al.* 1990, Weisbrot *et al.* 1993). If the EMFs induced the visible responses by direct interaction with the plasma membrane then some indication of this could be seen in the structural section of this work.

Section 5.2: MEMBRANE STRUCTURE TRIALS.

5.10. THE BACKGROUND FOR THIS SECTION OF RESEARCH.

(i) Experimental aims.

This section of the thesis research was intended to determine whether external EMFs modified the structure of the plasma membrane in *Vicia faba* root cells, or that of organelles and other functionally related structures. Prior to this investigation no published works were available on the effects of ELF-EMFs on membrane structure in plants, as such the background for this research comes from an investigation which utilised animal tissue.

(ii) Relevant published work.

A study into the effects of ELF-EMFs on membrane structure was performed by Paradisi *et al.* in 1993. In this study the investigators demonstrated that the application of sinusoidal, 50 Hz fields of 25 gauss, induced the development of mushroom-like swellings (or blisters), called blebs on the outer surface of the plasma membrane of K562 Leukaemic cells. The formation of blebs is believed to be a fairly general cellular response to stress, which is seen in the early stages of toxic injury (Paradisi *et al.*). Yet the investigators reported that the exposed cells displayed no change in their ability to grow and proliferate, so clearly the cells were not catastrophically injured by the exposure.

(iii) Parallels with this work.

The work performed by Paradisi *et al.* involved exposing cells to a higher intensity EMF than the 1 gauss used in this research. This combined with the utilisation of animal cells makes for significant differences between the two studies. Never-the-less Paradisi's work is worth considering, as the researchers did see a structural response to EMFs by the plasma membrane.

5.21. THE EXPERIMENTAL PROCEDURES UTILISED.

(i) The organelles scored.

The endoplasmic reticulum and the mitochondria were used as internal

controls because they were clearly visible and widely distributed in the experimental cells. Any difference in the distribution between the treatments would have bè evident.

The test organelles were chosen for their functional relatedness to the plasma membrane and each other. The golgi stacks because they were a visible component of the golgi apparatus, which is involved in the sorting and recycling of cellular membrane from the secretory and endocytic pathways of cells (Mellman and Simons 1992, Mollenhauer *et al.* 1991, Rothman 1985). Intracellular (or cytosolic) vesicles were scored because they may be derived from the golgi apparatus as well as to differentiate them from pinocytotic vesicles. Extracellular vesicles were scored because they must traverse the plasma membrane to exit the cell, while extracellular vesicle clusters were scored as they are composed of extracellular vesicles. Pinocytotic vesicles offer a nonspecific route of entry for extracellular liquids into cells (Wattenburg 1992) making their inclusion in the scoring necessary.

(ii) Micrograph site selection.

The scoring of organelles was performed at randomly selected junctions of three cortex cells. Enabling a structural similarity to be maintained between the micrographs, while also including as many cells as possible in the scoring process.

(iii) Statistical analysis of organelle counts.

A simple t test was used to analyse this data, since no more sophisticated analysis was required to compare the two treatments.

5.22 ANALYSIS OF THE ORGANELLE FREQUENCY DATA.

(i) Analysis of the internal controls.

The endoplasmic reticulum and the mitochondria were distributed in similar frequencies in both treatments (see Table 4.6), demonstrating that the test and control seedlings were comparable. As such any difference in organelle distribution between the two treatments would be attributable to the difference in

treatment, ie. the EMFs.

(ii) Analysis of the test organelles/structures.

Of the five test organelles scored three (intracellular vesicles, extracelluar vesicles, clusters of extracellular vesicles) were identical in distribution, to within 1%, between treatments (see Table 4.6). The t tests of this data (Table 4.7), affirms this result, which further confirms the similarity in the cells in the two treatments.

The only differences of greater than 1% in the frequency of organelles between the treatments was seen in the scoring of the golgi stacks and the pinocytotic vesicles (see Table 4.6). Regrettably the necessity of pooling the results for each plant within the treatment, brought about by the low counts in each micrograph, lessened the capability of the t tests to resolve differences in the data from the two treatments (see Table 4.7). The t tests suggests that the frequency differences in the golgi stacks seen in Table 4.6, were not significant. The t tests returned a significance for the pinocytotic vesicles of just above the 5% level (5.6%) at which probabilities are accepted as statistically significant. That such a low probability value was obtained despite having pooled the data would seem to indicate that the "true" significance of this data was of a much higher magnitude. A supposition which will be maintained henceforth.

5.23. ELF-EMFS AND MEMBRANE STRUCTURES.

Exposure of *Vicia faba* roots to 1 gauss, 50 hertz square wave EMFs did not induce the type of membrane aberrations seen by Paradisi *et al.* Rather the exposure enhanced that rate of occurrence of a pre-existing membrane function.

(i) Pinocytotic vesicle traffic.

Pinocytosis is a cellular mechanism with two functions, the first of which is to retrieve the segments of specialised secretory vesicle membrane, which the golgi apparatus recycles into new (Wattenburg 1992). It is doubtful this is the type of pinocytosis seen here for two reasons, firstly because no association was seen between the pinocytotic vesicles and the golgi apparatus and secondarily

because the cells under investigation were not from secretory tissue where exocytosis is sufficiently common for such vesicles to be abundant.

The second type of pinocytosis also serves a double function; in some instances pinocytosis is used to carry substances bound to membrane receptors on the outside of the plasma membrane into the cell (Keeton 1993). This mechanism is also unlikely to explain the enhanced pinocytotic activity seen here, as the seedlings were incubated in a simple media lacking in chemicals requiring this type of uptake. Furthermore such vesicles have a nap or blurring on the external surface of the membrane (Keeton 1993), which is not apparent on the membrane surface of these vesicles (see Appendix 7).

The remaining function of pinocytosis is the non-specific bulk uptake of extracellular fluids. Once a vesicle is inflated with fluid it buds away from the plasma membrane to be transported wherever in the cells its contents are required (Dillon 1981). This process most reasonably describes the pinocytotic activity seen during this research, as the cells under investigation were rapidly expanding cortex cells from the root zone of elongation. Therefore the bulk uptake of water and extracellular solutes would facilitate the rapid expansion of the young cells. Pinocytosis for bulk fluid uptake is consistent with the presence of remnants of pinocytotic vesicles being visible in the vacuoles of experimental cells.

(ii) Implications of this enhanced vesicle traffic.

The results of Table 4.6 suggest a 15% increase in pinocytotic vesicle traffic in root-tip cells exposed to EMFs. Such an increase in the rate of fluid uptake into exposed cells would enable the cells to take on water and expand more rapidly than the comparable controls. Whether this response could be correlated to enhanced growth depends on whether the response was a transitory response one, or a genuine adaptation to altered environmental parameters. Enhanced growth could only result if newly differentiating cells behind the meristem continued to respond to the external electromagnetic field in this manner. Even so then the enhanced growth would, most likely, be confined to the rapidly expanding cells in the zone of elongation. Since this process is cell

expansion not growth as such.

Once a cell attains its full size the enhanced level of pinocytotic vesicle traffic should fall back to control levels, as the expansion of the vacuole becomes restrained by pressure from the cell wall. If the enhanced uptake of fluids persisted beyond normal barriers then the type of damage seen by Paradisi *et al.* could result. A fuller understanding of this phenomena will only be achieved by more extensive investigation.

Previous work by Goodman, R. *et al.* 1992a saw an increase in levels of tubulin in *Drosophila melanogaster* salivary gland cells exposed to ELF-EMFs. This finding might have unexpected relevance to this research, since pinocytotic vesicles are transported within a cell along a framework of microtubules. Therefore the enhancement of tubulin levels seen by Reba Goodman and associates might potentially have been reflecting an enhancement in pinocytotic vesicle activity. Unfortunately there is no data available with which to verify this, but further work in this area may bear fruit.

5.24, PINOCYTOTIC VESICLE TRAFFIC AND MEMBRANE TRANSPORT.

(i) Could this be how EMFs modified alanine uptake?.

The enhanced transportation of extracellular medium into exposed cells by pinocytotic vesicles could potentially have contributed to the movement of alanine into cells exposed to EMFs with a square waveform. Since the enhanced level of pinocytotic vesicle traffic was induced by a square wave field. It would be interesting to determine whether exposure to sine wave field would have produced an inhibitory effect on pinocytotic activity, since such fields inhibited alanine uptake.

It is unlikely however that enhanced (or decreased) pinocytotic vesicle traffic fully explain the modified levels of alanine uptake seen during the functional trials, as chemical inhibition trials demonstrated that the majority of the uptake into the root-tips was due to the action of ATPase carriers.

(ii) Could this be how EMFs modified H^+ efflux?.

It is difficult to envisage how the modified transport of extracellular fluids

into cells could induce the modification to the concentration of H^+ ions in the extracellular media. Since pinocytotic vesicles move into not out of the cell and no elevation was seen in the frequency of any other type of vesicle.

CHAPTER 6: CONCLUSIONS.

6.1. THE EXPERIMENTAL CONDITIONS.

Every reasonable precaution was taken to control, or define the environmental and experimental conditions under which these trials were performed, thus independent replication of this work under identical conditions can be performed to verify these findings. Furthermore the use of intact seedlings has circumvented the complications of ferromagnetic contamination currently plaguing *in vitro* investigations of electromagnetic bioeffects (Kobayashi *et al.* 1995).

6.2. MEMBRANE TRANSPORT.

The results of this work were suggestive of an EMF interaction with at least two membrane transport channels. One channel is responsible for the active energy consuming uptake of alanine into cells by ATPase carriers. The second receptive channel is in involved maintaining the transmembrane electrochemical gradient (Koryta 1991) by exportation of hydrogen ions. It would seem likely that this transport channel also involves ATPase carriers.

The electromagnetic field interactions with membrane traffic, both uptake and efflux occurred within specific windows characteristic of the system. Electromagnetic field effects on the movement of alanine into cells was seen to occur within frequency and waveform windows. 50 hz fields inhibited alanine uptake, while squarewave fields stimulated alanine uptake. The amplitude of the EMF did not affect a response in the movement of alanine. Inhibition of hydrogen ion efflux due to EMF exposures occurred primarily within an amplitude window, with a lesser effect involving the waveform also occurring.

It is unlikely that both the uptake of nutrients into cells and the maintenance of the electrochemical gradients across cell membranes could be disrupted without some wider disruption to cellular functions, at least in the short term. Several major cellular enzymes are implicated in the control cycle of membrane traffic (Yingst 1988), so that feed-back from modified membrane traffic to these enzymes could be widespread throughout the cell. Wider responses within the cell could in turn be induced either directly by actions of enzymes in

the membrane traffic control cycle (Markov and Pilla 1994), or via activation of signal transduction pathways (Goodman, E. *et al.* 1995, Lednev 1994, Litovitz *et al.* 1994, Luben 1994).

6.3, PINOCYTOTIC VESICLE TRAFFIC.

The effect EMFs of pinocytotic vesicle traffic within *Vicia faba* root-tip cells appear to involved an amplification of a pre-occurring cell function, rather than induction of a new function. There was no significant alteration in the rate of occurrence any of the other membrane related structures counted. Enhanced pinocytotic vesicle traffic in the roots of exposed seedlings may enable the roots to elongate more rapidly than the controls. Most likely this enhanced "growth" would be restricted to cells in the zone of elongation, since these cells were rapidly taking up water from the environment by pinocytosis prior to their exposed to ELF (AC) EMFs.

6.4, CONCLUDING COMMENTS.

The effect of EMFs on pinocytotic vesicle traffic within *Vicia faba* root-tip cells appear to involve an amplification of a pre-occurring cell function, rather than induction of a new function. There was no significant alteration in the rate of occurrence of any of the other membrane related structures analysed. Enhanced pinocytotic vesicle traffic in the roots of exposed seedlings may enable the roots to elongate more rapidly than the controls. Most likely this enhanced "growth" would be restricted to cells in the zone of elongation, since these cells were rapidly taking up water from the environment by pinocytosis prior to their exposure to ELF (AC) EMFs.

6.4, CONCLUDING COMMENTS.

These results demonstrate that three different types of membrane transport in root-tip cells of *Vicia faba* are sensitive to low intensity (0.1, 1 & 10 gauss) electromagnetic fields of 50 and 60 Hz range. These transport mechanisms include alanine uptake by ATPases (both stimulation and inhibition effects), hydrogen ion efflux via ATPases (inhibition only), and the bulk uptake of water

into expanding cells by pinocytotic vesicles (stimulation only), but only within very specific windows of frequency, waveform and amplitude.

Thus the functioning of the plasma membrane of *Vicia faba* appears to be quite susceptible to modification of its routine functioning by EMFs. As the plasma membrane is a major site for the transduction of environmental information into the cell it is hard to see how such modifications could fail to have widespread implications throughout the organism, at least in the short-term.

Many of the mechanisms of the plasma membranes have been shown to bear considerable similarities, even between organisms from diverse phyla. Although, it would be extremely difficult to determine whether effects such as those seen in this study could have ramifications for human health. It is becoming increasingly clear, however, that the plasma membrane of a cell, and in turn the entire organism, is a major receptor of environmental information.

EPILOGUE

This 1974 quote by James B. Beal to my mind summarises, most eloquently, the situation electromagnetic research still finds itself in today.

"In general it seems, any-newly opened area of science meets considerable resistance to acceptance. The scientific sense of security is threatened; the ''snicker effect'' and condemnation without investigation is prevalent. This defensive prebiasing, and lack of imagination, has continually provided a situation where science gets in its own way.
Here is an observation of the situation in verse:

The platypus is a funny bird.
The way it's built is all absurd-
With duck-like bill and feet to match,
Milk for young which from eggs do hatch;
Speed underwater and beautiful furs,
Home underground and poison spurs.
I've heard it said, and it's true, I know-
It took ***fifty*** *years to prove it so!*
So remember the Platypus, my friend,
When you find a fact which won't fit in.
Just remember before you go,
To write it down as ''I told you so.''

J.B.B, May, 1969

It is interesting to note that yesterday's superstitions may become tomorrow's science when the proper inquiring mind, and suitable tools, techniques, and terminology are developed."

APPENDICES

APPENDIX ONE

MAGNETORECEPTION IN NATURE

Appendices Table 1, Responses to magnetic fields.

Organism	Responses from organisms: Magnetorecep	Receptor location	Response(s)
Mud flat Bacteria (Towe and Moench 1981)	A chain of cuboidal shaped magnetite particles	The chain runs along the axis of the cell	-Moves toward magnets. -Align themselves against two dimensional fields.
Bacteria (Chin-Yaun and Chia-Wei 1994)	As a single or a double chain of single domain particles	The chain runs along the axis of the cell	-Away from the equator movement is towards the pole. -On the equator move both ways.
Marine diatoms *Amphora soffeaeformis* (Male 1993)	Unknown	Unknown	-Movement is enhanced by calcium uptake in magnetic fields
Eugleniod protozoan *Anisonema platysomum* (Torres De Araujo *et al.* 1986)	A chain of $\approx 3x10^3$ Magnetite particles, of differing orientation. (functioning as permanent dipoles).	In or near the cell walls, occupying 0.2% of the cells total volume.	-Away from the equator movement is towards the pole. -On the equator move both ways.

Responses from organisms			
Marine Mollusc (nudibranch) *Tritonia diomedea* (Lohmann 1987)	Unknown	Unknown	-In the dark orientation was to the east. -In the dark and shielded from geomagnetic field orientation was random. -Therefore orientation is affected by cues from the lunar cycle and the geomagnetic field.
Colonial insects -Honey bees (Hsu and Chia-Wei 1994)	Enervated trophcytes (magnetite paticles)	Surrounding the abdominal segments	-The bee's "dance" is aligned against the local magnetic field.
-Hornets (Kisliuk and Islay 1977)	Unknown	Unknown	-A horizontal magnetic field laid over a hive caused all but pupal hornets to die. Upon emerging from pupae young hornets were sluggish for a few days. After which, often abnormally orientated combs were built.

Responses from organisms			
Fish Chinook Salmon (*Oncorhynchus tshawytscha*) (Kirschvink *et al.* 1985, Mann 1968)	A series of chains of uniformly sized single domain magnetite particles	Embedded in the ethmoid tissue in the front of the cranium.	-Smolt use celestial and magnetic field cues to navigate.
Sock-eye Salmon (Kirschvink *et al.* 1992, Quinn and Brannon 1982)	Magnetite crystals have a clear structure, morphology, size and crystallographic orientation within an organic matrix.	Unknown	Navigation
Salamanders *(Eurycea lucifuga)* (Phillips, John B. 1977)	Unknown	Unknown	-Salamandas were trained to choose which corridors to move along in response to the angle of the electromagnetic field surrounding the corridor.

Responses from organisms			
Homing Pigeons (Walcott 1974)	Unkown	Head, as attaching a magnet to the head negates the effects of external magnetic fields	-Use a combination of celestial and magnetic cues to navigate. -When the sun is obscured by cloud then the geomagnetic field is used in navigation. -But their primary navigational aid is by the position of the sun.
Migratory Sparrows *(Passerculus sandwichensis)* (Able & Able 1995)	Unknown	Unknown	-Navigate by a combination of celestial and magnetic cues.
Cetaceans -Whales	Unknown	Unknown	-Whale strandings found that the rate of whale strandings could be correlated to the intensity of the local magnetic field. With stranding rates being highest in areas of low magnetic intensity.

	Responses from organisms		
Humans (Kirschvink *et al.* 1992a)	Magnetite-maghemite crystals	Unknown	Navigation?

It should remembered that the geomagnetic field is static, while artificial electromagnetic fields produced by electrical appliances and electrical transmission equipment are alternating. Therefore the mechanisms of interaction and the reactions of organisms may differ.

APPENDIX TWO

FIELD FREQUENCIES:

Electromagnetic waves are characterised by their frequency and wavelength. This table describes the physical characteristics of various spectrum of electromagnetic waves.

Appendices Table 2, Electromagnetic field frequencies and wavelenghts

BANDS	FREQUENCY	WAVE LENGTH (m)
(ELF) Extremely low frequency	1 - 30 Hz	10^8 - 10^7
(SLF) Super low frequency	30 - 300 Hz	10^7 - 10^6
(ULF) Ultra low frequency	300 Hz - 3 KHz	10^6 - 10^5
(VLF) Very low frequency	3 - 30 KHz	10^5 - 10^4
(LF) Low frequency	30 - 300 KHz	10^4 - 10^3
(MF) Medium frequency	300 KHz - 3 MHz	10^3 - 100
(HF) High frequency	3-30 MHz	100 - 10
(VHF) Very high frequency	30 - 300 MHz	10 - 1
(UHF) Ultra high frequency	300 GHz - 3 GHz	1 - 0.1
(SHF) Super high frequency	3 - 30 GHz	10^{-1} - 10^{-2}
(EHF) Extremely high frequency	30 - 300 GHz	10^{-2} - 10^{-3}

The descriptions of wavelengths and associated frequencies in this table are taken from Sliney *et al*'s. 1992 paper. However, the definition of extremely low frequency fields used through-out this work comes from Reba Goodman *et al.* 1993, where she defines ELF fields as being those with a frequency of 1 - 300 Hz. This definition better encompasses the range of electromagnetic fields people come into contact with during their normal daily activities.

APPENDIX THREE

MAGNITUDES OF NATURAL & ARTIFICIAL FIELDS.

Between 1961 and 1967 an international effort assembled data acquired from around the world and produced "The International Geomagnetic Field" (IGRF) a series of global maps defining the properties of the geomagnetic field as of 1965. The map featured in Appendices Fig.1 is one of the maps produced as a result of this cooperative effort. It should be noted that the values of the geomagnetic field as shown on the map are no longer current. This is due to the drift of the magnetic values over time. This drift is considered to be sufficiently rapid as to necessitate revision of geomagnetic maps every 5-10 years. A full description of the scale of this drift and other phenomena related to the geomagnetic field can be found in Durward 1985.

Although the geomagnetic field is generally considered to be static, some low level fluctuations at low frequencies and intensities can occur as shown in Appendices Table 2.

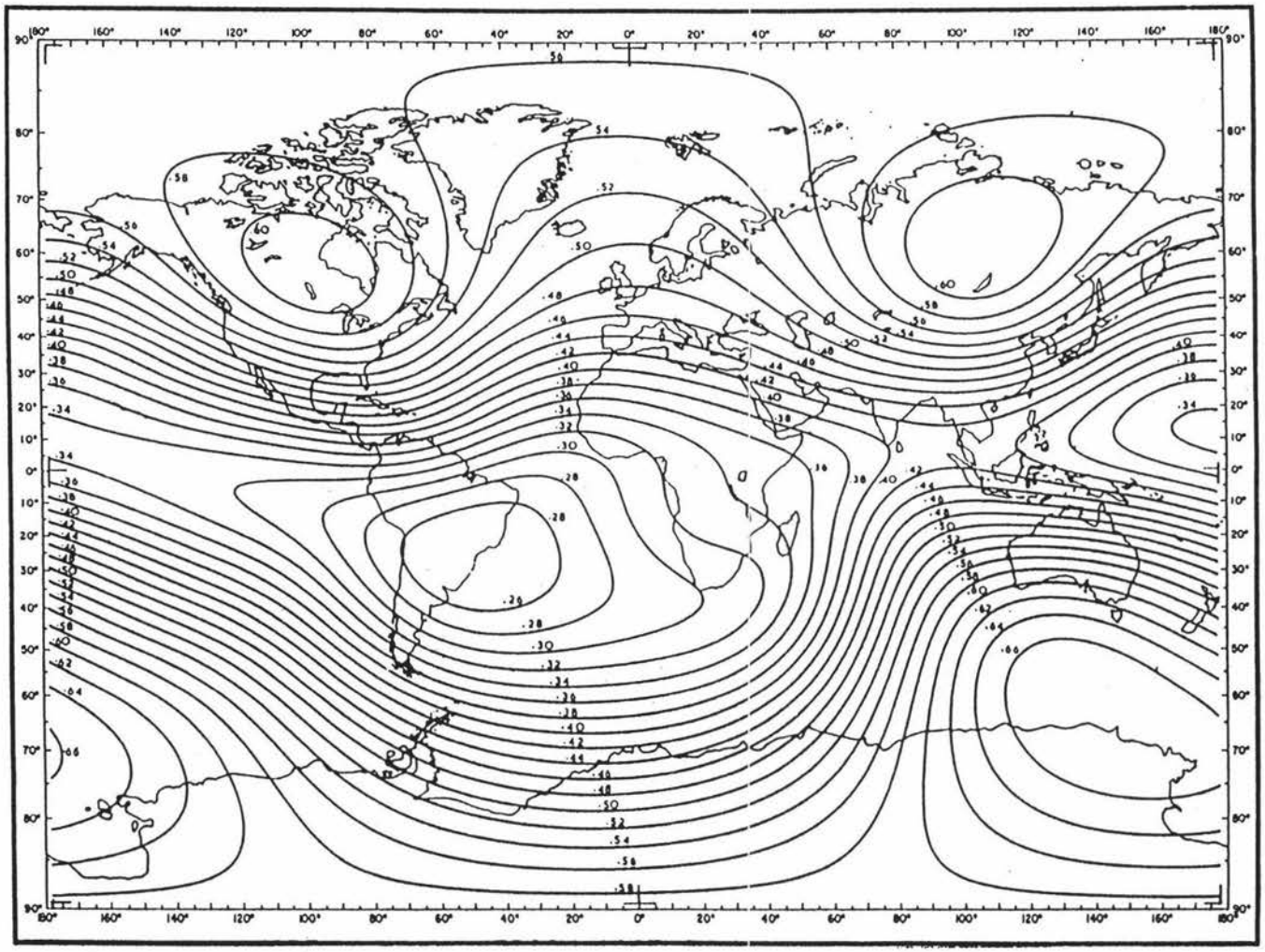
Appendices Table 3, Variations of the geomagnetic field.

Frequency in Hertz	Magnetic Field Strength
Static	0.3 - 0.6 Gauss
0.002 - 0.1	0.003 - 0.006 Gauss
0.1 - 5 (short duration)	Varies widely
5 -7	0.001 Gauss
*50 - 60	0.000 000 1 Gauss
3 000	0.000 000 001 Gauss

**See Appendices table 3, over page, for comparisons with EMFs generated by household appliances.*

Time varying electromagnetic fields originating from man-made sources generally have much higher intensities than do naturally occurring fields. This is especially true of fields in the region of 50 - 60 hertz. See Table 3, on the next page.

Appendices Fig.1, The total geomagnetic field intensities, in gauss.



Appendices Table 4, Magnetic field strengths from man-made sources.

Appliances	@3 cm		@30 cm		@1 m	
	min	max	min	max	min	max
Can openers	10.00	20.00	0.035	0.30	0.0007	0.001
Hair dryers	0.06	20.00	0.0001	0.07	0.0001	0.003
Shavers	0.15	15.00	0.0008	0.09	0.0001	0.003
Circular saws	2.50	10.00	0.1	0.25	0.0001	0.001
Drills	4.00	8.00	0.02	0.035	0.008	0.002
vacuum cleaners	2.00	8.00	0.02	0.2	0.0013	0.02
Mixers	0.60	7.00	0.006	0.10	0.0002	0.0025
Fluorescent desk iamp	0.40	4.00	0.005	0.002	0.0002	0.0025
Garbage disposal unit	0.80	2.50	0.01	0.02	0.0003	0.001
Microwave ovens	0.75	2.00	0.04	0.08	0.0025	0.006
Fluorescent lights	0.15	5.00	0.002	0.04	0.0001	0.003
Electric stoves	0.6	2.00	0.005	0.04	0.0001	0.001
Portable heaters	0.10	1.80	0.0015	0.05	0.0001	0.0025
Blenders	0.25	1.30	0.006	0.02	0.0003	0.0012
Television sets	0.025	0.50	0.0004	0.2	0.0001	0.0015
Electric ovens	0.01	0.50	0.0015	0.05	0.0001	0.0004
Washing machines	0.08	0.50	0.0015	0.03	0.0001	0.0015
Irons	0.08	0.30	0.0012	0.003	0.0001	0.0025
Fans and blowers	0.02	0.30	0.0003	0.004	0.0001	0.0035
Coffee makers	0.18	0.25	0.0008	0.0015	0.0001	0.01
Dish washers	0.035	0.25	0.006	0.03	0.0007	0.003
Toasters	0.07	0.18	0.0006	0.007	0.0001	0.001
Crock pots	0.015	0.08	0.0008	0.0015	0.0001	0.001
Clothes dryers	0.003	0.08	0.0008	0.003	0.0002	0.0006
Refrigerators	0.005	0.017	0.0001	0.0025	0.0001	0.001
Average strength	**0.91**	**4.56**	**0.0072**	**0.062**	**0.0004**	**0.0034**
Average max -min	**0.46**	**-**	**0.035**	**-**	**0.0019**	**-**

APPENDIX FOUR

LIQUID GROWTH MEDIA: as used by Watson and Fowden 1975

1 litre distilled water

0.2 grams $CaCl_2$ -(calcium chloride)

0.2 grams KNO_3 -(potassium nitrate)

0.02 grams Na_2HPO_4 -(disodium phosphate)

APPENDIX FIVE.

UNITS OF MEASUREMENT

1. Radioactive units:

Curie, Ci. This system for measuring levels of radioactivity is named after the French physicist Pierre Curie (1859-1906). The system of Curies has been used to measure radiation in concurrence with SI units, but the system is not coherent with the SI units system. The curie (Ci) is a unit of radiation that approximately equals the level of radioactivity emitted by 1 gram of radium-226. While still in widespread usage, this system is replaced by measurement system based by becquerels as a unit for measuring radioactivity.

Becquerels, Bq. The SI unit for measuring radionuclide decay rate. One becquerel (Bq) is equal to one spontaneous nuclear transition per second. In lay-man's terms that means, that on average one nucleus in the sample breaks down per second per second emitting waste energy in the form of radioactivity.

Curie-Becquerel conversions, 1 Ci = 3.7×10^{10} Bq.
The concertration of [^{3}H]-alanine used in this research was 1 μCi/ml, or 37 MBq/ml

Disintegrations Per Minute (DPM). DPM is a measure of the number of spontaneous nuclear transitions in a liquid sample as counted by a scintillation counter.

The scintillation counter records the number of light flashes per unit of time scintillation viL. The scintillation counter then compares the total number of light flash per minute against internal programs which compensates for the scintillation cocktail used and the radio-isotope in the sample to calculate the actual DPM of the sample.

2. Electromagnetic field measurements:

Gauss, (G). Units for measuring electric and magnetic fields, based on the c.g.s

units system electrostatic and electromagnetic units. Despite being replaced by the SI unit system, gauss are still widely used to express levels of magnetic flux density.

Weber, Wb. The SI unit for expressing *magnetic flux.* The unit system is named after the German physicist Wilhelm Weber (1804-1891). One weber is equal to the flux that, linking a circuit of one turn, produces in it an electromagnetic field of one volt as it is reduced to zero at a uniform rate in one second. What should be noted in this definition is, that it refers to a system containing only one circuit (loop) of wire conducting electricity.

Tesla, T. The SI unit for expressing *magnetic flux density.* The unit system is named after Nikola Tesla a Croatian born electrical engineer who did most of his work in the US. One tesla is equal to the 1 weber of magnetic flux per square metre, i.e. $1\ T = 1\ Wb/m^{-2}$. The difference in the definition of this system is that it refers to a system containing more than one circuit (loop) of wire conducting electricity, such as a coil, which produces magnetic field.

Gauss-tesla conversion, 1 gauss = 1×10^{-4} tesla.
The three intensities of electromagnetic radiation to which samples were exposed in the course of this research which have been expressed as gauss convert to tesla thus:

0.1 gauss = 10 μT
1.0 gauss = 100 μT
10 gauss = 100 μT or 1 mT

3. Conductivity:

Siemens, S. A measure of the ability of an aqueous solution to conduct electricity.

APPENDIX SIX

ELECTROMAGNETIC FIELD AND COIL PARAMETERS

Coil description:

Height:	50 mm.
Diameter:	60 mm.
Total turns:	150.
Layers:	2 layers of 75 turns each.
Current capacity:	2 Amperes maximum (continuous).
Wire:	22 gauge (0.642 mm) enamelled copper.

Coil modelling:

The magnetic field produced by the induction coil (described above) was modelled using the electromagnetic field simulation programme, MagneSim, developed by Brent Foster and Wyatt Page for Resonance Research[1]. MagneSim models the magnetic fields produced by circular form, air-cored induction coils including: short solenoid; long solenoid; Helmholtz and Maxwell pairs. The numeric solution is accurate to within ± 1% of the actual values as determined by measurement.

(i) The plane of calculation modelled.

The magnetic field produced by a circular form induction coil exists as a three dimensional phenomenon the space surrounding the coil. This circular symmetry about the Z (or long) axis means that it is only necessary to determine the field in a two dimensional plane. This plane may then be rotated about the Z axis to model the field in three dimensional space. The calculation plane is shown in Appendices Fig. 2, below.

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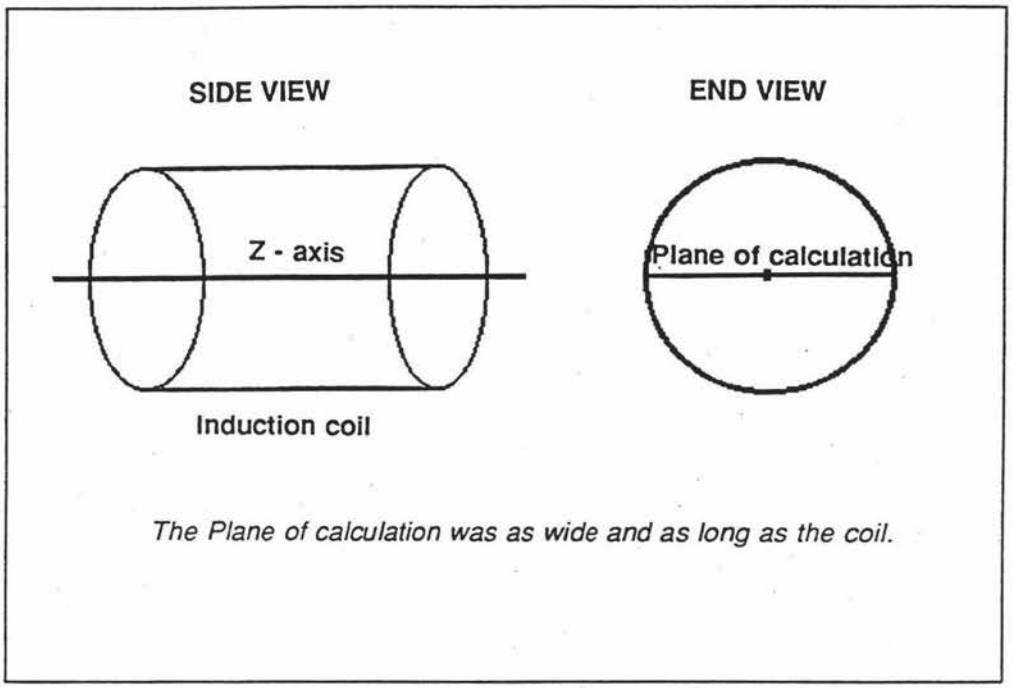
1. *Resonance Research is a small group of researchers investigating the biological effects of extremely low frequency electromagnetic fields. For further information contact: Bruce I.Rapley, Suite 2, 37 Ferguson St, Palmerston North, New Zealand, Phone (0064) 06 3571079.*

Appendices Fig. 2, MagneSim's plane of calculation.



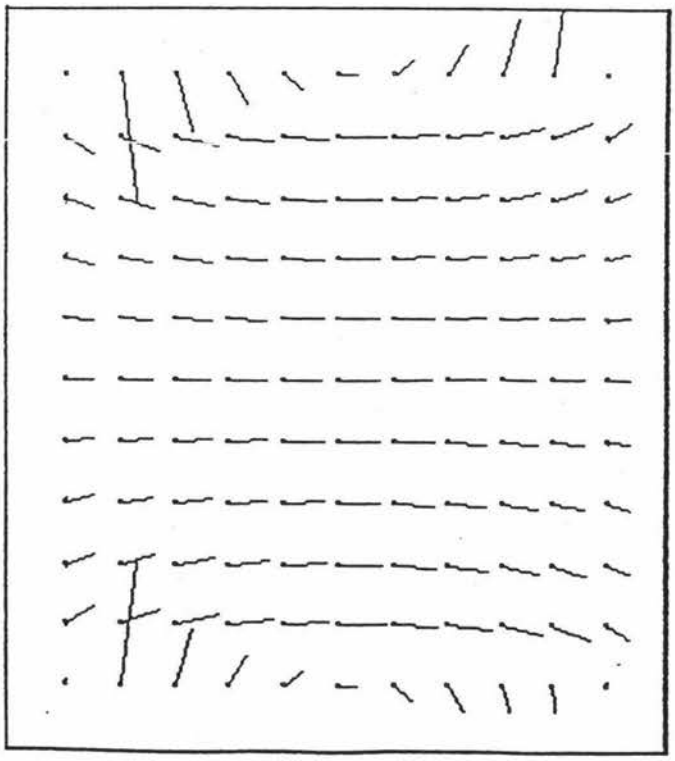
(ii) The calculation matrix.

MagneSim models the magnetic field of a coil in an 11 by 11 matrix of points on a two dimensional plane about the Z axis, thus bisecting the coil in the long axis. All points are equally spaced along their axis. However the x and y axes may be any arbitrary length. The calculation matrix may exist on any part of the calculation plane and is not restricted to having the coil in the centre. Indeed it is possible to choose an area anywhere, either within or outside, the coil. The only restraint is that the is that the points are automatically equally spaced in the x and y direction respectively. If a calculation point of the magnetic field in the calculation plane falls directly on the geometric x-y position of a turn of wire, MagneSim returns a "?", as the magnitude is, by definition, uncalculatable.

(iii) The electromagnetic field vector.

To visualise the magnetic field, MagneSim draws a plot of the magnetic field matrix points on the calculation plane. The field at each point is represented by a "lollipop", that is, a point with a stick attached. The "point" of the lollipop defines the exact geometric position on the calculation plane where the field is calculated, while the "stick" represents the field magnitude and the direction in vector form. The length of the stick represents the magnitude of the field, so that the largest field value represents two matrix units against which all other field values are autoscaled. The direction of the vector is graphically displayed directly in Cartesian coordinates. A zero degree vector is thus represented by a line drawn from a dot directly parallel to the Z axis pointing to the right. The lollipop plot of the above coil is shown in Appendices Fig. 3.

Appendices Fig. 3, Modelled directions of vectors as Lollipop plots.



(iv) The magnitude of the electromagnetic field.

The magnitudes of the magnetic field vectors for the coil used in this work are shown in Appendices Fig. 4. The inner box represents the area inside the

coil occupied by the beaker in which the seedlings were incubated.

Appendices Fig. 4, Modelled magnitude of vectors in gauss.

?	27.248	13.252	8.004	5.326	4.399	5.326	8.004	13.252	27.248	?
7.785	9.517	10.381	10.846	11.089	11.165	11.089	10.846	10.381	9.517	7.785
7.012	8.388	9.397	10.045	10.402	10.516	10.402	10.045	9.397	8.388	7.012
6.677	7.848	8.809	9.493	9.894	10.025	9.894	9.493	8.809	7.848	6.677
6.518	7.589	8.501	9.179	9.590	9.727	9.590	9.179	8.501	7.589	6.518
6.470	7.510	8.405	9.077	9.489	9.627	9.489	9.077	8.405	7.510	6.470
6.518	7.589	8.501	9.179	9.590	9.727	9.590	9.179	8.501	7.589	6.518
6.677	7.848	8.809	9.493	9.894	10.025	9.894	9.493	8.809	7.848	6.677
7.012	8.388	9.397	10.045	10.402	10516	10.402	10.045	9.397	8.388	7.012
7.785	9.517	10.381	10.846	11.089	11.165	11.089	10.846	10.381	9.517	7.785
?	27.248	13.252	8.004	5.326	4.399	5.326	8.004	13.252	27.248	?

The overall average field intensity within the beaker was 9.6933 gauss when the meter was set for a 10 gauss field. The actual field intensities of the 1 and 0.1 gauss fields were a decimal scale of this value.

(v) Direction of the magnetic field vectors.

The directions of the magnetic field vectors for the coil used in this work are shown in Cartesian coordinates in Appendices Fig. 5, below. Again the inner box represents the area of the coil occupied by the beaker in which the seedlings were incubated.

Appendices Fig. 5, Modelled directions of Cartesian Coordinates.

?	278	288	302	325	0	35	58	72	82	?
330	341	348	353	357	360	3	7	12	19	30
340	346	350	354	357	360	3	6	10	14	20
348	351	353	356	358	360	2	4	7	9	12
354	355	357	358	359	360	1	2	3	5	6
0	0	0	0	0	0	0	0	0	0	0
6	5	3	2	1	0	359	358	357	355	354
12	9	7	4	2	0	358	356	353	351	348
20	14	10	6	3	0	357	354	350	346	340
30	19	12	7	3	0	357	353	348	341	330
?	82	72	58	35	360	325	302	288	278	?

Ten electronmicrographs of randomly selected root sections, from four treated (EMF) and four control seedlings were scored for the presence of seven organelles. For uniformity and clarity the following definitions of the organelles were rigidly adhered to during this evaluation.

APPENDIX SEVEN.

DEFINITIONS OF ORGANELLE AS COUNTED.

Ten electronmicrographs of randomly selected root sections, from four treated (EMF) and four control seedlings were scored for the presence of seven organelles. For uniformity and clarity the following definitions of the organelles were rigidly adhered to during this evaluation.

Endoplasmic reticulum.

Only the rough endoplasmic reticulum (ER) was seen in counted cells. A positive count was logged if even a short segment of ER was visible within the cell, see micrograph **A**.

Extra cellular vesicles.

The major criteria for counting these organelles was that the vesicle was outside the plasma membrane (see micrograph **B**). The size, shape, or number of vesicles around the cell was not considered, as long as the vesicles outside the cell were scattered rather than clustered. Clusters of extracellular vesicles were counted separately.

Golgi stacks.

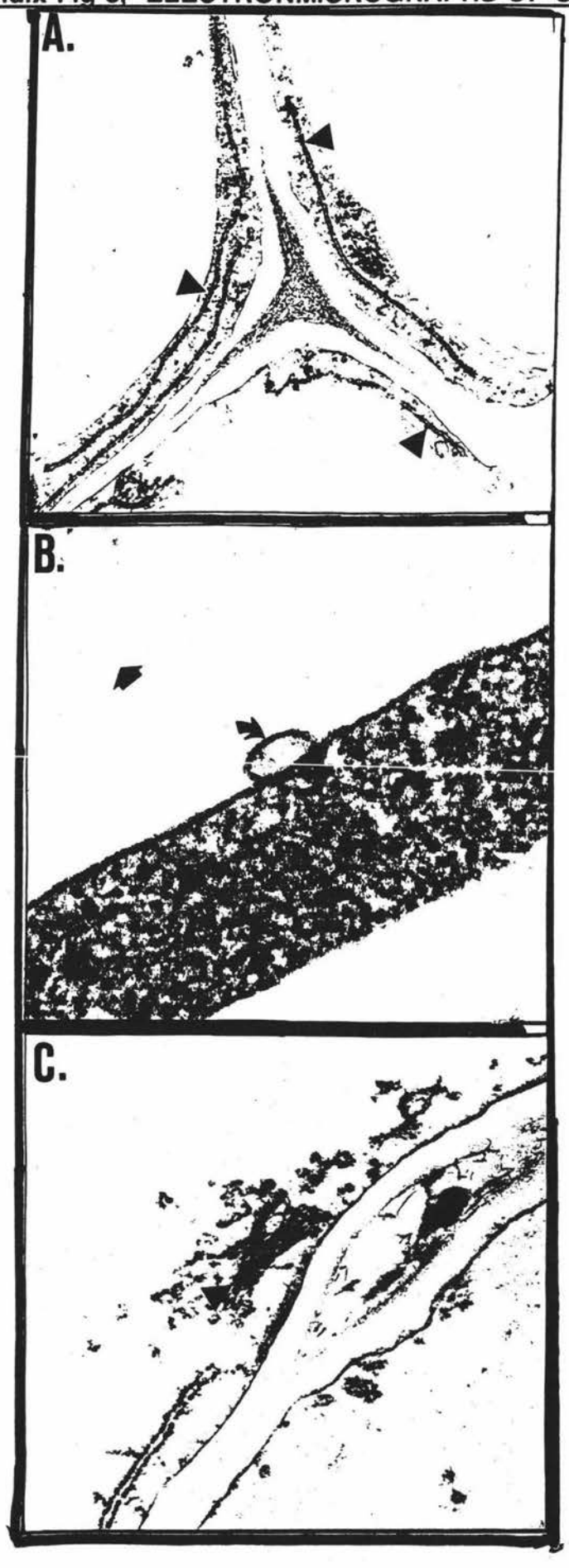
Of the various structures associated with the golgi apparatus only the stack of flattened golgi cisternae were readily recognisable. A typical stack of flattened golgi cisternae with clearly discernable cis and trans surfaces, can be seen in micrograph **C**.

Intracellular vesicles.

This term was aplied to vesicles within the cytoplasm of the cell, which had a clearly defined outer margin. Usually, but not exclusively, the contents of these vesicles were unstained and electron translucent (see micrograph **D**). While a vesicle with lightly stained contents can be seen micrograph **E**. Note that in both

examples the margin of the vesicles are crisp and clear.

Appendix Fig 5. ELECTRONMICROGRAPHS OF ORGANELLES.



A. Endoplasmic reticulum

Mag. x15,300. Lengths of endoplasmic reticulum (▶), can be seen in all three of these cells.

B. Extracelluar vesicles.

Mag. x103,600. ➔ Indicates the extracellular vesicle against the outer surface of the cells plasma membrane. ➧ Indicates the position of the cell wall which is only faintly visible.

C. Stack of golgi cisternae.

Mag. x31,800. ▶ Indicates the stack of flattened golgi cisternae. Cis side facing into the cell, while the trans side is toward the plasma membrane.

This crispness around the margin of these vesicles was the only readily apparent distinguishing feature differentiating these intracellular vesicles from vesicles derived from the plasma membrane (as discussed later in this section). This method of delineation between the two classes of vesicles had the drawbacks that the margins of vesicles which are small and electron opaque, or had a clathrin or nap coat were difficult to visualise. Therefore vesicles such as this were omitted from the count.

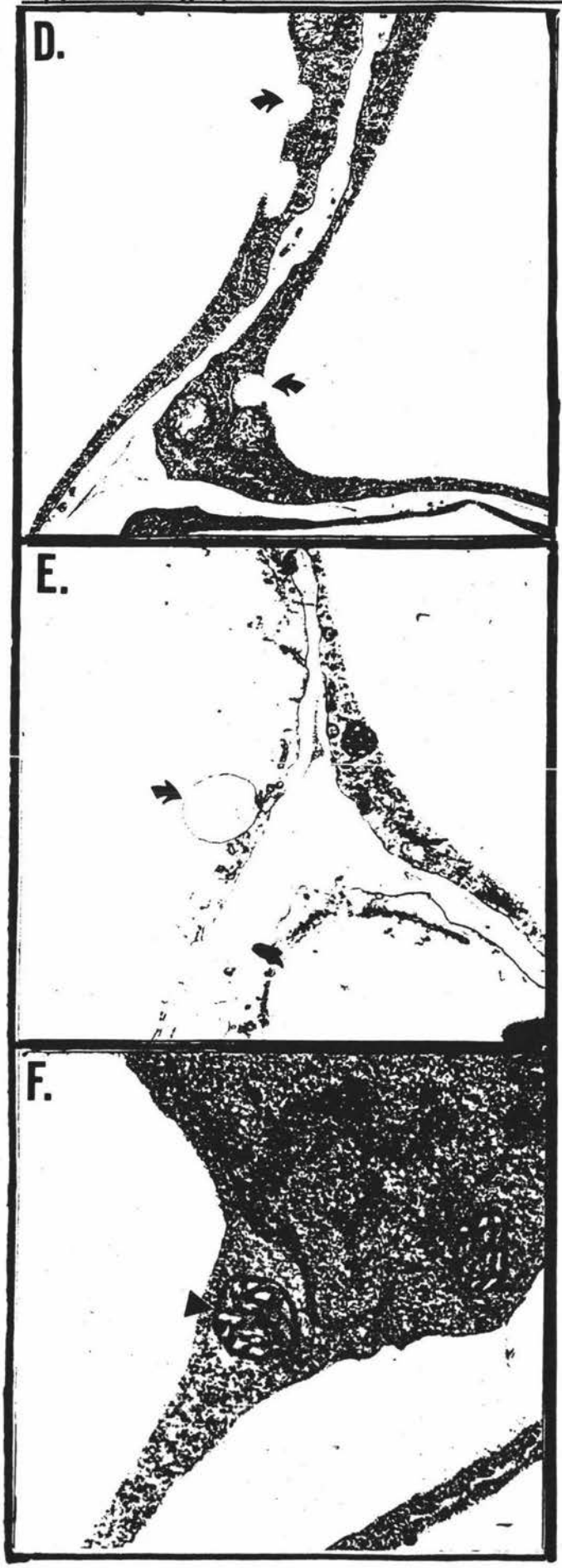
Mitochondria.

The mitochondria of *Vicia faba* have tubular cisternae snaking through their interior, as can be seen in micrograph **F**. Thus the mitochondria can be readily differentiated from other organelles as such as plastids. A positive count was logged only if the tubular cisternae were visible inside the organelle.

Pinocytotic vesicles.

These vesicles develop when an invagination forms on the plasma membrane and balloons into the interior of the cell (see micrograph **G**). At this phase it is redily apparent that membrane bounding the developing vesicle is double layered (see micrograph **G**). Close examination of the vesicle's membrane often shows some sites where the two layers of the membrane appear to be separating (see micrograph **G**), while at other places, the membrane may be poorly defined or fuzzy (see micrograph **G**).

Once fully formed, these vesicles bud away from the plasma membrane. At this stage the membrane bounding the vesicle often appears to have become blurred and indistinct as can be seen micrograph **H**. The blurring of the vesicles membrane is not always as advanced as that of the vesicle in micrograph H. But some reduction in the exactness of the membrane bounding these vesicles is always apparent. This blurring of the outer membrane was the major diagnostic feature used to differentiate pinocytotic vesicles (PV) from vesicles derived within the cell (intracellular vesicles) as described earlier.



D. Intracellular vesicles.

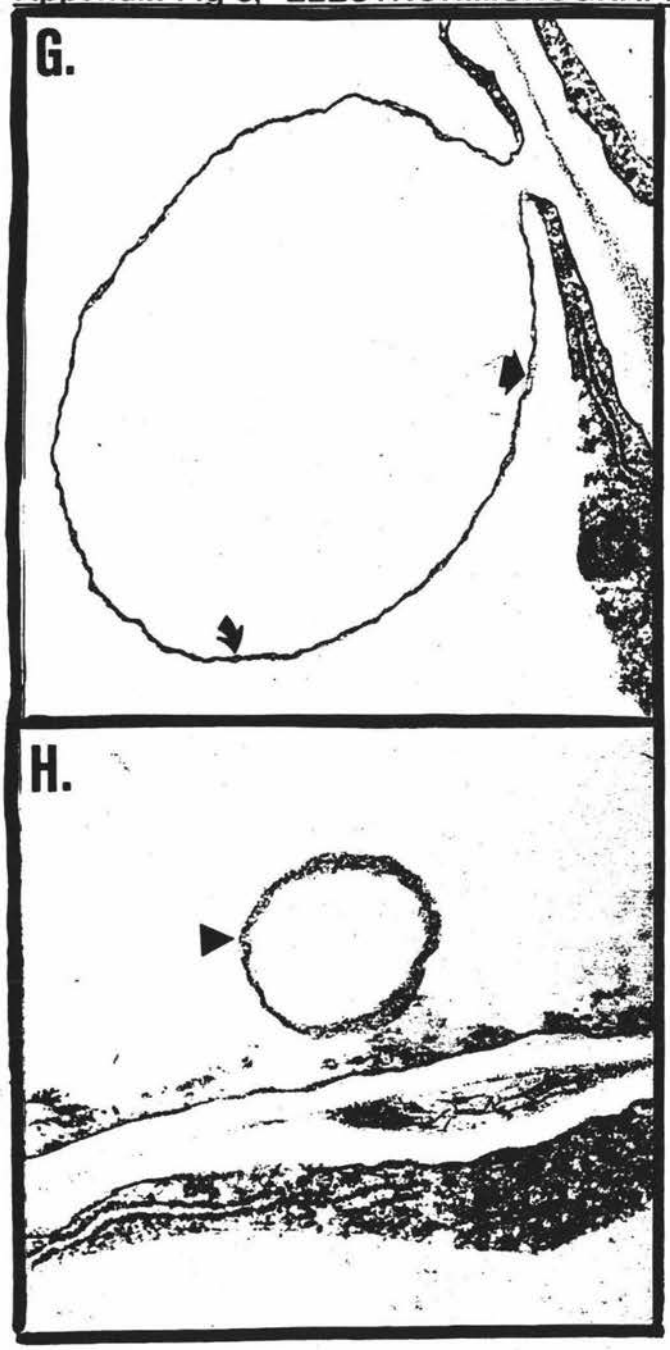
Mag. x11,200. Vesicles can be seen (➧) in the cytoplasm of two different cells. Their contents are unstained and electron translucent. Not the distinct margins around the vesicles.

E. Intracellular vesicle.

Mag. x11,200. The contents of this "vesicle" are stained and so electron opaque (➧). Again the vesicle has clearly differentiated margins.

F. Mitochondria.

Mag. x31,800. The mitochondria (▲) can be seen near the cells nucleus. Tubular cisternae are visible snaking through the interior of the mitochondria. The double layered membrane around the mitochondria are well visualised.



G. Pinocytotic vesicle.

Mag. x31,800. Here a vesicle can be seen ballooning into the cell from the plasma membrane. ➘ *Indicates a position where two layers of the vesicles membrane appear to be separating. While* ➧ *indicates a section of membrane which is blurred and becoming indistinct.*

H. Pinocytotic vesicle.

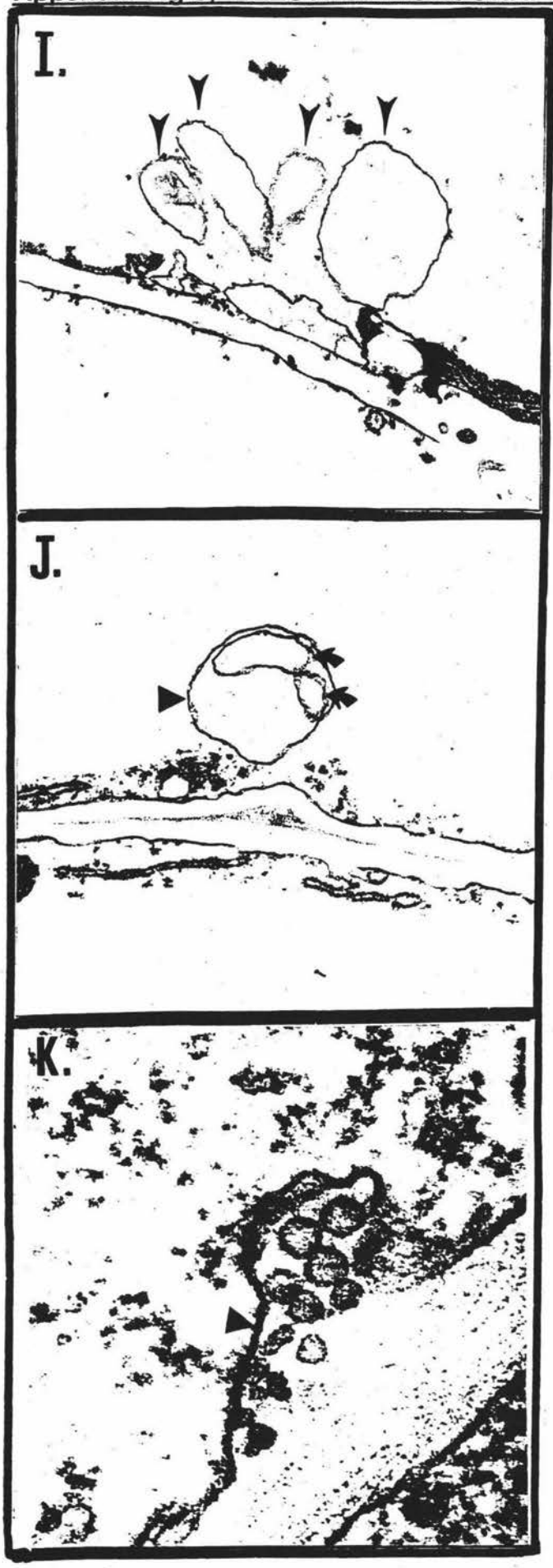
Mag. x11,200. This (▶ *) pinocytotic vesicle has budded away from the plasma membrane and is now positioned within the cells vacuole. Note the advanced blurring of the membrane.*

Considerable variability was seen in PV size, distribution and in the numbers of vesicles present in cells. Some cells had clusters of PVs (see micrograph **I**), while a few PVs could be seen to have smaller (daughter) PVs within them (see micrograph **J**). However most of the cells scored did not have PVs at all.

Extracelluar vesicle clusters.

Were defined as any group of three or more vesicles in close proximity and located outside the cell's plasma membrane. Sometimes the clusters of vesicles were within an infolding of the cell's plasma membrane, as is seen in micrograph **K**. The vesicles were not necessarily as regular in shape as those seen in micrograph K. Many times the vesicles within the cluster were quite irregular in both size and shape.

Appendix Fig 5. ELECTRONMICROGRAPHS OF ORGANELLES.



I. Pinocytotic vesicles.

Mag. x15,300. Note how these four pinocytotic vesicles form a cluster against the inside of the plasma membrane. Which is suggestive of several vesicles forming in close proximity from a small patch of the cells plasma membrane.

J. Pinocytotic vesicles.

Mag. x21,200. Here two vesicles (◀) appear to have formed within a larger pinocytotic vesicle (▶). These daughter vesicles also have heavily blurred membranes.

K. Extracelluar vesicles.

Mag. x103,600. The plasma membrane indicated ▶, of this cell has invaginated to form a pocket. Inside of the pocket is a cluster of extracellular vesicles.

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