The biological effects of electromagnetic fields

by Paula Hyson Kosted

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biochemistry
Montana State University

Abstract:
The effect of extremely low frequency electromagnetic fields on different biological systems has been studied with variable results. Biological effects of these fields have been systematically measured in procaryotic organisms that have proven to be reproducible bioassays.

Alfalfa sprouting experiments measured the amount of sprouting enhanced by the calcium resonance frequency. Alfalfa seeds sprouted in an incubator at an elevated temperature of 34 degrees C, subjected to the calcium resonance frequency, showed less stress than the control.

The controls dried up instead of sprouting while the seeds placed in the fields continued to grow for up to 7 days.

A diatom motility assay measured the response of this organism when exposed to an electromagnetic field on an agar plate with various millimolar concentrations of calcium.

The organisms that glided and left a countable trail were tallied in a total of a hundred individuals. These experiments showed a 30% increase in the number of diatoms moving when exposed to the fields as compared to the control. However, apparently due to genetic variability in populations of this organism, the results were variable.

The Ames Salmonella reversion assay was used to test for DNA damage, error prone repair, misrepair, and subsequent backmutation or reversion caused by the fields. When tested in electromagnetic fields at various frequencies, Salmonella appeared to show a biological response. This response was not robust but some statistical significance was seen.

Escherichia coli strain, GE94, a lacZ fusion to a recA promoter can be used as a bioassay system for DNA damage and other physiological stresses such as inhibition of DNA replication. This fusion allows the cell to respond to a DNA damaging compound or physical process that inhibits replication. The cell produces β-galactosidase proportional to the amount of physiological stress which includes DNA damage.

The E. coli experiments performed in the electromagnetic fields showed no enhancement of growth and a variable production of β-galactosidase depending on the field exposure. Experiments were performed on bacteria which were calcium depleted to add extra stress to the system. The results of these experiments showed a weak response with some statistical significance.

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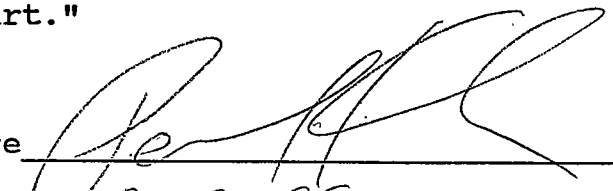
Signature 

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ABSTRACT

The effect of extremely low frequency electromagnetic fields on different biological systems has been studied with variable results. Biological effects of these fields have been systematically measured in procaryotic organisms that have proven to be reproducible bioassays.

Alfalfa sprouting experiments measured the amount of sprouting enhanced by the calcium resonance frequency. Alfalfa seeds sprouted in an incubator at an elevated temperature of 34 degrees C, subjected to the calcium resonance frequency, showed less stress than the control. The controls dried up instead of sprouting while the seeds placed in the fields continued to grow for up to 7 days.

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Escherichia coli strain, GE94, a lacZ fusion to a recA promoter can be used as a bioassay system for DNA damage and other physiological stresses such as inhibition of DNA replication. This fusion allows the cell to respond to a DNA damaging compound or physical process that inhibits replication. The cell produces β-galactosidase proportional to the amount of physiological stress which includes DNA damage.

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INTRODUCTION

Electromagnetic Field Effects

History

All organisms possess endogenous electric and magnetic fields and currents which are mechanistically linked to physiological control of cell membrane functions, glandular secretion, tissue growth and repair, and neural and neuromuscular activity. These physiological functions rely on electrical signals generated within cells through various processes involving chemical gradients, proteins, enzymes, ions, and ion channels. The functions of electrical signals have been studied in neuronal pathways, cell membranes, and bones in various organisms. Researchers in many disciplines including brain research, physiology, neurobiology, embryology, biophysics, behavioral, and muscle and bone research have studied this electrical activity in various organisms to attempt to understand particular life processes.(1-3)

It has been found that fish, birds, and some bacteria respond to external electrical or magnetic fields for navigation, object sensing, and communication.(3) It has been reported that the human brain contains iron magnetite which could conceivably respond to magnetic fields, just as in the case of the bird as a homing device.(4,5) These magnetic particles, referred to as magnetosomes, are

incorporated into the cell as iron magnetite. Magnetosomes have been found in magnetotactic bacteria which move along the lines of magnetic fields and are therefore influenced by the earth's magnetic field.(6,7)

The research presented here is based on experiments previously performed on diatoms, plants, cancer cells, and bone which were subjected to extremely low frequency electromagnetic fields(ELF).(8,9) In those experiments, the organism, tissue, or bone was exposed to an ELF field which was tuned to a resonance frequency which contains an AC and DC component for the magnetic field specifically tuned for a particular ion such as calcium, magnesium, or potassium.(8,10-12) These frequencies were chosen because these ions play important roles in many cell processes. This model, developed by Bruce McLeod, proposes that ions or ion channels are stimulated by the resonance tuned electromagnetic field. The model is based on calculations describing the movement of a charged particle in a magnetic field. From the calculations made by McLeod, the resulting mathematical expression describing this relationship between the charged particle and the magnetic field allows the ion's mass and charge plus a chosen AC frequency describe the DC magnetic component.

In the experiments performed in McLeod's laboratory using the resonance fields, diatom motility and root mass increased, bone fractures healed, and cancer cells

proliferated when placed in resonance tuned ELF fields. One aim of this research is to determine how these resonance frequencies are affecting the ions and/or channels and activity of protein performance or production.(13) Another goal of this research is to develop other reproducible methods to show the effects of these fields and to attempt to describe some mechanism of action.

The advent of high voltage electrical power plants brought about the first concerns of effects on humans or other life forms by electric and magnetic fields. This facilitated research commissioned by power companies starting in the 1960's. The United States Navy became involved in ELF research when a long wavelength transmitter which required burying a long antenna was proposed for submarine communications. Concerns about effects on organisms within the land surface affected by the antenna's fields and currents prompted the Navy to review research on electrical effects on organisms. Overwhelmed by the vast array of literature available on the subject, the Navy became involved in its own research on ELF and commissioned research by government agencies and universities.(3,14)

Physiological Electric and Magnetic Processes

Examples of the electrical nature of biological processes are numerous and should be reviewed with respect to this subject. The brain produces electrical and magnetic

rhythms which have an average frequency of 20Hz. These rhythms can be detected by electroencephalograms and magnetoencephalograms. Neurons propagate direct current pulses which act as electrical signals to muscles or glands. Cell membranes maintain an electric field gradient across the membrane due to the potential difference between the inside and outside of cells. Growth and differentiation in cells are associated with intracellular DC potentials and electrical currents. The protein constituent of bone, collagen, is piezoelectric, meaning electrical potentials are created when bone is under stress or pressure of physical force, such as when running or exercising and at times of growth and repair.(1-3)

The metabolic processes involved in maintaining a functional cell include the movement of electrically charged particles and ions. The ions most prevalent in biological systems are calcium, sodium, potassium, and magnesium. The rapid exchange of sodium and potassium ions creates a voltage spike in nerve cells known as a action potential. Transmission of this voltage spike from cell to cell maintains the electrical communication necessary for the proper function of the nervous system.(2)

Calcium, Ions, and Ion Channels

Biological systems require calcium for proper functioning. Calcium acts as a second messenger and is thus

a regulator in many cell processes such as cell motility, muscle contraction, cytoplasmic streaming, chromosome movement, neurotransmitter release, endocytosis, and exocytosis. Calcium is required for brain cell processes including energy metabolism and body thermoregulation. Calcium must bind prothrombin for proper blood clotting. Calcium levels regulate gap junctions which allow movement of ions and metabolites from one cell to the next. Muscle contraction is triggered by the release of calcium from the sarcoplasmic reticulum. The calcium then binds to troponin C, creating a conformational change necessary for actin and myosin to interact. Release of acetylcholine depends on the presence of calcium in the extracellular fluid.(2)

Ions, such as calcium, pass through membranes by means of channels or ion carriers. Membrane channels are pores spanning the membrane formed by proteins interacting to make a helical structure. Ions can move through this channel due to the hydrophilic environment created by the carbonyl groups from the peptide backbone of the protein. This environment greatly reduces the energy barrier across the membrane, allowing ions with sufficient thermal kinetic energy to pass through the channel. Channel forming proteins increase the permeability of the membrane to ions. Resistances are on the order of $10^8 ohm/cm^2$ for artificial membranes and the addition of channels can reduce this resistance by a factor of 10^6.(1,2,15,16)

An electrochemical gradient drives ions through the channels. This gradient is driven by chemical concentration differences and electrostatic forces. A membrane or lipid bilayer is typically 50A thick and carries a resting potential of 40-100mV, where the cell interior is at a lower potential than the exterior. This represents a voltage gradient of about 10^6V/m. Ion carriers use active transport to move ions across the membrane but energy is required to transport ions against the electrochemical gradient.(1,2,17,18)

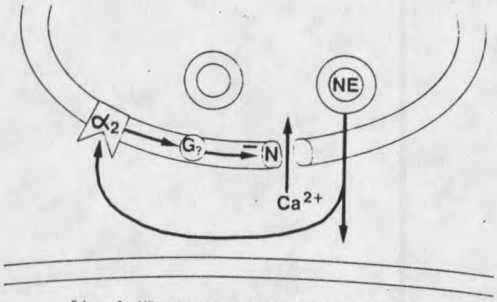
Calcium channels have been characterized according to electrophysiological and pharmacological properties. Subtypes have been established according to their voltage threshold for activation and by their inactivating characteristics. Low threshold inactivating calcium channels are referred to as T, dihydropyridine sensitive high threshold noninactivating channels as L, and high threshold inactivating channels as N.(19)

Electrophysiological experiments such as patch clamping are used to study gating and ion selectivity of channels. Gating kinetics are understood in terms of several closed and open states, while selectivity of the channel for calcium appears to be due to reversible binding of calcium to sites within the pore together with ion-ion interactions.(20)

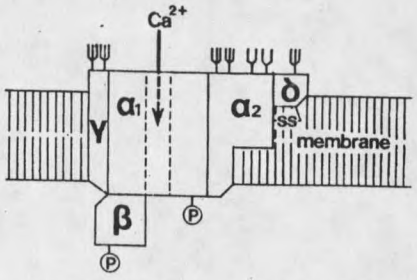
Most of what is known about calcium channels comes

from the study of skeletal muscle membranes. The skeletal muscle T-tubule membrane system was found to contain the richest source of receptors for calcium channel blockers, particularly 1,4-dihydropyridines(DHP). Two main types of calcium channels found in skeletal muscle membrane are two different T and one L type channel. Both T type channels are blocked by DHPs but have different voltage sensitivities of activation and different inactivation kinetics as determined by patch clamping.(20) Figure 1 shows various types of calcium channels.(20)

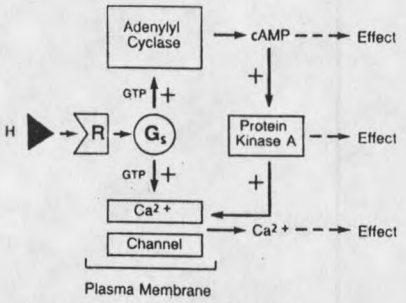
This background material is presented to indicate how important endogenous electrical currents are to living systems and how their proper maintenance requires the presence of ions such as calcium. The movement of ions through channels allows the proper physiological environment for cell function to be maintained. Slight fluctuations from equilibrium are sensed by the cell's regulatory mechanisms and a response is initiated toward regaining equilibrium, either through channel opening, ion binding, or enzymatic reactions. Stress on a system can also induce responses so that cell homeostasis may be maintained. It has been suggested that ion movement triggered by stress or a nonequilibrium state, such as during growth and repair, is enhanced when subjected to an ELF field.(21)



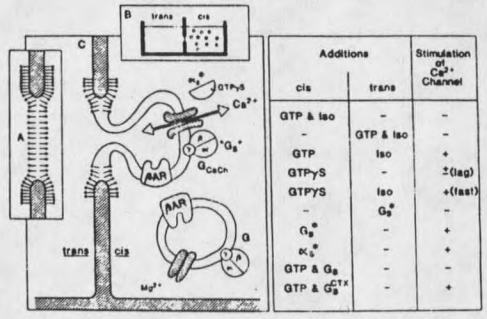
Scheme for NE-mediated autoinhibition. Depolarization causes opening of Ca channels leading to Ca entry. The Ca influx through N-type Ca channels is dominant in triggering release of vesicular sympathetic transmitter (NE) by exocytosis. Released or circulating NE can bind to an α_2 receptor to activate a G-protein leading to inhibition of N-channel activity, Ca entry and release. This scheme allows for discrete, localized regulation of Ca influx and release



Proposed model for calcium-channel structure. Sites of cAMP-dependent phosphorylation (P), glycosylation, and interaction with the membrane are illustrated.

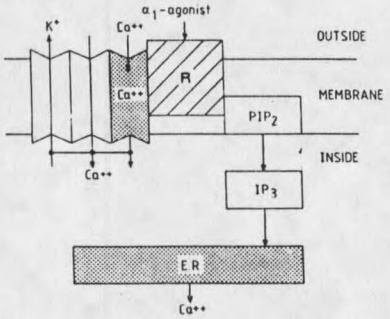


Proposed mechanism of G, effects on Ca^{2+} channels.



Additions		Stimulation of Ca^{2+} Channel
cis	trans	
GTP & Iso	-	-
-	GTP & Iso	-
GTP	Iso	+
GTPγS	-	±(lag)
GTPγS	Iso	+(fast)
-	G_s^*	-
G_s^*	-	+
α_s^*	-	+
GTP & G_s	-	-
GTP & G_s^{CTX}	-	+

Experiments that showed stimulation of skeletal muscle t-tubule Ca^{2+} channels on activation of coincorporated t-tubule G protein or addition of activated G_s



Processes activated during α_1-adrenoceptor stimulation in smooth muscle cells of taenia caeci. The following processes are assumed to take place. (1) Stimulation of the α_1-adrenoceptor (R) activates mobilization of calcium from a plasma membrane-bound store and facilitates the formation of inositol trisphosphate (IP_3) from the phosphatidylinositol bisphosphate (PIP_2) pool. (2) Calcium movement toward the cytoplasm is subsequently followed by replenishment of the calcium store from the extracellular space. Mobilization of calcium is coupled with the opening of potassium channels, causing potassium efflux and is linked with activation of calcium-dependent calcium channels, the latter being nonfunctional in the absence of external calcium. (3) IP_3 facilitates the release of calcium from the endoplasmic reticulum (ER).

Figure 1. Various types of calcium channels

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Previous Experiments

Bone Healing

Electric and magnetic fields have been used to heal chronically nonhealing bones for many years. This practice has been used by a large number of the orthopedic surgeons in the United States. However, the mechanism which acts at the cellular level associated with bone growth and repair is still unknown. In experiments on severed rabbit fibulas, it was found that by tuning the ELF magnetic fields to the magnesium ion resonance frequency provides the most striking results. The bone not only heals but also increases in thickness and mass.(22)

Plant Systems

The calcium ion is a major component of root cell growth and development. Because vegetative root systems have an enzymatic dependence on calcium, the calcium resonance frequency was used in experiments on plants. After exposure to the calcium resonant ELF magnetic fields, an increase of up to 25% in the root mass of plants was reported(23,24). A marked difference was noted when the plant was stressed by reducing the temperature in the growth chamber. Not only was the root mass significantly elevated but the plant mass and height also displayed significant

increases as compared to controls.(24)

Diatom Experiments

The diatom, Amphora coffeaeformis, is a microscopic unicellular algae which imbeds silica into its cell wall to form a bivalve shell-like structure.(25,26) As early as the Precambrian era, unicellular organisms in aquatic habitat developed two types of motility, flagellar and gliding motility.(25) The diatom being studied displays gliding motility. This is defined as the active translocation of an organism in contact with a solid or semisolid substrate or through a highly viscous matrix without a microscopically detectable organelle for locomotion or a visible change in shape. Different types of movement have been observed in the diatom under different environmental conditions. Jerking, twitching, or sliding motions are displayed in response to different environmental stimuli such as light, chemical, galvanic, mechanical, gravitational, and thermal cues.(25)

Motility enhances the organisms ability to seek a more favorable microhabitat for growth and reproduction. Motility is sometimes associated with secretion of mucous. Specific mechanisms of movement by algae have only been elucidated in a few organisms. Three types of motility known to occur in algal cells are calcium dependent actin-myosin, light dependent microtubule, and cytoplasmic

streaming.(25) While a raphe system is known to be present in A. coffeaformis, the exact mechanism of locomotion for this diatom has not yet been determined. The raphe consists of a complicated slit structure in the silicate frustule which allows active movement.(26,27)

While studying the adhesion properties of Amphora coffeaeformis, Cooksey et al. discovered that the diatom's movement was calcium dependent.(28,29) Because this organism was dependent on calcium for movement, experiments using the magnetic field frequency for calcium resonance were performed to determine its effects on this organism. Studies done by Smith and McLeod(8,10,11) showed enhanced movement of the diatoms when placed in an ELF field tuned to the calcium frequency and other ion specific cyclotron resonance frequencies. Movement of the diatoms on an agar plate was detected by using a phase contrast microscope. The mucous secreted by the diatom leaves a trail behind it which remains visible until the plate becomes too dry. Movement was most notably enhanced during the log phase of the growth curve of the diatoms. As seems to be the case with plants and bone, it appeared that the ELF effect in diatoms was greater under stress conditions.(30)

Due to the calcium dependence of this organism for motility, it was thought a calcium ion channel was being stimulated to allow calcium movement across the membrane and/or into the active site of a protein.(30) Motility could

then be enhanced either by setting up an electrical response such as hyperpolarization or actually causing binding of calcium in a calcium dependent protein such as troponin C which is required for movement. If for instance, the actin-myosin system is responsible for the movement, the additional calcium available by release from the sarcoplasmic reticulum or the enhanced binding of calcium to troponin C, could cause increased motility.(25,31,32)

Bacterial Systems

Bacteria require calcium for an enzyme dependent cell division reaction as well as many other functions.(33) In order for the cell to divide, calcium stores must be readily available for use with this enzyme. If bacteria become too stressed to divide because of lack of calcium, high or low temperature, or lack of nutrients, the cells will discontinue division and filamentation will occur. Filamentation is the stringing together of the cells that can't complete division, in other words, many cells string together with no clear single cell differentiation between them. The cell wall may have begun to form but the pinching off of one cell from the next one does not occur. Because growth and differentiation of cells is a stressful environment in itself, any other stress added to a normal system can very easily cause filamentation. If a cell remains in a healthful environment, the cells will reach

their normal logarithmic growth phase with complete cell division occurring. However if calcium channels could be triggered by a magnetic field, cell growth and division could be affected. Either a slowing down or stoppage of growth or a speeding up or enhanced growth should be seen. Effects on enzyme reactions involved in growth and repair may be effected by the nonbinding or enhanced binding of calcium.(33,34)

ELF effects on the growth of bacteria would be evidenced by changes in the growth curves, size and number of colonies, or protein expression. Similar results would be seen if activation or blockage of ion channels occurred through chemical means. If the ELF fields are actively affecting channels or protein binding, these results would mimic known responses to chemical stimulation.

For this research, bacteria with DNA mutations introduced into them were used for this study. These bacteria are Salmonella typhimurium and Escherichia coli. In the case of the Salmonella strains, specific genetic damage due to stress or mutagenic conditions could result in changes in the numbers of colonies grown.(35) With the E. coli strains, enzyme production could be triggered by physiological stress or more specifically, DNA damaging conditions(36). Stress, DNA damage and resulting RNA and protein synthesis, induced by the ELF fields, can be monitored by observing these bacterial bioassay systems.

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Stress

Stress is any environmental factor which does not allow an organism to grow and function properly. Stress can be induced by many means such as depleted or excessive light, heat, nutrients, ions, or water. An organism's response to these stresses can cause an inhibition or acceleration of processes needed for growth or repair. Actual blockage of a channel by competitive ions could result in an organism being nonmotile. Ion dependent proteins could be inhibited or unexpressed. Inhibition or over expression of proteins such as enzymes, which regulate homeostasis, could cause further imbalances. The table below lists some of the multigene systems which are triggered by various types of stress. (See Table 1)

TABLE 1. Multigene systems

Multigene system	Environmental stimulus	Regulatory gene(s)	Regulated genes
Nitrogen utilization	Ammonia limitation	*glnB, glnD, glnG, glnL*	*glnALG* plus others
Carbon utilization	Carbon/energy limitation	*cya, crp*	*gal, deo, ara, mal, dsd, tna, lac,* plus others
Phosphate utilization	Phosphate limitation	*phoB, phoM, phoR, phoU*	20+ genes
Stringent response	Amino acid/energy limitation	*relA, relB, relX, spoT, gpp,* plus others	Many
Heat shock response	Heat, certain toxic agents	*htpR* (*rpoH*)	17 genes
SOS response	UV and other DNA damagers	*recA, lexA*	17 genes
Adaptive response	Methylating agents	*ada*	3+ genes
Translation apparatus	Growth rate-supporting ability of medium	Many	200+ genes
Osmotic stress response	High osmolarity	*envZ, ompR* *kdpD*	*ompF, ompC* *kdpABC* plus others
Oxidative stress response	H_2O_2, other oxidants	*oxyR*	12+ genes
Anaerobic respiration	Presence of electron acceptors other than O_2	*fnr* (=*nirA*, =*nirR*)	20+ genes

Responses to stress can be monitored in various ways. In our systems, a response to stress can be shown by a change in movement, production of enzymes, or production of colony growth. Many systems have built in mechanisms to allow organisms to react to changes that they detect as harmful to themselves. These mechanisms, such as the SOS repair response, are global responses of the cell to some form of stress with SOS stress signals being predominantly linked to DNA damage and/or inhibition of DNA replication. The cell reacts to this recognition of stress by producing a series of proteins which can come to the aid of the cell for repair of DNA. In both Salmonella and E. coli, this response can be easily monitored.(37-40)

Mutation

Mutation is defined as a permanent change in DNA due to an error in replication or as a result of error prone repair in DNA causing changes in the organism which are inherited by subsequent generations. Mutations can be harmful and even lethal to an organism. Spontaneous mutations occur once in every 10^9 to 10^{10} base pairs. Errors in replication of DNA are few due to a multitude of proofreading and error free repair mechanisms within the cell.(2) A major protein involved in both error free recombinational repair and error prone SOS repair is the recA protein.

During replication proofreading removes incorrect nucleotides as soon as they are incorporated into the growing DNA strand. Hydrogen bonding errors allow an incorrect nucleotide to be added to the DNA chain at the rate of 10^{-4} to 10^{-5}. In prokaryotes, the incorrect nucleotide can be removed by the 3′ exonuclease activity of DNA polymerase III, which then also allows for replication to resume. In eucaryotes, a B-polymerase enzyme helps in the repair of DNA damage with the help of an associated nuclease. This is because the polymerase lacks nuclease activity, unlike the procaryotic polymerase. (2)

Effects of ELF fields on DNA or RNA during the reproduction cycle of a bacteria could cause replication errors to occur which could impair or improve growth. These mutations could cause repair of previously damaged systems or induce damage which could trigger repair systems to respond at a higher than normal level. The Salmonella strain of bacteria that has been engineered to detect mutagenic properties in chemical agents was chosen to test the possible mutagenic properties of ELF. Another bacterial system which will detect DNA damage and/or physiological stress is the GE94 strain of E. coli which has a gene fusion of a recA promoter to the reporter gene β-galactosidase which allows for detection of DNA damage and/or stress. Damage is reported by overproduction of this enzyme linked to the SOS repair regulons.(36,41)

SOS Response

Many procaryotic and eucaryotic organisms contain mechanisms to respond to stress and genetic damage. In the systems studied here, the SOS response is the focus of the physiologically related or DNA damage induced response by these stress related signals.(38-40,42,43) In the Salmonella and E. coli bioassays being used, the response by the cell to stress or mutation events that occur during the DNA replication cycle of the bacteria, are reflected by induction of the SOS system. The Salmonella genes mucA and B, carried on the pkM101 plasmid, are under SOS regulation and are analogous to the umuC and D SOS genes of the E.coli.(42)

The SOS response is a global reaction by an organism to many types of stress such as DNA damage, inhibition of DNA replication, inadequate or inappropriate growing conditions, or in this case magnetic fields.(44) The system controls up to 17 proteins which are responsible for returning the cell to homeostasis. The proteins that are synthesized are responsible for repairing any damage done to the DNA or shutting down synthesis of DNA for as long as stress is felt. This allows the organism to react to possible genomic changes before they become too frequent in the system and cause cell death.(39,40,44-46)

Some of the genes involved in the SOS response are the din genes or damage inducible, sul or cell division gene,

and the rec or recombination genes. The recA gene is responsible for the proper recombination of the DNA(39), sul allows proper cell division(47), and din repairs the DNA damage produced if it is not too destructive or wide spread.(44) Since these are global responses, they are regulated by the same activator and repressor on different operons in the organisms' SOS regulon. The activator is recA+ and the repressor is lexA.(39,48) When the system detects stress in the form of DNA damage, it develops a build up of the activator of the SOS operon or recA+. The recA then binds to lexA and the protease action on lexA, inactivates lexA as an SOS repressor and allows production of the protein. An effect on a specific protein such as sulA could activate the SOS response if cell division problems were occurring from starvation or lack of appropriate ion availability as well as perceived DNA damage by the presence of single stranded DNA.(49-51).

While the recA activates all of the 17 different proteins in the SOS system, it can react when specific genes such as sulA or the din proteins have been affected. If the organism was starved or ion concentrations were low, it would respond by stopping reproduction of any more cells until a healthy environment was reestablished. In this case it would trigger the sulA gene to stop cell division. If the cell's environment improved, the sulA synthesis would be shut down and proper cell division would again take

place.(36)

For the purpose of this study, the E. coli genes in the SOS response system have been fused with an enzyme for the reporting of SOS activity. Researchers have developed many gene fusions within the SOS repertoire to report on specific activity of the genes spliced. Our research uses the recA:lacZ.(52) When the SOS response is triggered by the production of the recA protein, the lexA repressor is cleaved by the activated proteolytic recA protease and the proteins affected are turned on. Instead of the proteins being able to help relieve the stress, they now produce the enzyme β-galactosidase. This is called a reporter gene. The gene would normally produce an appropriate protein response but instead produces the enzyme in a quantitative response equal to the amount of DNA damage. The amount of genetic effect corresponds to the amount of enzyme produced.(53-55) In the case of GE94, there is a recA promoter lacZ fusion in addition to a normal recA operon.

Cell division is a calcium mediated process. If calcium was low, the cell will respond by shutting down reproduction.(33,36) This will turn on the sulA gene to shut down cell division. If the sulA gene is spliced to the lacZ gene it will produce β-galactosidase instead. If there is a mutational event where the DNA is damaged the recA gene and din genes would be turned on, and therefore the enzyme would be produced in accordance with the amount of damage.

The recA:lacZ fusion reports the global, not the individual, responses to DNA damage. When the SOS regulon receives a DNA damage signal, the entire regulon of the SOS system is turned on. The SOS response regulon appears to be an additional or back up defense even when other direct paths to repair DNA are being used.

The SOS response is an error prone process.(38,44) When DNA damage is found by the repair system and it attempts to fix the flawed piece of DNA, it sometimes makes mistakes. When the damaged piece of DNA is removed and new bases move into the spot, improper base pairing can take place causing a mutation.(38)

Electromagnetic Fields

The magnetic fields used for these experiments are produced from Helmholtz coils constructed by Dr. Bruce McLeod. The coils produce two magnetic fields, one time varying, AC, and one static, DC. A frequency generator supplies the current for the AC field while a DC power supply provides the DC current to the coils.

The AC fields are monitored by an oscilloscope and calibrated by a magnetometer. The DC and magnetic fields are monitored by a magnetometer. These instruments are used to monitor the field in a specific area in an incubator where the temperature can also be controlled. Very little drift or change in the fields is noticed through time. This

monitoring is necessary to regulate the fields closely and to show that outside sources of electric or magnetic fields do not cause changes in the fields.

The Helmholtz coils are used to generate a known magnetic field that is uniform throughout some region in space. They were originally used to generate a zero field region where the geomagnetic field is cancelled. In recent years Helmholtz coils have been used in experiments to determine the effects of extremely low frequency electromagnetic fields on biological organisms.(8,12) A typical coil system is shown in the figure below.(See Fig.2)

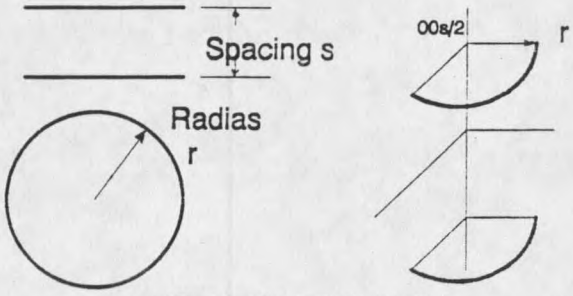

Figure 2. HELMHOLTZ COILS

Two coils of "magnet wire" (copper with enamel coating acting as the insulation) have N turns in each coil. For our purposes, 400 turns of #28 wire are used for the laboratory exposure coils. The coils are spaced at some distance s, where s is usually equal to the radius, r. If the spacing equals r, the region between the coils where the magnetic field is nearly uniform is a cylinder whose radius is approximately 0.6r and height is 0.6r.

The fields are set to an ion frequency which is determined using the equation $2\pi F=(q/m)B$ which refers to the Lorentz force used when calculating cyclotron resonance.(56)This can be rearranged to $f = qB/2\pi m$ where

f = frequency

B = dc magnetic flux density(Tesla)

q = charge of electron, $1.6x10^{-19}$coulomb x n # of charges

m = mass of ion q/m = coulomb/kg

Once a working frequency is chosen, this equation is used to determine a magnetic field strength for each individual ion according to its mass and charge. The required DC B field is set by use of the magnetometer.

The ion cyclotron resonance theory developed by Liboff(57) and McLeod(56) predicts ion movement to be stimulated through channels or ion binding to active sites of proteins. The theory can show the movement of ions by specific pathways thus predicting the passage through channels or not. While this theory proposes activation of ions through channels or into active sites, the actual response in experiments performed does not match the calculated speed of movement of ions through channels. In McLeod's theory, the ions move 10^4 times slower than some known systems. One reason for this slower response is that the channels being activated are not previously identified channels, but are a type of emergency channel.(56)

These emergency channels may only be triggered by small fluctuations created by the magnetic fields on the normally voltage gated channels. Another possible explanation of this slow movement might be that the magnetic fields are actually inducing some sort of chaperon effect on the channel, or inducing some other protein response to activate a channel.

STATEMENT OF THE PROBLEM

Electrically generated electric and magnetic fields in our environment are the cause of much anxiety to people living and working in conditions that expose them to high levels of these fields. Historically these fields have been shown to have effects on bone growth and other aspects of biological growth. If this is the case, what effect do these fields have on people exposed to them everyday of their lives? If a child grows and develops in an area where exposure to electromagnetic fields is high, is that child at risk of developing certain types of diseases such as cancer? What is the risk of long term exposure to these fields? The research described in this paper is directed toward creating methods for beginning to answer these questions.

The mechanisms of bone growth and therefore repair of broken bone have not been elucidated. Yet magnetic fields have been used very successfully by orthopedic surgeons for healing chronic nonhealing fractures. Is there a chance that while bones are being repaired by ELF fields, other areas exposed at the same time are harmed? If so, how is this type of damage detected and eliminated? If not, why does "normal" bone and tissue not respond? Stress on the system appears to play a role in this dilemma.

Reproducibility of results in any scientific experiment, especially when using living organisms, is essential. The experiments and epidemiological studies

performed in this field show that reproducibility of results is a continual problem. It has been suggested this is due to genetic traits that are inherent in certain populations of biological systems. While one set of chickens respond to electromagnetic fields in a particular way, other populations may have no response at all. This could be due to genetic adaptations of some populations in one manner while another population may have a different and more prevalent set of traits.

Experimenters studying the effects of ELF fields on biological systems often mention the "window" where an effect may be seen. This window might coincide with a biological activity of unknown origin which is synchronous only some of the time when an effect is seen. If the system being used is not stressed adequately for the fields to be felt, and thus compensates for the stress, then no response is observed. It has been shown in many different systems that stimulatory or inhibitory effects are seen when a biological system is exposed to an ELF field, but the greatest effects are seen when something essential to diet or homeostasis is challenged or lacking, thus stressing the system. If an organism can eliminate the stress successfully by endogenous compensating mechanisms and achieve equilibrium or homeostasis before the effect of the field is involved, no result may be seen in an experiment.

Environmental factors such as the geographical origin

of a population and exposure to a certain field or its actual family genetics may play a significant role in the measurable effects of ELF fields. Just as certain people have a higher risk of heart disease or cancer because their familial DNA contains genetic defects, all biological systems have these same tendencies. For this reason, the effects of these fields should be examined on the most simple possible model. Through the use of bacteria that can be manipulated to produce known responses to a stimulus, a reproducible result can be obtained.

If reproducibility can be obtained for one system, the possibility exists for greater control over other, more complex systems. Since magnetic fields heal bone effectively, other positive effects of EMFs may be found. Repair responses may be triggered to allow for DNA damage to be reversed before lethal or damaging effects to an organism are incurred. If repair responses can be manipulated through the use of ELF fields, then diseases such as cystic fibrosis, which are caused by the improper opening and closing of ion channels, may be treatable. An experimental method that will allow reproducibility of results is a major goal of this project.

Magnetic fields may affect systems in more than one way. It is possible repair systems, channels, enzymes, ion binding proteins, membrane permeability, or free radical formation are all affected to a greater or lesser extent by

ELF fields. Much more research must be carried out to determine exactly which mechanisms are affected in any given system.

A major criticism of the research involving ELF fields is that no mechanism of action can be pointed out in any system. By using bacteria with specific systems that respond to stress in a known manner, a mechanism for the stress reaction to the electromagnetic fields may be found.

EXPERIMENTAL

Alfalfa Seed Sprouting

Alfalfa seeds were used to determine the effect of the fields on seed germination. Ten grams of seeds were placed in a tall covered plastic container with 20mls of distilled water and placed in incubators, one containing a Helmholtz coil with the fields tuned to the calcium resonance frequency at 60Hz AC and 78.4uT DC. The temperature in the incubators was maintained at 34 degrees to stress the seeds with heat. The containers with the seeds were weighed after 24 hours to determine any change. For up to 7 days, a total of 20mls of water was added after weighing. Any variation on the amount of sprouting or drying was noted each day.

Diatom Motility Experiments

Motility experiments involving the diatoms, Amphora coffeaeformis, were developed by Bruce McLeod, Stephen Smith, and Barbara and Keith Cooksey to detect what effects, if any, tuned magnetic fields have on calcium mediated motility.(8,10,11) The diatoms, as described previously, have a raphe system used for movement, however the actual mechanism which allows movement is not known. It has been demonstrated that diatom motility is calcium dependent.(29) The calcium ion is possibly participating in a myosin motility system.(25)

The diatoms used for these experiments were three strains, #2039-Texas, Cooksey-IIIB, and Fritsen-IIIB. These types refer to who was maintaining the population and if the population was a clone or mixed population. The Texas strain was a mixed population while the IIIB types were clones maintained by the person named. These cultures were maintained on an agar slant at room temperature.

To prepare a sample for experimentation, a scraping from a slant was mixed into 100ml of 0.25mM ASP2 medium. This medium was a standard synthetic salt water medium with calcium added.(58) The population was monitored daily to determine the number of diatoms present in the container by counting diatoms with a hemocytometer under a phase contrast microscope. The hemocytometer has a 400 square grid. The number of diatoms on the 400 squares were counted and multiplied by 10,000 to obtain the number of diatoms per milliliter of solution. These numbers were plotted on log paper to observe whether a normal growth curve has developed. Once the normal growth curve was established, the diatoms were ready to be used for an experiment.

To perform the experiment, 30,000 cells/ml were placed in a test tube with the final volume of 5ml ASP2 media and allowed to grow. This was done everyday for a week with the growth determined daily from each tube. Two or three days of growth was shown to be optimum for movement. Two and three day old diatoms were spun down in an ultracentrifuge,

the media removed, and the diatoms were washed twice with a minimal media to remove all calcium. The diatoms were then counted and reconstituted with the minimal media to obtain a final concentration of $1.5x10^5$cells/ml.

Agar plates were made with minimal salts and specific molarity of added calcium were prepared ahead of time and stored in the refrigerator. Prepared plates were warmed to room temperature before use. Lines were drawn on the underside of the bottom plate as a guideline on which to lay the diatoms. Immediately before use, the tube containing the diatoms was vortexed gently. A small drop of the liquid culture was placed at one end of the line, the plate was tilted to allow the drop to run down the line drawn on the plate and then reversed to ensure even dispersion of the diatoms. The plates were placed in the magnetic fields under a light for 30 minutes then the diatoms were killed by formaldehyde in a vacuum.

Diatom movement was indicated by mucous trails left on the plate media. These trails showed as white lines compared to a dark background under a phase contrast microscope. After the diatoms were killed, plates were placed under the phase contrast microscope and the number of diatoms that could be counted as individual visible trails were recorded.

Great care was taken so as to count only individual diatoms. Diatoms tended to clump in large numbers. Any