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
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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.

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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.

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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.
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
Bozeman, Montana
January 1995
APPROVAL

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Paula Hyson Kosted

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF TABLES ...................................... vi</td>
</tr>
<tr>
<td>LIST OF FIGURES .................................... vii</td>
</tr>
<tr>
<td>ABSTRACT ........................................... ix</td>
</tr>
<tr>
<td>INTRODUCTION ....................................... 1</td>
</tr>
<tr>
<td>Electromagnetic Field Effects ..................... 1</td>
</tr>
<tr>
<td>History ........................................... 1</td>
</tr>
<tr>
<td>Physiological Electric and Magnetic Processes ........ 3</td>
</tr>
<tr>
<td>Calcium, Ions, and Ion Channels .................... 4</td>
</tr>
<tr>
<td>Previous Experiments ................................ 9</td>
</tr>
<tr>
<td>Bone Healing ....................................... 9</td>
</tr>
<tr>
<td>Plant Systems ..................................... 9</td>
</tr>
<tr>
<td>Diatoms Experiments ................................ 10</td>
</tr>
<tr>
<td>Bacterial Systems .................................. 12</td>
</tr>
<tr>
<td>Stress ............................................. 14</td>
</tr>
<tr>
<td>Mutation ......................................... 15</td>
</tr>
<tr>
<td>SOS Response ..................................... 17</td>
</tr>
<tr>
<td>Electromagnetic Fields ............................ 20</td>
</tr>
</tbody>
</table>

| STATEMENT OF THE PROBLEM ........................... 24 |

| EXPERIMENTAL ......................................... 28 |
| Alfalfa Seed Sprouting ............................ 28 |
| Diatom Motility Experiments .................... 28 |
vi

LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Regulon</td>
<td>14</td>
</tr>
<tr>
<td>2. Alfalfa Sprouting Results</td>
<td>51</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Various types of calcium channels</td>
<td>8</td>
</tr>
<tr>
<td>2.</td>
<td>Helmholtz coils</td>
<td>21</td>
</tr>
<tr>
<td>3.</td>
<td>Effect of AC Field on Alfalfa Sprouting</td>
<td>45</td>
</tr>
<tr>
<td>4.</td>
<td>Effect of DC Field on Alfalfa Sprouting</td>
<td>45</td>
</tr>
<tr>
<td>5.</td>
<td>Effect of Calcium Resonance Field on Sprouting</td>
<td>47</td>
</tr>
<tr>
<td>6.</td>
<td>Comparison of Different Fields on Sprouting</td>
<td>47</td>
</tr>
<tr>
<td>7.</td>
<td>Calcium resonance Field Effect over 7 Days</td>
<td>48</td>
</tr>
<tr>
<td>8.</td>
<td>Three strains of Amphora coffeafomis</td>
<td>55</td>
</tr>
<tr>
<td>9.</td>
<td>IIIIB strains of Amphora coffeafomis</td>
<td>55</td>
</tr>
<tr>
<td>10.</td>
<td>Comparison of age and motility at various Ca++</td>
<td>56</td>
</tr>
<tr>
<td>11.</td>
<td>Comparison of motility in 2/3 day old diatom</td>
<td>56</td>
</tr>
<tr>
<td>12.</td>
<td>Diatom movement on 0.0mM calcium with fields</td>
<td>58</td>
</tr>
<tr>
<td>13.</td>
<td>Diatom movement on 0.25mM calcium with fields</td>
<td>58</td>
</tr>
<tr>
<td>14.</td>
<td>Diatom movement on 0.5mM calcium with fields</td>
<td>59</td>
</tr>
<tr>
<td>15.</td>
<td>Diatom movement on 2.5mM calcium with fields</td>
<td>59</td>
</tr>
<tr>
<td>16.</td>
<td>Diatom movement on 5.0mM calcium with fields</td>
<td>60</td>
</tr>
<tr>
<td>17.</td>
<td>Diatom movement on various Ca concentration</td>
<td>60</td>
</tr>
<tr>
<td>18.</td>
<td>Salmonella reversion, K+ resonance frequency</td>
<td>69</td>
</tr>
<tr>
<td>19.</td>
<td>Effect of K+ resonance on various ul of azide</td>
<td>69</td>
</tr>
<tr>
<td>20.</td>
<td>Dose response, various azide concentrations</td>
<td>74</td>
</tr>
<tr>
<td>21.</td>
<td>Effect of Ca++ resonance frequency on reversion</td>
<td>74</td>
</tr>
<tr>
<td>22.</td>
<td>Effect of 20Hz AC field on reversion</td>
<td>76</td>
</tr>
<tr>
<td>23.</td>
<td>Effect of 26.1uT DC field on reversion</td>
<td>78</td>
</tr>
</tbody>
</table>
24. Various field conditions developed in sham......80
25. Various field conditions developed in coil......80
26. Potassium resonance frequency on reversion.......82
27. Effect of 51.0uT DC field on reversion...........84
28. Effect of 60Hz AC field on reversion.............84
29. B-galactosidase production, 78.4uT, + calcium....91
30. B-galactosidase production, 60Hz, + calcium.....91
31. B-galactosidase production, calcium resonance...93
32. Growth curve with calcium..........................93
33. B-galactosidase production, 78.4uT, no calcium..96
34. B-galactosidase production, 60Hz, no calcium....96
35. B-galactosidase, Ca++ resonance, - Ca++...........97
36. Growth curve, no added calcium.....................97
37. Experimental over control ratio, DC, +/- Ca++...99
38. Experimental over control ratio, AC, +/- Ca++...99
39. Experimental over control ratio, AC/DC, +/- Ca..100
40. All - Ca++ ratios.....................................100
41. B-galactosidase, 78.4uT, +/- calcium.............101
42. B-galactosidase, 60Hz, +/- calcium...............102
43. B-galactosidase, Ca++ resonance, +/- calcium....103
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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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. 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 an 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 $10^6\text{ohm/cm}^2$ 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 50 Å thick and carries a resting potential of 40-100 mV, where the cell interior is at a lower potential than the exterior. This represents a voltage gradient of about 10^6 V/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 autophosphorylation. 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 neurotransmitter (NE) by neurons. Release or concurrent NE-evoked signal transduction requires activation of 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 mechanism of G, effects on Ca" channels.

Experiments that showed stimulation of skeletal muscle tubule Ca" channels on activation of coincorporated i-tubule G protein or addition of activated G,.

Processes activated during a-adrenoceptor stimulation in smooth muscle cells of taenia caeci. The following processes are assumed to take place: (1) Stimulation of the a-adrenoceptor (R) activates mobilization of calcium from a plasma membrane-bound store and facilitates the formation of inositol triphosphate (IP,). (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, 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 organism's 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. 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.

While studying the adhesion properties of Amphora *coffaeaformis*, Cooksey et al. discovered that the diatom's movement was calcium dependent. 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 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.

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. 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.\textsuperscript{(25,31,32)}

\textbf{Bacterial Systems}

Bacteria require calcium for an enzyme dependent cell division reaction as well as many other functions.\textsuperscript{(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 affected 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.
monitored by observing these bacterial bioassay systems.

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>
<thead>
<tr>
<th>Multigene system</th>
<th>Environmental stimulus</th>
<th>Regulatory gene(s)</th>
<th>Regulated genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen utilization</td>
<td>Ammonia limitation</td>
<td>glnB, glnD, glnG, glnL</td>
<td>glnALG plus others</td>
</tr>
<tr>
<td>Carbon utilization</td>
<td>Carbon/energy limitation</td>
<td>cya, crp</td>
<td>gal, deo, ara, mal, dsd,</td>
</tr>
<tr>
<td>Phosphate utilization</td>
<td>Phosphate limitation</td>
<td>phoB, phoM, phoR, phoU</td>
<td>tna, lac, plus others</td>
</tr>
<tr>
<td>Stringent response</td>
<td>Amino acid/energy limitation</td>
<td>relA, relB, relX, spoT,</td>
<td>Many</td>
</tr>
<tr>
<td></td>
<td></td>
<td>gpp, plus others</td>
<td></td>
</tr>
<tr>
<td>Heat shock response</td>
<td>Heat, certain toxic agents</td>
<td>htpR (rpoH)</td>
<td>17 genes</td>
</tr>
<tr>
<td>SOS response</td>
<td>UV and other DNA damagers</td>
<td>recA, lexA</td>
<td>17 genes</td>
</tr>
<tr>
<td>Adaptive response</td>
<td>Methylation agents</td>
<td>ada</td>
<td>3+ genes</td>
</tr>
<tr>
<td>Translation apparatus</td>
<td>Growth rate-supporting ability of medium</td>
<td>Many</td>
<td>200+ genes</td>
</tr>
<tr>
<td>Osmotic stress response</td>
<td>High osmolarity</td>
<td>envZ, ompR</td>
<td>ompF, ompC</td>
</tr>
<tr>
<td>Oxidative stress response</td>
<td>H₂O₂, other oxidants</td>
<td>oxyR</td>
<td>kdpABC plus others</td>
</tr>
<tr>
<td>Anaerobic respiration</td>
<td>Presence of electron acceptors other than O₂</td>
<td>fnr (=nirA, =nirR)</td>
<td>12+ genes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>20+ genes</td>
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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
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. 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. 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. 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)

![Diagram of Helmholtz Coils](image)

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.\textsuperscript{(56)} This can be rearranged to $f = qB/2\pi m$ where

- $f =$ frequency
- $B =$ dc magnetic flux density (Tesla)
- $q =$ charge of electron, $1.6 \times 10^{-19}$ coulomb $\times$ n # of charges
- $m =$ mass of ion $\quad q/m =$ 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\textsuperscript{(57)} and McLeod\textsuperscript{(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.\textsuperscript{(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. 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
diatoms which moved away from a group were not considered an individual. Single diatoms completely separated away from any clumps were counted and recorded as to whether they had made a movement trail. This was done until 100 individual diatoms had been counted. A ratio of moving to total number of diatoms was then calculated. This was repeated for every line on every plate. Exposed and nonexposed plates of varying calcium concentrations were assessed for diatom motility effects.

BIOLOGICAL ASSAYS FOR MUTAGENICITY

Salmonella reversion assays

The Salmonella reversion assay is a mutagenicity test utilizing various strains of Salmonella typhimurium. These strains were developed by Dr. Bruce Ames of the University of California, Berkeley to test suspected DNA damaging or mutagenic agents and specific DNA damaging conditions such as oxidation, UV light, and SOS inducing activity. Through manipulation of certain genes and plasmids which inactivate three enzymes, these strains become unable to produce their own histidine. Strains have a specific mutation in the histidine operon which prevents the production of histidine in each of the bacterial strains. These strains then require added histidine for normal growth. The hisG46 mutation, for example, is in the hisG gene, the first enzyme in histidine synthesis. This
mutation substitutes a proline (GGG/CCC) for leucine (GAG/CTC) in the wild type organism. The strains which contain this base pair mutation, TA1535 and TA100, detect mutagens which cause base pair reversions, at the GC nucleotide pairs. The hisD3052 mutation is in the hisD gene coding for histidinol dehydrogenase. The strains with this mutation, TA1538, TA1978, and TA98, detect frameshift mutagens. An ochre mutation, TAA/ATT, in the hisG 426 gene detects oxidative mutagens such as formaldehyde, hydroperoxides, bleomycin, X-rays, UV light, and cross-linking agents such as psoralens and mitomycin C. (43) This mutation is contained in the TA102 strain. (59)

Other mutations in the test strains increase the sensitivity of the bacteria to detect mutagens. An rfa mutation, which is contained in the gene for outer cell wall synthesis, causes the partial loss of the lipopolysaccharide structure making the outer cell wall more permeable to large molecules which normally cannot penetrate the cell wall. The uvrB mutation is a deletion of the gene coding for the DNA excision repair system which then enhances the ability of the bacteria to detect mutagens. (35, 43) This deletion also included part of the biotin synthetic pathway.

Plasmids have been introduced into the bacteria which contain ampicillin and tetracycline resistance genes which become useful selectable marker strains. The R-factor plasmid, pKM101, increases chemical and spontaneous
mutagenesis by enhancing an error prone DNA repair system which is normally present in E. coli and Salmonella typhimurium by adding two genes mucA and mucB which are analogs of the genomic umuC and umuD of the SOS regulon. The strains TA100, TA102, and TA 98 contain this plasmid. TA102 also contains a multicopy plasmid, pAQ1, which has the hisG428 mutation and a tetracycline resistance gene. The R-factor strains are reverted by mutagens that are weakly detected or not detected at all in the non R-factor strains.(35,43)

The bacterial tester strains are histidine dependent and without the reversion of the gene for histidine production, the bacteria cannot grow into colonies when plated out on minimal media. When the mutant bacterial strains are treated with a mutagen this will allow for more revertants to occur and an increased number of colonies will form compared to the spontaneous mutation. This is called back mutation or reversion and the colonies that grow are called his revertants.

The DNA base pairs that are revertible are either AT or GC mutations. The strains containing AT base pair mutations are more sensitive to point mutations and small deletions from oxidative damage while the GC mutation allows detection of base pair substitution as well as deletions.

Utilizing magnetic fields tuned to specific resonance frequencies of ions such as calcium, potassium, and
magnesium, the effect on growth or reversion of the bacteria was tested. The strain tested was TA100 due to its sensitivity to nonoxidative properties. It has been shown by Zeiger et al that the TA100 strain will detect 80% of all mutagens, and if used in conjunction with up to four more strains about 95% of mutagens will be detected.\(^{60}\)

To assay for a mutagenic effect using these Salmonella strains, assurance that their plasmids are intact is a must. The genetic backgrounds are routinely screened. Strains that are uvrB, rfa, and carry the pkM101 plasmid can be checked for sensitivity to UV light, detergents, and resistance to ampicillin. The strains are provided by Bruce Ames on request. The bacteria arrive soaked into a paper disk in disposable plastic bags. When received they must be cultured overnight in a nutrient broth at 37 degrees Celsius and then stored in DMSO in individual aliquots in an ultracold freezer. When use of a strain is required, a sample is removed from the freezer and streaked out on an ampicillin minimal glucose plate and allowed to grow for 48 hours. Only the bacteria that have intact plasmids will grow into colonies. One colony is then removed from the ampicillin plate and cultured in nutrient broth overnight at 37 degrees. All procedures are done under sterile conditions to avoid contamination.

Top and bottom agar is prepared and sterilized before use. The bottom agar is plated out into 100mm diameter
disposable plastic petri dishes. The top agar is made of agar and sodium chloride to which a histidine/biotin solution is added before use. A mutagen is preincubated with the bacteria in a minimal M9 media before it is placed in a top agar and plated onto a bottom agar. This is done by melting the top agar, adding the his/bio solution and allowing it to cool to 44 degrees as per the Ames' procedure.

The bacteria (100μl) was placed into a sterile test tube with 100μl of M9 buffer and 2μl of 3x10^-6 M sodium azide, which was the mutagen. This mutagen was chosen for two reasons, its use as a positive control by Ames (43) and its use as a test mutagen for field studies by Tabrah (61). Two sets of bacteria and buffer +/- mutagen were preincubated for 30 minutes in a sham coil and the coil set to specific ion conditions. The sets of samples were then brought out of the incubator and 2mls of 44 degree top agar was added to each tube, vortexed, and then poured onto the bottom agar. The plates were allowed to set up and then placed back into the incubator +/- coil for 24 hours while reproduction of the bacteria on the plate was ongoing and could be effected by the coils. The plates were removed from the incubator with coils and put into a normal incubator for another 24 to 72 hours to develop. It had been shown this preincubation with the mutagen was essential for obtaining reproducibility.
The effect of the fields is indicated by either an increased or decreased amount of colonies formed compared to the control. The mutagenicity of the fields indicated by a turning on or shutting down of the DNA repair mechanisms would reflect the organisms' response to DNA damage. Certain repair mechanisms that can be triggered can also cause DNA damage. The SOS response repair system is known to cause DNA damage by error prone repair. Error prone repair refers to DNA damage caused by an attempt by the cell to repair damage already occurring. When the repair mechanism is turned on by the cell, it removes the damaged section of DNA and fills in with a complementary sequence of DNA. Sometimes during this repair, the wrong bases are reinserted into the spliced DNA. This in turn results in a mutation and can go undetected by other repair systems and replication of this mutated DNA is now carried by subsequent generations of organisms.(62-64)

**SOS Repair Response**

The bacteria Escherichia coli as well as Salmonella is versatile in the field of microbiology because it can be engineered to contain plasmids or many kinds of genomic mutations. For our purposes, the strain GE94 has been chosen for its DNA repair reporter system.(36) This strain contains a gene fusion between the lacZ operon and the recA SOS repair response gene. The lacZ operon normally responds
to lactose, introduced as an energy source, by producing β-galactosidase. This enzyme breaks the lactose down to its component parts of galactose and glucose, so the glucose can be used as an energy source.

The recA gene is used by the cell as a response to DNA damage to trigger the SOS repair regulon. When the recA promoter fusion is made the cell responds to DNA damage by producing the enzyme, β-galactosidase. Depending on the amount of DNA damage done, the cell will produce a proportional amount of β-galactosidase.

Typically this experiment is performed on a large volume of bacteria grown from a single colony in an Erlenmeyer flask while being aerated, usually by gyrotory motion in a temperature controlled water bath overnight. From the overnight culture, a dilution of 100μl of culture to 20ml of media are grown to the density of 0.2 to 0.3 at 600nm. The mutagen to be tested is added to 5ml portions of this culture and allowed to grow until absorbance reaches approximately 0.6 at 600nm. Differing amounts of mutagen are added to each 5ml aliquot to produce a dose response curve, comparing production of enzyme, β-galactosidase, to concentration of mutagen.

Due to constraints of field size and lack of adequate aeration systems, the method was changed to use smaller amounts of culture. This allowed the removal of individual samples at the appropriate time interval without removing
the entire sample from the field each time a sample was to be taken. This also changed the experiment from an aerobic to an anaerobic experiment. This removed the possibility of oxidative damage being done to the DNA. Therefore any DNA damage produced or lessened would be a consequence of the field effect. Control experiments were performed first to ensure proper growth and production of β-galactosidase under anaerobic conditions.

To perform the assay, a culture was grown in nutrient broth overnight at 37 degrees. A new culture was started from the overnight culture by adding 1ml of the culture to 50mls M9 media. This media was used to control exact quantities of calcium, magnesium, and other nutrients. The 1:50 dilution of culture to media was stirred constantly while 1ml aliquots were removed and placed into 2ml microcentrifuge tubes. Twenty four tubes were prepared for each incubator. These tubes were capped creating anaerobic conditions. The tubes were placed in the incubators, one containing Helmholtz coils to induce the electromagnetic fields and one containing no coil as the control. The tubes were removed two at a time at 30 minute intervals. The culture in the tubes was measured for absorbance at 600nm in a Varian recording spectrophotometer to detect growth. The samples were then placed in a refrigerator to deter further growth until the enzyme assay was performed the next day.
The β-galactosidase was measured in each sample the next day. This was done by removing 0.2 to 0.7ml of culture and adding an appropriate amount of Z-buffer to bring the final volume to 1ml. To this one drop of chloroform and one drop of 0.1% SDS were added, vortexed, and allowed to stand for 20 minutes. Next the substrate, 200μl ONPG, was added and the amount of time the reaction has run is timed until 800μl of 1M sodium carbonate was added to stop the reaction. Using ONPG, o-nitrophenyl-β-D-galactoside, as the substrate results in a yellow colored ortho-nitrophenolate ion. After the sample was spun down in a microcentrifuge to eliminate cell debris the amount of enzyme was measured at 420nm in a spectrophotometer. The units of activity are calculated in Miller units by the equation:

\[
\frac{(1000 \times \text{OD}_{420\text{nm}})}{(\text{time} \times \text{volume of cells} \times \text{OD}_{600\text{nm}})}
\]

For our purposes of testing DNA damage caused by magnetic fields, the samples of culture were placed in two different incubators. One incubator contained a Helmholtz coil while the control incubator did not. The Helmholtz coils were tuned to the calcium resonance frequency at 78.4μT DC field and 60Hz AC field.

Growth curves of exposed and nonexposed samples can be plotted to detect the effect on growth from the fields. The β-galactosidase can be plotted to show actual induced or inhibited production by the magnetic field.
Statistical Analysis

Robust differences in responses between exposed and nonexposed organisms would be ideal for the purposes of showing an effect of EMF on biological organisms, however this is not the case with the systems chosen here. Typically for a mutagen to show an effect in the Ames test, the number of revertants in the exposed plates should be twice the number of the control plates. This doubling did not occur with the magnetic fields and therefore, statistical significance must be employed as the indicator of an effect.

To determine whether these experiments have biological significance, two different statistical tests were performed. The Student’s $t$ test (paired two sample for means, one tailed) and the ANOVA analyses were used. Because reproducibility has been a problem in experiments with field effects, statistical significance must be established for validation of these experiments.

The ANOVA analysis was employed first and due to large deviations in the results, was not successful in showing significance. Therefore a $t$ test was sought out to determine significance. The $t$ test used, paired two sample for means, was chosen due to the timed SOS experiments. This test allows the means from each set of experiments to be compared. Large deviations in the results also gave cause to using a comparison of the means.
RESULTS AND CONCLUSION

Alfalfa Seed Sprouting Experiments

Seed sprouting and growing has been the subject of many ELF experiments with interesting results. In the 1960’s, experiments were performed on seeds in storage bins to determine if their storage lifetime could be extended.(3) Experiments were also performed on different types of seeds to see if germination could be speeded up. Under certain field conditions, the seeds deteriorated more quickly due to lipid breakdown.(3) Significant EMF effects have been seen in experiments utilizing radish seeds performed by Stephen Smith.(23) Seed experiments performed in the McLeod laboratory using fava bean seeds under much different conditions than those imposed by Smith have shown little effect.

While Smith’s experiments have shown an enhancement in the growth of radish plants, especially in root weight, it was thought that seed size might contribute to the effect felt by the fields. While radish seeds are small, fava beans are much larger. Since the radish seeds respond to the resonant fields, it was thought that the even smaller alfalfa seeds might also respond.

Alfalfa seeds were purchased from the Bozeman Community Food Coop which supplies organically grown
products. Organically grown seeds were used to ensure no pesticides or fungicides were present on the seeds at any time.

As sprouting of seeds takes only a short time, these experiments could be performed in just 4 days. The seeds were sprouted in plastic containers with only water introduced into the experiment. No dirt, vermiculite, or growing substance was necessary for the seeds to sprout. This gave a good controlled experiment where no ions or nutrients could leach out of dirt or vermiculite to affect the results.

Twenty milliliters of distilled water were added to ten grams of seeds each day at the same time. In the first set of experiments the seeds or sprouts were rinsed with 20mls of water. The excess water was removed. The weight of the seeds was measured before the water was added and after. The seeds absorbed the water over the first 24 hours and began to sprout on the second day. As the sprouting continued the weight dropped in most experiments.

The seeds were subjected not only to resonant calcium fields where both AC and DC fields are used together, but the individual AC and DC fields were used also. This could show the effect of the DC and AC fields by themselves on the sprouting of the seeds and show if combined fields had an enhanced effect greater than the individual fields. All experiments were performed utilizing 60Hz AC fields. The DC
magnetic field monitored by the magnetometer was set to 78.4μT as determined by the calcium ion resonance frequency. The control fields were the natural fields generated by the earth's magnetic fields and the incubator. The incubator added little to the magnetic fields. The fields were monitored in all directions over two days for each and there was no fluctuation in the fields. The measured DC magnetic field was 44.4μT, which is the earth's field in the Roger's laboratory, and no AC field was observable by the instruments.

Heat was used as a source of stress for this experiment, with an initial temperature of 37 degrees C. The purpose of the first experiment was used to determine an appropriate stress temperature. Thirty seven degrees proved to be too hot for sprouting. At this temperature, the seeds in the coil dried up very quickly to less than the original 10g weight, while the control was double the weight of the experimental seeds. Neither sample was sprouting after the second day. The temperature was then dropped to 34 degrees C where it was observed that the exposed and control seeds continued to sprout past the second day.

In preliminary experiments it was shown that the sprouts dried up after 3 days in the control incubator while the sprouts in the field continued to grow for 6 days. Using the calcium resonant frequency, by the fourth day the control sprouts weighed almost the same as the original
twice as much as the controls after 4 days.

In the first experiments, the weight of the sprouts was only recorded on the fourth day, as alfalfa seeds are supposed to sprout between four and six days under normal conditions at room temperature. It was noted that the controls dried up after day three, so the weights were then recorded on a daily basis. As this was a preliminary trial for these seeds, only two experiments were performed for each field condition. The first two experiments listed for all conditions were rinsed on each day with distilled water and allowed to drain until no more water dripped off. In the third experiment with both AC/DC fields on, the 20mls of water was added each day and the seeds were not rinsed off.

With the AC field only (Fig. 3), there was little to no effect on the sprouting of the seeds. The large differences seen in the 2 separate experiments at 48 and 72 hours was due to the containers holding the seeds being stacked. The top container allowed more evaporation of the water while the bottom container retained more water. Both sets of seeds continued to lose weight after day 1, until they dried up to the original weight or less. The effect of the DC field only seemed more enhanced (Fig. 4). The seeds in the field did not lose weight as fast and on the fourth day there was a 50% increase in the weight of the coil over the control. Using the resonance frequency with both AC and DC fields on, the first two experiments gave consistent results.
Comparison of Sprouting in Alfalfa Seeds

**Figure 3.** Effect of AC Field on Alfalfa Sprouting

**Figure 4.** Effect of DC Field on Alfalfa Sprouting
when weighed on the fourth day (Fig. 5). The results showed that the seeds in the coil weighed almost two and a half times as much as the controls when only rinsed with water each day. Figure 6 shows the effect of all the field conditions compared to each other.

When 20 mls of water was added to the seeds each day, with no rinsing, they continued to grow in both the coil and control. While the control lost weight until the fourth day and then started growing again, the seeds exposed to the fields started regaining weight on the third day. The weights on the fourth day showed an enhancement of growth in the coils by 30%.

This experiment using the addition of 20 mls of water everyday in the AC/DC fields was continued until the seventh day when the sprouts in the field rotted very quickly (Fig. 7). From the fourth to the sixth day, both sets of sprouts continued to gain weight. The fifth day showed the sprouts in the field to have a 33% increase in weight over the control and the sixth day showed a 44% increase in the exposed sprouts. On the seventh day the control sprouts had gained weight again, while the exposed sprouts had rotted.

This final experiment using the calcium resonance fields showed a shift in the growth of the exposed seeds. These seeds absorbed water better, sprouted faster, and appeared to grow faster than the controls. More sprouting was seen in the field in all experiments but especially in
Comparison of Sprouting in Alfalfa Seeds

Figure 5. Effect of Calcium Resonance Field on Sprouting

Figure 6. Comparison of Different Fields on Sprouting
this final experiment. The fact that these seeds continued
to sprout and gain weight when having a fixed amount of
water added versus being rinsed each day shows that the
experiments using the rinsing method put too much stress on
the sprouts for water when the temperature was so high.

This shift in growth is also seen by Smith when
using radish seeds, but in the opposite fashion. When
radish seeds are exposed to the same calcium ion resonance
field conditions but are planted in Perlite (expanded
silica), the seeds in the field germinate after the control
seeds have sprouted. Once the seeds have come up in the
field, they grow quicker and exceed the growth in the
control. No stress factors were added beyond the growth
stress in Smith's experiments whereas heat was used as a
stress factor in these experiments.

![Figure 7. Calcium resonance Field Effect over 7 Days](image)

Figure 7. Calcium resonance Field Effect over 7 Days
The alfalfa seed experiment results indicate the seeds in the field were less stressed than the seeds in the control incubator at 34 degrees C. Higher plants contain a vacuolar membrane which is triggered to release calcium in response to inositol 1,4,5-triphosphate acting as a possible second messenger. It was found by patch clamping that beet vacuoles contain IP$_3$ gated calcium channels. These vacuolar membrane channels might be stimulated by the fields to release calcium. If this is the case, the calcium which normally modulates many developmental processes is enhancing sprouting. These calcium channels, activated by the field, release calcium which works as a nutrient or possibly as mediator for an enzyme.

The seeds not subjected to the fields might be experiencing some form of heat shock. If so, the plant would respond by discontinuing sprouting or growth. The seeds subjected to the fields seem to thrive under what appears to be very stressful conditions for the control seeds. The calcium channels may be stimulated strongly enough to overcome the stress of the heat during the sprouting process.

While the vacuolar calcium channels are IP$_3$ gated, instead of the channels being directly stimulated, the IP$_3$ might be responding to the field to activate the channels. Another possibility is that the calcium itself is activated by the field to pass through the channels. Another option
indicates the seed may or may not be able to release its calcium in response to stress.

Vacuolar calcium channels might be activated by the cells to respond to the stress by releasing the calcium stores available in the vacuole. Once calcium is released, a proton-pumping ATPase activity allows the reuptake of the calcium back into the vacuole for later use again. This activity may be stimulated by the calcium resonance fields and move the calcium back into the vacuole for further use in dealing with stress. Another possibility is that the calcium is blocked from reuptake by the field effect not allowing ATPase activity. Constant excess calcium may cause pH and other imbalances which could cause further damage to the seeds or sprouts.

Experiments to block the uptake of the calcium by the calcium ATPase pump might indicate if this is a proper area to pursue for further research. If the calcium could be blocked from being moved back into the vacuole, the seeds in the fields would not continue to sprout, just like the controls. EDTA could sequester the calcium after release therefore not allowing reuptake. Experiments should be performed to determine if release of calcium is irreversible for the stressed seeds.
RESULTS OF SEED SPROUTING EXPERIMENTS  
all weights in grams

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Diatom Motility Experiment

The results of previous experiments by McLeod and Smith using the diatom Amphora coffeaformis had shown an increase in diatom motility when exposed to the cyclotron resonance frequency of calcium. Compared to the control, the diatoms exposed to the fields required one tenth the amount of added calcium to achieve optimum movement. At 0.25mM calcium, the exposed diatoms had a ten-fold increase in movement over the controls.

Using the conditions of the experiments performed by McLeod and Smith(8,10,11), other researchers had been unable to reproduce the effects of the calcium fields on diatoms(68-70). Therefore the goal of the first experiments performed was to attempt reproduction of these results. New strains were acquired to test their motility properties to see if this phenomena existed in other diatoms. Starting with the original strain, IIIBC, provided by Barbara Cooksey and also two additional strains, IIIBF and Texas Star #2039, the growth curves of the diatoms were established and reproduced. Figure 8 shows the growth curves of the 3 diatom strains, notice the difference between the Texas strain and the IIIB strains. Figure 9 shows the IIIB strains and their growth similarities.

To test if the new strains were similar in properties of the Cooksey IIIB strain, the first experiments were performed with the Texas #2039 strain. The growth curves
of the Texas strain showed much higher population of diatoms compared to the other strains (Fig. 8). It was thought faster growth might afford a better chance to observe an effect due to the stress put on the organism from growing so quickly.

The field conditions were set using 16Hz AC at 42μT peak to peak, with the DC magnetic field set to 20.9μT for the calcium resonance frequency. The control field conditions showed no measurable AC field and 41.8μT DC field, which is the earth's field in the McLeod laboratory. The controls had high numbers of movement relative to the original Smith experiment. The number of moving cells remained constant through the ranges of calcium concentrations and with cell ages of 2, 3, and 4 days old. The counts showed numbers 2 to 15 times higher than the Smith experiments.

These results were so contrary to the original results, that new experiments were then performed using the Fritsen IIIB strain. This strain grew to a much lower level than the Texas strain, and much more similar to the growth curve of the Cooksey strain (Fig. 9). As with the Texas strain, the motility of the controls was higher than previously reported. On 0.0mM calcium plates the movements were a minimum of 5 times higher but the number of movements did increase with the increasing calcium concentrations. A few plates were subjected to the coils, but the activity was
decreased in the coils by one half to one third. While this result did show a change in the fields, it did not mimic the Smith experiments so was not pursued further.

Neither of the new strains responded in the same manner as had the original Cooksey IIIB strain in the previous experiments. The IIIBC strain was grown on a daily basis for the experiments thereafter. After having all excess calcium washed off, the diatoms were streaked onto the agar plates containing various amounts of calcium. The calcium plates used had between 0.0 and 5mM calcium incorporated into them and were warmed to room temperature before use.

The results of the experiments showed that while the diatoms on the plates exposed to the resonant frequency did have an enhanced amount of movement, it was not the large difference seen before by Smith. In the experiments performed by Smith, the number of diatoms moving on the 0mM to 0.25mM calcium plate was consistently below 5 movements per 100 diatoms counted. The numbers found in these experiments showed between 10 to 20 movements per 100 diatoms on the 0.0mM calcium plates and the numbers increased with increasing calcium concentrations. Figure 5 shows the response of various ages of cells to 0.0mM calcium plates.

The experiments were performed on diatoms from one to seven days old to test which age was most responsive. These
experiments were performed in the sham incubator. (Fig. 10)
From several experiments, it was determined that the two and
three day old diatoms were more motile. (Fig. 11)

Growth Curves
Three Strains of A. Coffeaeformis

Figure 8. Three strains of Amphora coffeaeformis

Figure 9. IIIB strains of Amphora coffeaeformis
A. coffeaformis III BC
Motility vs Molarity Exp. 2

Figure 10. Comparison of age and motility at various Ca++

Motility vs Molarity
A. coffeaformis III BC

Figure 11. Comparison of motility in 2 and 3 day old diatoms
Experiments were then performed using the fields to determine which ages of diatoms were more responsive. Figures 12 through 16 show results of the different calcium concentrations, varying between 0.0mM to 5mM, on diatoms of different ages subjected to the calcium resonance fields. Experiments performed on two day old diatoms gave the most enhanced results when subjected to the fields and were used for all other experiments. Figure 17 displays a typical experiment performed on two day old diatoms and their response to the fields. The results shown in these figures are not an average number of movements for many experiments but experiments performed on a single day. These figures merely represent the daily experiments performed, due to the variation in the results being too large for all concentrations other than the 0.25 and 5.0mM calcium plates.

In these experiments, the difference between the exposed and nonexposed plates averaged 33.76% with 2 day old cells on 0.25mM calcium plates. The plates with the diatoms exposed to the calcium resonance field had increased movement. The Student’s t test showed a P value of .0002 for the 0.25mM plates. While this response was not the doubling effect found in the field as in Smith’s experiment, it was obvious there was a significant difference in the field at 0.25mM calcium. In the 5.0mM plates there was an average response to the field of 32.9%. The diatoms again had increased movement when exposed to the resonance field.
A P value of .0038 was obtained for the 5.0mM calcium plates. The deviation in the results for the other concentrations of calcium was too large to be significant.

0.0mM Calcium Plates
Motility of IIIBC Cells, May 29, 92

Figure 12. Diatom movement on 0.0mM calcium with fields

Motility of IIIBC Cells, June 21, 92

Figure 13. Diatom movement on 0.25mM calcium with fields
Figure 14. Diatom movement on 0.5mM calcium with fields

Figure 15. Diatom movement on 2.5mM calcium with fields
5.0mM Calcium Plates
Motility of IIIBC Cells, June 21, 92

Figure 16. Diatom movement on 5.0mM calcium with fields

2 day Old Cells
Motility of IIIBC Cells, May, 29, 92

Figure 17. Diatom movement on various calcium concentrations
The diatom movement observed in these experiments did not reproduce Smith’s experiments. (8,10,11) The reason Smith’s results were not reproducible was attributed to different growing conditions and/or genetic variability in the Cooksey IIIB organism. It is common for these types of organisms to become genetically different through time. (25) Differences in environmental factors during growth could have contributed to this drift. While the original strain was grown and maintained by Barbara Cooksey in her laboratory for the use in the Smith experiments, the strains used for these experiments were grown and maintained in the McLeod laboratory. There may have been lighting conditions, heat, and media differences not accounted for. The maintenance protocol for the diatoms was unknown from the first experiment, therefore the possibility of not duplicating procedures was high.

It is also thought that the fields in which the diatoms are grown can have an effect. It is possible that the diatoms’ field environment was different for the two experiments. These were never measured or monitored in either laboratory before the experiments were begun. Other evidence suggests stray field effects or even “normal” magnetic fields inherent to the particular place may play a part in the variability of the results.

Research done by Mark Davies (70) in Plymouth, England indicates growing conditions may predispose the diatoms to
enhanced motility. Davies attempted to reproduce the Smith experiments. He could not reproduce Smith's results and began growing his diatoms in a magnetic field which was the same as the resonant fields they were exposed to during the calcium experiment. His results then showed a 30% enhancement in movement when the diatoms were exposed to the fields during an experiment. Davies' level of response was similar to the experiments conducted in Bozeman and may indicate again the genetic variation referred to earlier. Davies was also using the Cooksey strain but only obtained a measurable response when the diatoms were grown in the field.

Another attempt to reproduce the Smith experiment by Prasad et al(69) indicated no effect on the diatoms by the magnetic field at the same settings as used by Smith. Reese et al(68) attempted to reproduce Smith's experiment but did not use the full range of calcium concentrations on their plates. Using the 0.0mM, 0.25mM, and 2.5mM calcium concentrations, they obtained statistically significant effects with only the 0.25mM calcium response. Their research did confirm the existence of the 21µT, 16Hz magnetic field effect on the diatoms.

The Reese results more closely approximate the results found here. Their results showed a much higher number of background movements as ours also did. The enhancement of movement in the field for the 0.25mM calcium plates was
similar for both experiments. The deviations in both sets of experiments were large.

The mechanism by which the diatoms move is unknown except a raphe is present and the movement is calcium mediated. No cilia or flagella are known to be present, therefore ruling out the possibility that tubulin is involved. This leaves a myosin system to examine as a possible mechanism of movement.

Observing the diatoms under the phase contrast microscope, an apparent movement is seen. This movement looks like toes wiggling on one end of the slit of the raphe. From the site of movement, the trail of mucous issues from the diatom. The wiggling movement may be only some contraction of a secretory gland to extrude the mucous but propels the diatom at the same time.

The movement being calcium dependent infers the action is driven by a myosin system as in muscle contraction. Assuming this is the case, an explanation for the enhanced activity of the diatom when exposed to the calcium resonance field is plausible.

Calcium is the physiological regulator of muscle contraction, in both skeletal, striated, and smooth muscle contraction. In skeletal muscle, the sarcoplasmic reticulum regulates the concentration of calcium around the contractile fibers of muscle. The sarcoplasmic reticulum releases its calcium, which it maintains at high
concentrations during resting states, in response to IP₃ activation. When stimulated by a nerve impulse the calcium concentration is changed from low to high around the muscle fibers by release of calcium from the sarcoplasmic reticulum. The effect of calcium on actin and myosin is mediated by tropomyosin and the troponin complex. This release of calcium triggers a muscle contraction by occupying the low affinity binding sites of troponin C of the troponin complex.

The troponin complex and tropomyosin are located on the thin filament of actin. Tropomyosin is a two stranded alpha-helical rod aligned parallel to the long axis of the thin filament. Troponin is a complex of three polypeptide chains referred to as TnC, TnI, and TnT. TnI binds to actin and TnT binds to tropomyosin. TnC is a calcium sensing component. The troponin complex is located at intervals along the actin filament set by the length of the tropomyosin unit.

TnC contains two bound calcium ions in its high affinity binding sites at all times. When calcium is released by the sarcoplasmic reticulum, the troponin C binds two additional calcium ions in its low affinity sites. This binding induces a conformational change of the amino-terminal domain. This conformational change in TnC is transmitted to the troponin complex and on to tropomyosin. A shift in the position of tropomyosin allows the binding of
actin to myosin to create muscle contraction.

The sarcoplasmic reticulum resequesters the calcium by the action of an ATP-driven calcium pump. The cycle of conformational changes driven by the phosphorylation and dephosphorylation transports 2 calcium ions for each ATP hydrolyzed. The calcium-ATPase exhibits reversibility and transduces phosphoryl potential into an ion gradient or the opposite.

The actin-myosin system is ATP driven. Myosin itself is an ATPase. The hydrolysis of ATP by myosin provides the free energy that drives muscle contraction. Myosin has a heavy and light chain which display different activities. The heavy chain binds ATP and actin and the light chain is responsible for filament formation.

Actin also forms filaments by polymerizing and forming a helix. Actin increases the ATPase activity of myosin by binding to myosin once it has hydrolyzed an ATP and is in the myosin-ADP-Pi form. The actin accelerates the release of the ADP-Pi from the actomyosin-ADP-Pi complex. This then allows actomyosin to bind ATP and dissociate to actin and myosin again.

Striated muscle myosin is phosphorylated by a calcium stimulated kinase and dephosphorylated by phosphatase C. Phosphorylation doubles the actin stimulated ATPase activity of myosin suggesting a faster turnover of myosin.

In smooth muscle, contraction is controlled by the
degree of phosphorylation in its light chains and an increase in cytosolic calcium concentration mediated by calmodulin. Calcium calmodulin stimulates myosin light chain kinase. This kinase phosphorylates myosin and contraction occurs. A phosphatase dephosphorylates the myosin to a relaxed state.

Molluscan smooth muscle must bind calcium directly to a myosin light chain. In the absence of calcium, the interaction of myosin with actin is inhibited.

The number of diatom movements increase with increasing concentration of calcium. Experiments to remove available calcium could be performed to test whether the diatoms would stop moving if very little calcium was available. The calcium was removed from the diatoms in these experiments by washing. If more drastic steps were taken to remove more calcium, a distinction could be made as to if the movement was directly activated by calcium as in the molluscan system. If the movement was similar to the molluscan smooth muscle movement, it would be inhibited by no calcium.

If another actin-myosin system was being used by the diatoms, calcium stores would still probably be available for release even if all free calcium was bound up by EDTA. Removing all the calcium would be difficult as the entire chemistry of the organism would be affected and possibly die.
Ames salmonella Reversion Assay

The Ames Salmonella reversion assay\(^{(35,43)}\) was used to test the mutagenicity of electromagnetic fields. This test has been used since the 1970's for screening mutagens that could be potential carcinogens.\(^{(71,72)}\) It is used by most countries as the initial bioassay in the process to test for the mutagenicity of a chemical. It consistently gives reproducible results throughout the world from laboratory to laboratory and therefore was thought to be useful as a bioassay for the mutagenic effects of ELF.\(^{(73-76)}\)

The introduction of the Ames test into this research was suggested by the research of Frank Tabrah.\(^{(61)}\) Tabrah performed experiments using the TA100 Salmonella strain with field strengths of 2G, versus a 2mG field as a control. His results showed a 14% increase in the number of revertants in the 2G field. While it is known that fields of this strength can induce effects\(^{(3)}\), his experiment showed these results could be reproducibly demonstrated using the Ames test.

Ames and Tabrah used sodium azide as a positive control with the TA100 strain to ensure the plasmids were present and were giving a predictable number of revertants at defined azide concentrations.\(^{(35,43)}\) The reversion frequency of azide had been established by Ames in the 1970's. Azide was used as a positive control and stress inducer for these experiments. The amount of mutagen as a
stress inducer had to be determined and tested for best positive results. According to Ames, 1 µg of azide will quadruple the number of revertants on a plate. Azide was prepared and tested on TA100 and TA2638 at dosages of 0 to 100µl of 1µg/10µl concentrations.

A dose response curve was generated for the differing concentrations of azide versus revertants produced. The amount of azide to be used for further studies was determined from this curve. An amount was chosen so that the number of revertants would not be too large to count accurately with a colony counter.

The coils were initially set to the potassium frequency and only a range of up to 20µl of azide was tested to see if any response could be detected. (Fig. 18) Of the doses from 1 to 20µl of azide, only the 2µl dose gave positive results in every case. (Fig. 19) The results of all other doses of azide in or out of the field, were conflicting. (Fig. 20) One experiment would produce an enhanced effect and the next experiment would be the opposite.

The 2µl of azide generated an additional 75 revertants above the background number of 150 to 250 revertants. This number was measurable by the colony counter and was about a third of the background number of revertants. This also made it easy to count the number of revertants manually to
Ames Salmonella Mutagenicity Test

60Hz; Potassium Resonance (51.0 uT)

Figure 18. Salmonella reversion, K+ resonance frequency

Figure 19. Effect of K+ resonance on various ul of azide
check the accuracy of the settings of the colony counter. The colony counter was set according to colony size, then tuned for sensitivity according to the manual counts. Once the colony counter was calibrated for at least two plates, the sensitivity setting was kept constant thereafter and all plates were read.

In the initial experiments performed using the potassium frequency, the exposed plates with 2μl azide added showed an increase in the number of revertants by 11%. Additional experiments were performed to test if slightly more or less than 2μl azide would enhance the effect more. This gave mixed results as before with the other concentrations when 1.5, 2, and 2.5μl azide were used. All doses of azide gave conflicting results from experiment to experiment.

To attempt to bring the experiment under better control and get consistent results, a preincubation step was added to the experiment. The bacteria were placed into 100μl of buffer solution and subjected to a dose of azide for 30 minutes in the incubator prior to the mixing of the bacteria with the top agar for plating. Two sets of 12 test tubes, of which 6 tubes had azide added were prepared for each experiment. One set was preincubated in the control incubator and one set in the field incubator tuned to the frequency at which the experiment was to be performed. The preincubated sets were then split in half so each now
contained half preincubated in the control incubator and half preincubated in the field. One set of samples was developed in the control incubator and one was developed in the field incubator. Again using small doses of azide to keep the number of revertants countable, 1, 2, and 3μl of azide were tested. It was again found that the most consistent results were obtained with 2μl azide and all experiments thereafter were performed with 2μl azide.

The first experiments using the preincubation step were tuned to the calcium resonance frequency of 26.1μT DC at 20Hz AC. The measured control field conditions had no AC field and the earth’s DC field in the McLeod laboratory as before with the diatoms, 41.8μT. The results must be viewed in several ways. In each experiment performed, there were several different experiments which were broken down into subsets. The subsets were preincubation conditions, development conditions, and samples containing azide under these same conditions. Each experimental sample contained 3 tubes preincubated in the control incubator with no azide designated the controls, 3 tubes preincubated in the control incubator with azide added were positive controls, 3 tubes preincubated in the field with no azide, and 3 tubes preincubated in the field with azide added.

The first subset is the comparison of samples with no azide added, preincubated in the control incubator and developed in either the control or field incubator. This
allows the control number of revertants to be established, which should fall in the range of 150 to 250 for the Salmonella TA100 strain. This number can be compared to the plates developed in the field. The identical conditions were used on samples with azide added making up subset 2. This is the positive control. Subset 3 is comprised of samples with no azide, preincubated in the field and developed in the control or field incubator. The last subset is samples with azide added, preincubated in the field and developed in either the control or field incubator. This can be very confusing. Refer to the graphs to better distinguish these subsets.

For clarification on viewing the graphs for the Salmonella reversion assays refer to the preincubation conditions denoted -Coil for preincubated in the sham or control incubator and +Coil as preincubated in the coil or field. The addition of azide to the experiments is denoted -N3 for no azide and +N3 for azide added. The notation for azide conditions is in parentheses beside the preincubation conditions. The preincubation conditions with the azide conditions are labeled on the x axis. The legend below the x axis denotes the development conditions. The term Sham denotes the development of the samples in the control incubator. The term Coil refers to the samples developed in the field.

The results from the graphs are interpreted by viewing
the conditions listed on the x axis in a side by side manner, as differences in preincubation conditions, either developed in the control or field incubator. The development conditions are displayed by viewing the differences in the connected lines of the Sham or Coil development as per the legend. The control is designated -Coil(-N3) and the positive control is designated -Coil(+N3).

Using calcium resonance conditions (Fig. 21), the samples preincubated in the control incubator and developed either in the control or field incubators were evaluated first. The control (no azide, preincubated and developed in the control incubator) showed an increase in the number of revertants in the plates developed in the control incubator. These samples showed a 0.8% increase over the samples developed in the field. The positive control, containing azide, had a 10.7% increase in the number of revertants when developed in the field. Statistical analysis, using the paired two sample for means, one tailed t test, resulted in a P value of .01.

Evaluating the samples preincubated in the field shows the samples without azide had a 3.1% revertant rate increase when developed in the control incubator. The samples containing azide showed an increase in revertants by 4.5% when developed in the field. Comparing the difference preincubation had on the
samples, the plates without azide developed in the control incubator showed an increase of 2.8% in the samples

**Dose Response Curve**

![Graph showing dose response curve for various azide concentrations](image)

**Figure 20.** Dose response, various azide concentrations

**Effects of Ca Freq. 26.1uT DC, 20Hz AC**

![Graph showing effect of Ca++ resonance frequency on reversion](image)

**Figure 21.** Effect of Ca++ resonance frequency on reversion
preincubated in the field. With azide added, the plates preincubated in the field also showed an increase of 13.7%. This increase gave a P value of .056.

The samples developed in the field without azide added, showed a 0.5% increase when preincubated in the field. The samples containing azide showed an 8.26% increase also when preincubated in the field.

Overall it appears the resonance field produced an enhancement in reversion frequency when compared to the control. While percent differences in the results seems to indicate some effect from the field, the statistical data show no significance in the results except for the two experiments with P values given.

The next experiments were performed using only a 20Hz AC field (Fig. 22). The experiments were prepared in the same manner and evaluated as follows. The control samples preincubated in the control incubator showed an 11.4% increase (P = .009) in the plates developed in the field. The positive controls had a 2.1% (P = .08) increase in the samples developed in the field.

The samples preincubated in the field with no added azide showed a 9.2% increase in the plates developed in the field. The samples with azide added showed a 2.1% (P = .043) increase in the plates developed in the field.

For the difference in preincubation on the plates, the samples developed in the control incubator showed an
Salmonella Reversion Assay
Effects of no DC, 20Hz Coil

Figure 22.
Effect of 20Hz AC field on reversion
increase of 10.15% and an 8.75% over the samples
preincubated in the field either without or with azide added
respectively. The samples developed in the field also
showed an enhancement of 7.98% and 8.75% in the plates
preincubated in the fields, without or with azide added
respectively.

The 26.1μT DC field alone was tested next (Fig. 23). The control samples preincubated in the control incubator
showed a 4.5% (P = 0.056) increase when developed in the
control incubator. The plates with azide added showed a 6%
(P = 0.007) increase when developed in the field.

The samples preincubated in the field showed a 7.4%
increase in the plates developed in the control incubator
when no azide was added. The plates with azide showed a
1.4% increase when developed in the field.

When samples without azide were developed in the
control incubator, there was a 4.5% increase in the number
of revertants in the plated preincubated in the control
incubator. With azide added there was no difference. The
samples developed in the field showed a 7.4% (P = 0.018) and
4.85% increase in the samples preincubated in the control
incubator, without and with azide respectively. The
experiments performed utilizing the calcium resonant
frequency or the separate AC and DC fields showed no
statistical significance as determined by an ANOVA analysis.
The three separate field conditions used, calcium resonance,
Salmonella Reversion Assay
Effects of Ca DC, 0Hz AC Field

Figure 23. Effect of 26.1uT DC field on reversion of revertants +/− 2uL Azide.
20HZ AC, and 26.1μT DC, are shown in figures 24 and 25. These show the effects of preincubation in the different field conditions when developed either in the sham (Fig. 24) or the field (Fig. 25).

These same sets of experiments were again performed using the potassium resonance frequency (Fig. 26). The AC field was 60Hz and the DC field was 51.0μT. Comparing the samples developed in the control incubator, the plates preincubated in the control incubator, both with and without azide had a 2.6 and 6.9% increase respectively. Of the samples developed in the field, the plates preincubated in the field had a 9.3% increase without azide and a 6.2% increase in the plates preincubated in the control incubator with azide.

The development of the plates in the field versus those developed in the control incubator showed a 10.2% increase between samples without azide preincubated in the control incubator. Those plates preincubated in the field without azide showed a 4.4% (P = .071) increase in samples developed in the field. The samples with azide added showed a 4.3% (P = .069) and 0.94% increase in the plates developed in the field for preincubation in the control incubator and field respectively. These differences were also smaller than the deviations for the sets of data between single experiments.

For experiments performed using the 51.0μT DC field
Effect of Preincubation +/- Coil
Developed in Sham at Various DC and AC

Figure 24. Various field conditions developed in sham

Developed in Coil at Various DC and AC

Figure 25. Various field conditions developed in coil
only (Fig. 27), the samples developed in the control incubator showed very small effects of opposite nature compared to the results from the combined fields. The samples without azide had a 3.2% increase in the plates preincubated in the control incubator and the samples with azide added had a 0.72% increase in the plates preincubated in the field. The samples developed in the field, both with and without azide, had an increase 11.2 and 7.3% (P = .023) respectively for the plates preincubated in the field. Comparing the same preincubation conditions developed in and out of the field, the samples without azide had a 6.0% increase in the plates preincubated and developed in the control incubator and a 4.8% (P = .053) increase in the plates preincubated in the field and developed in the field. The samples containing azide showed an increase in both cases for the samples developed in the field of 9.7% (P = .06) for those preincubated in the control incubator and 0.4% for those preincubated in the field.

The last set of experiments used the 60Hz AC field only (Fig. 28). The samples developed and preincubated in the control incubator without azide had an increase of 5.0% (P = .05) over the plates preincubated in the field, while the samples with azide had less than a 1% difference in revertants. The samples developed in the field, both with and without azide, had approximately a 3% difference, with the plates preincubated in the fields having the higher
Effect of Preincubation +/- Coil
Developed +/- Coil at Potassium DC, 60Hz

Preincubation +/- Coil = K+ DC

figure
Potassium resonance frequency on reversion
Revertants +/- 2ul Azide

Sham, 60 hz  --  Coil, 60 Hz
number of revertants.

Comparing the development conditions, the plates with no azide and preincubated in the control incubator had no change. The samples with no azide preincubated in the field had a 9% increase when the samples were developed in the field. In the samples with azide added, the plates preincubated in the control incubator but developed in the field had a 5.8% (P = .037) increase. The samples preincubated in the field and developed in the field had a 7.4% (P = .032) increase.

No statistical significance was seen except for one subset of experiments in the potassium frequency experiments by ANOVA analysis. With the t test, at least one set of points in every different subset of experiments was significant. The most significant finding with the t test was with the comparison of the positive control. When developed in and out of the fields, 5 out of 6 experiments showed small P values which averaged to .045. The calcium resonance fields had a P value of .01 and with just the DC field of 26.1μT the P value was .007. The 20Hz AC field had a P value of .08 for the same samples. The potassium combined and DC of 51.0μT had P values of .07 and .06 respectively. While all these results are not robust signs of significance, they do indicate a trend of response to the fields. The calcium resonant fields do have a significant response and the DC component has the most significant
Salmonella Reversion Assay
Effects of K DC, 0Hz AC Field

Figure 27. Effect of 51.0uT DC field on reversion

Effects of native DC, 60Hz AC Field

Figure 28. Effect of 60Hz AC field on reversion
response.

In the potassium experiments, the controls preincubated in the field and developed in either the control or field incubator all show significance. Here the separate fields show more significance with an average \( P \) value of .044, while the combined fields show a \( P \) value of .07.

Overall it is better to view these experiments in light of trends which occur. If the sets of experiments display the same trends even with small differences, this may be a hint as to how to pursue the next step in this research. It can be seen from the raw data that very few experiments behaved exactly the same from experiment to experiment. The calcium experiments have at least one case of this under each of the various conditions, where either all or the majority of the experiments showed an increase or decrease in the same direction. The potassium experiments seem to have much more random results.

It is very possible that the Salmonella experiments did not contain a strong enough stress factor from just the field. While in other experiments the calcium or other ions could be removed or depleted, this is not the case here. The media and agars involved with these experiments contain no added calcium. The organism cannot be stressed enough if the ion concentration cannot be lowered to less than normal levels.
To attempt to lower the level of available calcium, lanthanum could be added as lanthanum chloride. Lanthanum is a known calcium channel blocker. If the lanthanum lowers the number of revertants, then it might be inhibiting the reproduction of Salmonella by reducing the calcium stores available for cell division. This is one possible example of experiments that should be performed to determine if magnetic fields affect living organism through a mechanism of stress enhancement.

All experiments that have been performed using various systems seem to point to the necessity of calcium for a response to the effect of ELF. The calcium concentration must either be changed by binding it up so it cannot be available for use or it must be lowered physically by not adding it to a media necessary for the experiment. While the Salmonella experiments did not show a large effect of the field, this indicates that the possibility for mutagenicity from these fields is small. Consideration must be given to the fact that this system was not stressed with a calcium depletion. If it had felt an excess stress of calcium depletion, the results may have been different.

There are many other strains of Ames Salmonella that need to be used in testing field effects. It is possible that this strain does not give reliable results if these fields are mutagenic. There are many cases known where a particular strain of Salmonella will give false negative
results even for a known mutagen. (55) This usually is due to the lack of permeability of the cell wall to the chemical mutagen or lack of enzyme activation in the case of an indirect mutagen. Sometimes chemicals do not become mutagenic until activated by enzymes which respond to its presence as a foreign substance. The chemical, once detected by the body, will undergo a process to detoxify, and in this process some chemicals become active as mutagens.

The Ames test utilizes an activation by a liver homogenate in this manner. This activation step was not necessary here since the direct acting mutagen was used. It is also possible that the field strengths used were not appropriate for this bacteria. A series of experiments should be performed varying the AC and DC field combinations. The effect may be felt at a different harmonic of the same frequency. The first harmonic of calcium was used here but maybe a higher harmonic would give better results. Steps should be taken to pursue this in the manner performed by McLeod for the diatom experiments. (11)

Another possibility as to why little effect was seen in the Ames test is that the calcium channels for this bacteria may respond to quite a different frequency. If the postulated calcium channel gating due to magnetic fields is stimulating an emergency channel as inferred in McLeod's
paper(56) about the predicted diatom channels, then it might also mean the Salmonella have a different set of emergency channels which might be stimulated with some other variation of this frequency. If ion binding is affected in the diatom system for movement as in actin-myosin instead of channel stimulation, then the Ames strains might respond to calcium activation in a different manner than reversion.

Conversely, if an effect was detected by any of the Ames Salmonella strains, it would be expected that the number of revertants above the background would at least double. This would be a good indication of a mutagen, but as seen here, even without a doubling of revertants, some significant results were obtained.

The most important result achieved in the Ames assay was the significance of the positive controls developed in the differing fields. This shows the coils do have an effect. Adding azide as an extra stress factor did give significance to this experiment. If many more experiments were performed the statistics may become more reliable for other exposures to resonance fields.

The Salmonella strain used also contains the SOS response system, but the repair by the recA protein is allowed to take place. The actual DNA damage produced by the fields may induce misrepair or error prone repair by recA, therefore an enhanced number of revertants might indicate an increased amount of error prone repair.
The SOS Response

The SOS response makes use of the ability of the genes of E. coli to be readily manipulated into reporting information about cellular activity. The information reported from the fusions of SOS genes to the beta-galactosidase operon indicates DNA damage. The amount of DNA damage detected by the fusion genes is directly proportional to the amount of β-galactosidase produced. For these experiments the strains with recA and sulA fusions have been tested.\(^{(50,77,78)}\)

Identical sets of 24 eppendorfs containing 1ml of a 1:50 dilution of bacteria to M9 media were placed into incubators set at 37°C. One incubator contained the Helmholtz coils to produce the magnetic fields while the other incubator was the control containing no coil. Two eppendorf tubes were removed from each incubator at 30 minute intervals. The cultures in the tubes were checked for cell density at 600nm in a Varian spectrophotometer and then placed in the refrigerator. An enzyme assay was performed the next day on each tube culture.

The SOS response assay was performed on samples that were exposed to calcium resonance fields at 60Hz. Using the 60Hz AC field gives a calculated DC field of 78.4μT. The field conditions in the control incubator were as for the alfalfa sprouting experiments in the Roger’s laboratory, no measurable AC field and 44.4μT DC field. The media used for
growth experiments has added calcium as well as magnesium. Also in these experiments, calcium was withheld from the media mixture and the results with and without calcium were compared. As in the Salmonella experiments, separate AC and DC fields were tested and compared to the combined resonant field conditions.

The experiments with calcium added to the media were performed first. These experiments showed no effect on growth or production of β-galactosidase in the separate (AC or DC alone) fields as evidenced by the averaged values shown in the graphs (Figures 29 and 30). Varying conditions showed consistently random results. The differences in levels of enzyme between control and field were not significant as a random response was seen. The randomness of the response was demonstrated by no apparent pattern of increase or decrease of enzyme production when the fields were applied individually. For each experiment, the amount of enzyme produced increased as the growth of the bacteria increased. No single time interval exhibited a greater or lesser response than any other.

The calcium resonance frequency fields however, showed a statistical significance of P value .014. These results again validate the theory that the resonant fields do produce an effect. While the graph representing the β-galactosidase production in the fields does not appear to show much effect (Fig. 31), the statistical evaluation
shows otherwise. Figure 32 shows a typical growth curve for the experiments with calcium.

**Average B-galactosidase Production**

**DC Field 78.4uT +Ca**

**Average B-galactosidase Production**

**60Hz AC, +Ca**

Figure 29. B-galactosidase production, 78.4uT, + calcium

Figure 30. B-galactosidase production, 60Hz, + calcium
Graphs representing an average of three experiments for each field condition display visually the random nature of the response through time. The control and field exposed values for β-galactosidase fluctuate through time with no pattern of response to the field conditions. The background level of β-galactosidase at 30 minutes varies slightly between field conditions. This is indicative of the normal background levels known for the recA: lacZ fusion strain of E. coli.

In experiments with the calcium removed, again the various field conditions were tested to see what effect the individual or combined fields might have. All experiments were performed using a 60Hz AC and 78.4μT DC calcium frequency, individually or combined for the resonant effect.

With the calcium removed from the media, the growth curves from all experiments appeared to be unaffected. The cells grew reproducibly from day to day as evidenced by growth curves. These growth curves displayed the same characteristic log growth as had the previous growth curves. This indicated that the removal of calcium did not affect cell division.

However, upon closer inspection, the growth curves generated from the media with no calcium appeared to grow at a slightly faster rate and to higher cell densities. This may however be an artifact from the particular cell population or an indication of a stress response to lack of
Average B-galactosidase Production

AC/DC +Ca

Figure 31. B-galactosidase production, calcium resonance

Growth Curve GE94

Figure 32. Growth curve with calcium
calcium.

The β-galactosidase curves did appear to be different when calcium was removed. Instead of the constant rise in enzyme production displayed through time as before, the maximum amount of enzyme produced occurred at approximately 180 minutes in an experiment run for 360 minutes. This was apparent for all the experimental field conditions. While no indication of calcium depletion was apparent in the growth, the enzyme production seemed to show a faster response to DNA damage or the DNA damage occurred quicker. This could indicate lack of calcium generates less reliable reproduction which would lead to more DNA damage.

The graphs representing the β-galactosidase production through time appear as a hump instead of a line with steadily increasing slope. Notice also the background level of β-galactosidase at 30 minutes. Instead of the normal value of 200 Miller units, it starts from the value of 350 Miller units for each field condition. The shapes of the curves and the background values of enzyme indicate this is a response by the bacteria to a lack of calcium since this is apparent for all field conditions.(79)

From these graphs, it is obvious the DC field(Fig. 33) lowers the level of expression of β-galactosidase by approximately 10%. This corresponds to a slightly enhanced growth curve. This could indicate an enhancement also in the repair processes for the cell. The level of growth
increases due to less DNA damage being naturally induced in normal cell division. The DC field seems to reduce the stress on the cell during replication.

With the AC field (Fig. 34), the curves from the exposed samples were again random as to increase or decrease of enzyme production. No pattern was apparent and no time interval in particular showed any difference of significance.

Using the resonance frequency for calcium, the growth was not affected by the fields. However the enzyme production was increased in the fields for the time interval of approximately 90 to 210 minutes for all experiments. This response was small when compared to the controls but the pattern was apparent (Fig. 35). This is the opposite effect of the DC field. Therefore the AC field reverses the cell’s response to stress. The amount of stress felt by the cells is increased by adding the AC field to the DC field. Figure 36 displays the normal growth for no calcium conditions.

To compare the total amount of enzyme produced through time, the highest average value was subtracted from the value at 30 minutes for each different field condition. The net difference in values of enzyme produced both with and without calcium are similar. When calcium is depleted, there is an increase in the total amount of β-galactosidase produced in AC field and in the resonant fields. In the DC
Average B-galactosidase Production
DC Field 78.4uT -Ca

Figure 33. B-galactosidase production, 78.4uT, no calcium
- exp-Ca  - control-Ca

60Hz AC, -Ca

Figure 34. B-galactosidase production, 60Hz, no calcium
Average B-galactosidase Production
AC/DC -Ca

Figure 35. B-galactosidase, Ca++ resonance, - Ca++

Growth Curve GE94
AC/DC -Ca

Figure 36. Growth curve, no added calcium
field, the exposed samples produced less enzyme than the controls.

By viewing this net difference in β-galactosidase production with no added calcium, it shows a 36% decrease in the amount of enzyme produced over time in the DC field, a 33% increase when exposed to the AC field, and a 26% increase under resonant conditions as compared to the calcium added. This again points to the DC field being able to provide a more amenable environment for the bacteria.

To compare the results of calcium enriched or depleted media at the different field conditions, a ratio of the values from the exposed samples to those not exposed was determined. This is displayed as the experimental over control and is represented by a single line with points above or below a middle value of one. In this way, each exposed point in the curve generated by the increasing amount of cells, is compared to the corresponding control point. After this ratio is calculated for each individual experiment, the set of experiments under the same conditions can be compared on the same graph (Figures 37, 38, 39). Further, if the ratios of the individual experiments are averaged for each field condition, then all the different field conditions can be compared on the same graph (Fig. 40). In this way also, the results of the experiments with calcium can be compared to the results of those without. No experimental conditions showed significance by a t test when
Experimental/Control B-galactosidase
DC Field 78.4uT +/-Ca

Figure 37. Experimental over control ratio, DC, +/- Ca++

Average(n=3) AC Experimental/Control
Effect of 60Hz AC, 784pk to pk

Figure 38. Experimental over control ratio, AC, +/- Ca++
B-gal Average Experimental/Control
AC/DC +/-Ca

Figure 39. Experimental over control ratio, AC/DC, +/- Ca++

Experimental/Control B-gal -Ca
Comparing AC, AC/DC/ and DC fields

Figure 40. All - Ca++ ratios
**B-galactosidase Production +/- Ca++**

DC only

**Figure 4.1**

- coil +Ca
- sham +Ca
- coil -Ca
- sham -Ca

**Graph:**
- X-axis: Time (120, 150, 180, 210, 240, 270, 300, 330, 360)
- Y-axis: Miller units (0, 30, 60, 90, 120, 150, 180, 210, 300, 270, 330, 300, 360, 450)

**Legend:**
- ■ coil +Ca
- ● sham +Ca
- □ coil -Ca
- ◆ sham -Ca
B-galactosidase Production +/-Ca++

Figure 42.

exp+Ca
control +Ca
exp-Ca
control-Ca
Figure 43

B-galactosidase Production +/−Ca++

AC/DC

Miller units

100 150 200 250 300 350 400 450 500 550 600
30 60 90 120 150 180 210 240 270 300 330 360

time

— coil +Ca — sham +Ca — coil -Ca — Sham -Ca
ratios were evaluated. Figures 41, 42, and 43 compare the results with and without calcium for each different field condition.

Using the ANOVA and t test (paired two sample for means, one tailed) for statistical significance, none was found when using only the 60Hz AC field when comparing the \( \beta \)-galactosidase production under any condition, the P value for the calcium added was .292 and for calcium depleted P was .277. There was significance with the DC field with no calcium but not with calcium. The P values were .0028 and .44 respectively. The opposite was true in the combined resonant fields. The experiments containing calcium gave a significance in the \( \beta \)-galactosidase production with a P value of .014. A P value of .2 was determined for the calcium depleted experiments. The statistics indicate the resonant fields did have a significant effect on the production of \( \beta \)-galactosidase when calcium was present.

The effects of no calcium with just the DC field indicate the bacteria thrive during growth incurring less DNA damage. The growth phenomena has been seen before in several different types of bacteria including E. coli.(80) The fact that reduced calcium availability also helped growth and reduction of DNA damage could indicate the natural stores of calcium are more than sufficient for the bacteria to thrive. Calcium might be inhibiting other processes or competing with other ions when in excess. The
lack of calcium might make the cell more efficient with the use of other ions involved in enzyme or protein reactions.

The recA protein binds to single stranded DNA (ssDNA) in a cooperative manner with ATP when activated by the SOS response. The recA protein (RecA) requires magnesium to assist the binding of ATP. The ion is required for a conformational change to allow structural association with the binding of ATP to the recA protein. The binding of ATP induces a high affinity single stranded DNA binding state. The recA protein filament assembles unidirectionally on ssDNA. The recA protein catalyzes unidirectional strand assimilation.

The recA-ssDNA filament then binds duplex DNA. RecA scans the duplex DNA for sequence complementarity. The duplex DNA is partially unwound so its base sequence can be read. When a complementary sequence is found, the duplex is further unwound and the ssDNA pairs with the strand. The strands exchange in a process called branch migration. Once the damaged strand has been repaired, the hydrolysis of ATP allows the RecA to recycle.

Weinstock et al (81) reported that calcium chloride decreases the rate of ATP hydrolysis and thus interferes with the actual hydrolysis step. This showed that ATP binding but not hydrolysis was required for induction of the high affinity state. Under reduced hydrolysis conditions, the affinity of all recA protein molecules for ssDNA is
sensitive to the unbound ATP/ADP molar ratio in the cell. As this molar ratio decreases, the affinity of the bound recA protein changes abruptly from a high to low affinity state. The affinity of recA protein for ssDNA is decreased in the presence of ADP. (79)

To restate the previous paragraph in relation to our experiments, when calcium chloride is present, ATP hydrolysis is greatly reduced causing less binding of ssDNA to recA. When calcium chloride is removed, the ATP hydrolysis can proceed as normal and the recA binding affinity is increased. So calcium reduces the apparent affinity of recA protein for ssDNA in the recA protein-ATP-ssDNA complex. This being the case, if calcium was removed the affinity of recA for ssDNA would be increased.

This activity could explain the change in the shape of the β-galactosidase curves when calcium was removed from the media. The experiments with calcium added display a gradual increase in the response to DNA damage by the recA protein through time. The binding of the recA would be reduced due to the calcium present. With no calcium added the response of the cells to the DNA damage would be faster, resembling the curves without calcium.

When comparing the calcium depleted β-galactosidase experimental results to those with calcium there was a large significance, however this only indicated the overall difference in the experiments. No significance was found in
any of the calcium depleted experiments except when using the DC field.

To separate the various effects of the fields in experiments where no calcium was added, the ratio of experimental values to controls was determined for each point. These results are placed on a graph so the real differences between the conditions can be seen. It is apparent that the effect of each condition is observed in the time interval from 120 to 240 minutes. The graph shows the obvious decrease in enzyme production from the DC field by having values mostly below one, whereas the AC and resonant fields display positive peaks, above one, for this same time period.

While the AC field alone and that of the resonant field have somewhat similar results; neither represent more than a 10% increase over their respective controls. Yet when these results are compared to the DC field effects, the differences become enhanced. The most significant observation is the reversal of the effect when an AC component is added to a DC component. This suggests the AC component of the combined resonant field is a destructive force causing DNA damage to the E.coli cells.

While no statistical significance was found in the experimental results without calcium using only the AC field, a trend appears on the graphs which indicate a possible increase in DNA damage. This trend may be showing
the influence of the field on the ability of the recA protein being able to bind ssDNA better for an enhanced detection of β-galactosidase, not necessarily enhanced production.

The DC field experiments without calcium could indicate the activation of calcium therefore inhibiting recA binding and β-galactosidase production. The resonant calcium fields in the experiments with calcium produced a significantly higher level of enzyme. This shows there is an effect attributable to the fields on calcium activation. The recA protein was already challenged by the calcium being present and inhibiting binding of the ssDNA. The fields acted to enhance the detection of enzyme by the increased binding of the recA to the ssDNA. This response points to the calcium being moved away from the active site of the recA protein-ssDNA complex, allowing the magnesium salt of ATP to bind to the complex. The calcium may have been activated for other protein interactions or processes such as cell division.

The SOS response system has given some insight into the possible mechanisms of the effects of EMFs. While the exact reaction or mechanism being affected by the fields is still vague, the experiments on E. coli utilizing this system of DNA damage response have given a hint as to the direction of research to be pursued. As with the Ames Salmonella, many other strains of E. coli have been
developed to report effects of DNA damage. These other strains can also produce β-galactosidase in response to DNA damage. The Salmonella and E coli strains can be paired in the same manner as the strains we have used here to report on the same type of response from the cell. Continuing the process of finding an appropriate stress factor for the strains chosen could lead to finding the mechanism or mechanisms of action of the effects of the electromagnetic fields.
Reviewing the different processes involved with the experimental systems used here allows similarities to be found throughout the organisms. All living organisms rely heavily on calcium for many functions from cell division to movement. Calcium must be made available for whatever purpose by release from stores held until appropriate physiological conditions are met. The movement of calcium from the stored state is regulated by ions, messenger systems or nerve impulses. Typically the calcium held in stores is released through channels to the cytosol where it is needed for a process. The process involved may require only the presence of calcium for pH or energy purposes or for actual binding of calcium to a protein. Because pH and ion gradients or concentrations allow physiological conditions to remain responsive to changes in the cell's equilibrium, the process of recycling ions back to their original storage areas is very important. For calcium ions, it appears that most systems use a form of ATPase transport for sequestering the ions.

As seen in the plant, diatoms and recA systems, the calcium resonance frequency enhances the ability of the organism to react to a calcium linked process. In the case of the alfalfa seeds, growth is enhanced, and the diatoms show enhanced motility. Both of these systems contain processes directly regulated by calcium concentration. The
recA protein activity is inhibited by the presence of calcium. The calcium inhibits the protein's ability to be recycled for further use and the calcium resonance frequency appears to enhance this effect.

The calcium resonance frequency affects all the systems tested and while the results were not robust, some results were statistically significant. It appears the calcium ions are being manipulated in some manner by the fields. Most notably, the reversal in responses in the β-galactosidase assay when the bacteria was subjected to DC magnetic fields and then resonant fields when calcium is absent from the media. This indicates the DC magnetic fields are helpful in stimulating growth. In the recA system, when an AC magnetic field is added to the DC magnetic field, growth is no longer stimulated and the amount of enzyme produced indicates the AC magnetic field is destructive or allowing better detection of damage.

Humans have great ability to adapt to their environment. Only in the past century has man been subjected to the constant bombardment of electromagnetic fields from unnatural sources. The development of electricity and its common everyday usage has escalated with the development of technology. While man benefits from the ability to manipulate his environment even more because of this technology and the use of electricity, he should step back and question its effects on himself and other
organisms. This has not been done adequately to determine if electromagnetic fields can harm genetic material.

It is possible that man has adapted with the exposure to electromagnetic fields and can compensate biologically for its effects. However, if biochemical imbalances enhance the effect of the fields, care must be taken to protect sensitive systems that may be harmed. If there is a chronic imbalance in homeostasis for an organism, there could be an increased risk that electromagnetic fields will induce damage to the organisms DNA.

Care must be taken to sort out the real effects of electromagnetic fields on living organisms. Just as damage may occur due to exposure to fields of a certain frequency, repair also occurs as demonstrated by chronic nonhealing bone. If certain frequencies can repair bone, then other frequencies may repair other damaged or impeded biological processes. Continued testing of mutant strains of Salmonella and E. coli could help pinpoint the actual repair systems being triggered by the cells in response to particular magnetic fields. If these repair systems are found, then enhancement of their responses could allow further investigations into the mechanisms of action of the electromagnetic fields.
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