Biological Effects of Magnetic and Electromagnetic Fields

Edited by Shoogo Ueno
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PREFACE

The International Symposium on Biological Effects of Magnetic and Electromagnetic Fields was held from September 3-4, 1993 at Kyushu University in Fukuoka, Japan. Originally, it was only intended to be an informal gathering of many scientists who had accepted my invitation to visit Kyushu University after the XXIVth General Assembly of the International Union of Radio Science (URSI), held in Kyoto prior to our symposium. However, since so many distinguished scientists were able to come, it was decided that a more formal symposium would be possible.

It was a very productive symposium and, as a result, many of the guests consented that it would be a good idea to gather all the information put forth at the meeting and have it published. In addition, although they were unfortunately unable to attend the symposium, many other distinguished scientists had also expressed their wish to contribute to this effort and, in so doing, help to increase understanding in this, as yet, relatively immature field of science.

The question of both positive and negative effects of magnetic and electromagnetic fields on biological systems has become more and more important in our world today as they have become increasingly ubiquitous in the environment, medicine, research, and industry. Not only has it become an important tool for scientists to use in their research, but it is has also become necessary for us to understand how safe it is and what long-term effects it could have on our environment. Topics covered in this book include studies of biomagnetic and electromagnetic fields at the genetic, cellular, and hormonal levels, with both laboratory and epidemiological methods. They include investigations of intended direct effects such as neural stimulation, and unintended indirect effects from such sources as power lines, consumer electronics, and mobile telephones. They address our anxieties about effects such as immune system damage and cancer, and our hopes about potential beneficial uses.

I sincerely hope that our combined efforts in this book help to do more than just stimulate the scientific mind to investigate further. I hope it also makes us take a step back to see and realize how our research is not only limited to the laboratory but reaches out beyond those walls and into our everyday lives.

The symposium that gave birth to this book project was made possible by generous financial support from various companies and organizations, for which I am very grateful. I also thank co-sponsoring organizations for their support, and the members of the Bioelectromagnetic Society who contributed to this book. Sincere thanks are due to Prof. M.A. Stuchly and Prof. P. Bernardi, who organized a commission on "Electromagnetics in Biology and Medicine" at the Kyoto URSI meeting, and to Prof. Masao Saito, who was the coordinator in Japan of the commission. Their work made the planning of the Kyushu symposium possible.
My sincerest thanks to the staff and my colleagues at Kyushu University. I owe a debt of gratitude in particular to Keiji Iramina, Masakazu Iwasaka, and Terumi Asai. Last, but not least, I thank Susanna Heckmann and Deborah W. Mrongowius for all their efforts in putting this book together.

Shoogo Ueno
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INTRODUCTION

Biological effects of magnetic fields are classified into three categories; the effects of (1) time-varying magnetic fields, (2) DC or static magnetic fields, and (3) multiplication of both static fields and other energy such as light and radiation. For each category, a different strategic approach is required to shed light on the biomagnetic effects.

Time-varying magnetic fields produce eddy currents which stimulate excitable tissues at low frequencies. Magnetic brain stimulation can be realized by this effect. The first part of this paper focuses on magnetic nerve stimulation.

Biological effects of static magnetic fields have been poorly understood. Recognition of the role of diamagnetic, paramagnetic and ferrimagnetic materials in the body may help in unraveling the underlying mechanisms. The latter part of this paper focuses on the effects of magnetic fields on the behavior of diamagnetic water and paramagnetic oxygen. The embryonic development of frogs, blood coagulation, fibrinolytic processes and other biochemical processes are also observed under strong magnetic fields up to 8 T.

BIOMAGNETIC PHENOMENA

Biomagnetic phenomena in different intensities of magnetic fields and their frequencies are shown in Fig. 1. Effects of magnetic fields on living systems and biological materials have been observed mostly in the range of magnetic fields higher than the earth's magnetic field. Recently, superconducting magnets are being used in MRI (magnetic resonance imaging) systems, where human subjects are exposed to static magnetic fields of 1.5 T - 2.0 T. More intense magnetic fields (4 T order) are used in advanced MRI systems. We observed the phenomenon that surface of water is parted by static magnetic fields of up to 8 T. Fibrin
fibers, as diamagnetic living materials, are oriented parallel to a direction of static magnetic field of $1 - 11 \text{T}$.

The so called magnetophosphen e is a visual sensation caused by low frequency magnetic field exposure which was first reported by D'Arsonval (D'Arsonval, 1896). Lövsund et al reported that the threshold of magnetophosphen e is 10 mT at 20 Hz, and they claimed that the magnetophosphen e is a reasonable example of the study of potential biological hazardous effects of envirmental magnetic field effects (Lövsund et al, 1980). Magnetic stimulation of the human brain requires a 1 T order of pulsed magnetic fields for $100 - 200 \mu\text{sec.}$ Magnetic stimulation of the heart requires more strongly pulsed magnetic fields for $1 - 2 \text{msec.}$ In Fig. 1, the frequencies of the pulsed magnetic fields are converted from pulse width.


The study on biological effects of higher frequency magnetic and electromagnetic fields has become important in connection with the rapid increase of mobile telephones (Ghandi, 1992).

Using SQUID (superconducting quantum interference device) techniques, magnetic fields from the brain (Magnetoencephalogram, MEG), heart (Magnetocardiogram, MCG),...
Magnetic Fields on Biological, Physical and Chemical Processes

and lung (Magnetopulmogram, MPG) can be measured. This chapter, however, does not include these biomagnetic measurements.

MAGNETIC NERVE STIMULATION

Magnetic stimulation of excitable tissues has been studied by several investigators (D'Arsonval, 1896, Bickford et al. 1965, Irwin et al, 1970, Maass et al. 1970, Öberg, 1973). Among them, Maass and Asa proposed a transformer type of stimulation in which a nerve bundle was threaded through a core as the secondary winding (Maass et al, 1970). They demonstrated that the flux change in the core could be used to excite nerves. Öberg proposed an airgap type of stimulation, in which a nerve bundle was exposed to alternating magnetic fields (Öberg, 1973). Induced eddy currents in the membrane tissues could be expected to stimulate nerves. Although these types of magnetic nerve stimulation were experimentally demonstrated, the underlying nerve excitation processes following magnetic stimulation were not yet understood (Ueno et al, 1978).

We carried out an experiment to measure the action potentials of lobster giant axons under time-varying magnetic fields (Ueno et al, 1981, 1986). The axon membrane was excited by galvanic stimulation, and the action potential was recorded intercellularly with micro-electrodes. During the propagation of the action potential along the axon, alternating or pulsed magnetic fields were applied across the middle of the axon in order to study whether or not the magnetic fields had any effect on parameters such as conduction velocity and refractory period of the nerve fiber and amplitude, duration and shape of the action potentials.

The results obtained from the lobster experiments suggest that nerve excitation by magnetic field influence is mediated via the induction of eddy current in the tissue surrounding the nerve. The current density induced depends on geometrical factors as well as the resistivity of the tissue in which the current flows. In other words, for nerve excitation, the microscopic eddy currents that flow inside the microstructures of the axon membrane are not very important. What is important are the macroscopic eddy currents that flow along the nerve axon and in the tissues surrounding the nerve, as they contribute to the depolarization of the membrane.

After the study of single nerve axons, we proposed a new type of magnetic nerve stimulation (Ueno et al, 1984). An insulated magnetic core is implanted into the body with a nerve bundle positioned on the core aperture. The nerve can be stimulated by the eddy currents that flow in the body fluids around the core when the magnetic flux in the core is changed. There is no interlinkage between the core and the nerves. Therefore, this method is a good example for verifying that the nervous system responds to time-varying magnetic fields through eddy currents induced in the body.

Magnetic stimulation of the human brain was first reported by Barker et al. (Barker et al, 1985, 1986, 1987). Parameters of muscular potentials, such as conduction velocity, latency and amplitudes were studied by a number of researchers by recording the electromyographic (EMG) responses to stimulation of the motor cortex (Day et al, 1987, Hess et al. 1987a, 1987b, Mills et all, 1987, Rothwell et al, 1987). Single coils were used for these studies. Current pulses were passed through a single coil placed outside the head, and eddy currents induced into the head by the pulsed magnetic fields stimulated the brain. With this method, however, broad areas of the brain are stimulated simultaneously. We developed a method of localized magnetic stimulation of the human cortex (Ueno, Tashiro, and Harada, 1988).

The basic idea is to concentrate induced eddy currents locally in the vicinity of a target by a pair of opposing pulsed magnetic fields which can be produced by a figure-eight coil. Using this method, we were able to stimulate the motor cortex of the human brain within
a 5 mm resolution (Ueno, Matsuda, Fujiki, and Hori, 1989, Ueno, Matsuda, and Fujiki, 1989a). Since the concentrated eddy currents beneath the intersection of the figure-eight coil flow in a direction parallel to the tangent of both circular coils, vectorial stimulation can be achieved. The neural fibers can be excited easily when the fibers are stimulated by the eddy currents that flow parallel to the nerve fibers.

Based upon this principle, we obtained functional maps related to the hand and foot areas (Ueno, Matsuda, and Fujiki, 1989a, Ueno, Matsuda, and Fujiki, 1989b, Ueno, Matsuda, and Hiwaki, 1990). We have observed that an optimal direction of stimulating currents for neural excitation exists in each functional area in the cortex. We have also observed that the functional maps of the cortex vary with the orientation of the stimulating current. To explain the mechanism that is responsible for producing this anisotropic response to brain stimulation, we developed a model of neural excitation elicited by magnetic stimulation (Ueno, Matsuda, and Hiwaki, 1991).

The model explains our observation that the directions of the induced current vectors reflect both the functional and the anatomical organization of neural fibers in the brain. We need to emphasize that the point of excitation is not at a point under the intersection with the coil. Instead it is a few ten mm away from the point of intersection; in the case in which the coil diameter used is 50 mm, the distance between the coil and the nerve fiber is 10 mm.


PARTING OF WATER BY MAGNETIC FIELD: MOSE'S EFFECTS

Water, which is a diamagnetic material, is one of the most general and abundant diamagnetic fluid. It is an interesting problem for humans as to whether or not magnetic fields have any influence on water. Diamagnetic water is often used for calibration and correction in determining magnetic susceptibilities of materials with the magnetic balance system. However, little has been observed about the hydrodynamic behavior of water or diamagnetic fluids, in horizontal magnetic fields of 100 – 1000 T^2/m order.

We investigated the dynamic behavior of water in high gradient magnetic fields. We used a horizontal type of superconducting magnet 700 mm long with a 100 mm diameter wide bore. The superconducting magnet has a distribution of high gradient magnetic fields near the center of the magnet, as show in Fig. 2. When the magnet produced 8 T at its center, the maximum product of the magnetic field and the gradient was 400 T^2/m at z= ±75 mm, where the z-axis was directed to the bore axis. A water chamber, 50 mm wide, 60 mm high, and 700 mm long was filled with distilled water.

When the water chamber was inserted into the bore of the magnet, we observed a phenomenon in which the surface of the water was pushed back by magnetic fields of higher gradients. Two “frozen” cascades were formed; the surface of the water near the center of the magnet was parted, and the bottom of the water chamber could be seen. The water level at both ends of the chamber was raised (Ueno and Iwasaka, 1994a). We call this phenomenon the Moses' effects. This phenomenon reminds us of the Biblical story from Exodus, i.e., Moses parting the Red Sea.
We also found the Moses' effect with agarose gels. The agarose solution was allowed to gel in the superconducting magnet. Fig. 3 shows that the surface of the agarose gel was formed in the same manner as in the water experiment.

To measure changes in the water level in the magnetic fields, one of the edges of the water chamber was positioned at the center of the magnet, and the water level was observed by a video camera. Magnetic fields in the center of the magnet were changed from 0 to 8 T, and a decrease in the water level was observed. The changes in the lowest level of water was −22 mm when magnetic fields were changed from 0 T to 8 T as shown in Fig. 4. The decrease in water level was proportionate to $B^2$ at the center of the magnet. This result shows that the energy used for the formation of water-walls in a steady state is proportionate to the magnetic energy of the water (Ueno and Iwasaka, 1994 b).
A stress analysis was carried out to explain the mechanism of the Moses' effect. The stress tensor of a diamagnetic fluid (in indicial notation) is given by

\[ T_{ij}^m \cdot n_j = \left( p^m + \mu_0 H^2 / 2 + \mu_0 M H / 2 \right) n_i , \]

where \( n = (n_x, n_y, n_z) \) is a unit vector that is perpendicular to the water surface, \( p^m \) is the static fluid pressure, \( \mu_0 \) is magnetic permeability, \( \rho \) is mass per unit volume, \( g \) is gravitational constant, \( M \) is the magnetization of diamagnetic fluid and \( H \) is a magnetic field (Ueno and Iwasaka, 1994a).

On the other hand, the stress tensor that is outside of the diamagnetic fluid is given by

\[ T_{ij}^o \cdot n_j = - \left( p^o + \mu_0 H^2 / 2 \right) n_i , \]

where \( p^o \) is the environmental pressure.

On the surface of the water, \( T_{ii}^m \cdot n_i \) equal to \( T_{ij}^o \cdot n_j \). Equations (1), (2) and (3) can be combined to give

\[ z = h = (\mu_0 / rg) \int_0^H M \, dH - \chi_m \mu_0 H^2 / 2rg . \]

Decreases in water levels calculated by equation (4) are shown in Fig. 4 and Fig. 5.

The hydrodynamics of diamagnetic fluid in \( \sim 400 \, T^2 / m \) is comparable with that of ferromagnetic fluid in weak magnetic fields. Remarkable effects of high gradient magnetic fields are expected in blood circulation, cerebral spinal fluid system and other intra and extra cellular solutions.

The magnetic force acting on water molecules is given by

\[ F = \frac{\chi}{\mu_0} B \frac{dB}{dz} \]

where \( \chi \) is the magnetic susceptibility of water molecules, \( B \) is the magnetic flux density, and \( \mu_0 \) is the magnetic permeability of the vacuum. Molecular susceptibility of water is \(-12.97 \times 10^{-6} \) [cgs] at 293 K. The magnetic forces acting on 100 ml of water is shown in Fig.
6. A maximum magnetic force of \(0.288\, \text{[N]}\), which is about one third of the force of gravity, is obtained where \(B_x dB/dz = 400\, \text{T}^2/\text{m}\).

Magnetic levitations of diamagnetic materials such as water, ethanol and acetone in magnetic fields of more than 20 T produced by a vertical type of superconducting magnet were reported by Beaugnon et al (Beaugnon et al, 1991). Distortion in the surface of liquid helium by magnetic fields of 6.5 T was also studied (Frost et al).

Studying the role of diamagnetic fluids in gradient magnetic fields is important in understanding the mechanism of biological effects of magnetic fields. The phenomena observed in the present experiments will provide a new aspect to applied physics and biomagnetics.

![Figure 5. Surface profile of diamagnetic water in magnetic fields of up to 8 T.](image)

![Figure 6. Magnetic force acting on water.](image)

\[|F|_{\text{max}} = 0.288\, \text{[N / 100 ml]}\] at 20°C
REDISTRIBUTION OF DISSOLVED OXYGEN CONCENTRATION UNDER MAGNETIC FIELDS

It is an interesting question whether or not magnetic fields have any influence on dissolved oxygen in living tissues and cells. In several reviews and papers, the importance of the role of oxygen has been suggested by Aceto et al (Aceto et al, 1970). Since oxygen molecules and dissolved oxygen as paramagnetic molecules are an important role in living systems, possible biomagnetic effects can be expected if spatial distributions of dissolved oxygen concentration in the tissues are modulated by magnetic fields.

Based on this assumption, we have measured dissolved oxygen concentration under magnetic fields using an electromagnet with 1.0 T with a gradient of 10 T/m (Ueno and Harada, 1982), and a superconducting magnet with magnetic fields of up to 8 T and a gradient of 50 T/m (Ueno et al, 1994).

Redistribution of dissolved oxygen was observed during both oxygen desorption and absorption processes in a magnetic field of 1.0 T when the surface of the water contacted the atmosphere. It is understood that the oxygen in the atmosphere over the surface of the water is redistributed first by magnetic fields, resulting in oxygen desorption absorption rates being modulated by the spatial distribution of oxygen concentration in the atmosphere (Ueno and Harada, 1982).

To obtain the direct effects of magnetic fields on the redistribution of dissolved oxygen in water, we used a horizontal type of superconducting magnet (Ueno et al, 1994). A closed water chamber 100% filled with distilled water which was separated from the air atmosphere, was moved together with a DO (dissolved oxygen) sensor in the direction of the bore of the magnet. This experiment, however, might include the undesirable factor of electrochemical processes in the DO sensor possibly being affected by magnetic fields.

The present study focuses on the dynamics of dissolved oxygen in water under magnetic fields of up to 8 T, with the gradient of 50 T/m. The dissolved oxygen concentration is measured by a DO meter before and after magnetic field exposures in order to exclude undesirable interactions.

![Diagram](image)

*Figure 7. Experimental set-up for redistribution of dissolved oxygen in magnetic fields of up to 8 T.*
The experimental set-up is shown in Fig. 7. We used a horizontal type of superconducting magnet 700 mm long with a bore 100 mm in diameter. When the magnet produced 8 T at its center, the maximum product of the magnetic field and the gradient was 400 T·m at z=±75 mm, where the z-axis is the bore axis.

An acrylic water chamber, 700 mm long, 70 mm high, 50 mm wide was 100 % filled with distilled water, and the water chamber was sealed with an acrylic cover to separate the water from the air atmosphere. The water chamber consisted of 7 sections, and the length of each section was 100 mm. Partitions, i.e., dividers, were removable.

The dissolved oxygen concentration was controlled by introducing oxygen gases into the distilled water in the range from 8 mg/l to 26 mg/l. The water chamber without dividers was placed in the magnet's bore, and the water was exposed to magnetic fields of up to 8 T for 60 min. After the water chamber was taken out of the magnet, the water in the chamber was divided by six acrylic dividers into seven sections. The dissolved oxygen concentration at each section was measured with a Clark type DO (dissolved oxygen) meter.

To obtain dynamic behaviors of dissolved oxygen during and after magnetic field exposures, time-courses of changes in dissolved oxygen concentration were measured in the 4th section, changing the exposure periods.

Fig. 8 shows the redistribution of dissolved oxygen concentration by magnetic fields of up to 8 T for 60 min. The dissolved oxygen concentration before magnetic field exposures, i.e., an initial concentration, was controlled from 11 mg/l to 22 mg/l. The horizontal axis shows the section number of each section divided by acrylic dividers. The dissolved oxygen concentration in sections 3 – 5 at the central part of the magnet was increased.

Fig. 9 shows the normalized data of the redistribution of dissolved oxygen concentration. The data were averaged over 8 trials where the dissolved oxygen concentration at

Figure 8. Redistribution of dissolved oxygen concentration by magnetic fields of up to 8 T. The concentration of dissolved oxygen before magnetic field exposure was controlled by introducing oxygen gases into the water.

Figure 9. Effect of an 8 T magnetic field exposure for 60 min on distribution of dissolved oxygen concentration. The data were averaged over 8 trials where the dissolved oxygen concentration in sections 1 and 7 fell in the range 14 mg/l to 16 mg/l.
both edges of the chamber fell in the range of 14 mg/l – 16 mg/l after magnetic field exposures of 60 min. The results show that the increase in dissolved oxygen concentration was 5%.

Fig. 10 shows the effect of initial concentration on the redistribution of dissolved oxygen concentration. When the initial concentration changed from 8 mg/l to 26 mg/l, the change of dissolved oxygen was 0% to 13%.

The magnetic force acting on a group of oxygen molecules is described as follows.

\[ F = \frac{\chi}{\mu_0} B \frac{dB}{dz} \]  

(in z-direction)

where \( \chi \) is the magnetic susceptibility of a group of oxygen molecules, \( B \) is the magnetic flux density, and \( \mu_0 \) is the magnetic permeability of the vacuum. For oxygen molecules, \( \chi = 3449 \times 10^{-6} \) [cgs] at 300 K. The magnetic force acting on 1 molar of oxygen is shown in Fig. 11.

High gradient magnetic separation (HGMS) has made it possible to separate micrometer-sized paramagnetic particles from solution. For instance, Melville et al. succeeded in separating red cells from whole blood by HGMS when the hemoglobin was in the completely deoxygenated state (Melville et al., 1975). Friedlaender et al. captured small paramagnetic particles such as Mn₂O₃ and Cr₂O₃ by HGMS (Friedlaender et al., 1978).

The magnetic energy of an oxygen molecule is \( 3 \times 10^{-26} \) [J] at 1 T and \( 2 \times 10^{-24} \) [J] at 8 T, respectively. Thermal energy at 300 K is \( 4 \times 10^{-21} \) [J]. It is estimated that oxygen molecules drift in a particular direction in magnetic fields of more than 8 T when at least 2000 oxygen molecules aggregate.

Oxygen molecules are attracted by a maximum magnetic force where \( B_x dB/dz \) is largest in magnetic fields. When there are two maximum peaks in a magnetic force as shown in Fig. 11, dissolved oxygen molecules are trapped in the area between the two peaks.
resulting in the redistribution of dissolved oxygen concentration. In other words, spatial distribution of dissolved oxygen concentration conforms to a single peak distribution in a steady state, similar to the distribution of magnetic fields, but not to the distribution of magnetic force. In contrast, in the case with captures of paramagnetic particles by HGMS techniques, trapped particles accumulate in the vicinity of wires where the magnetic force is the strongest.

Effects of high gradient magnetic fields on biological systems are expected when a living tissue contains a high concentration of dissolved oxygen. For instance, the oxygen effect is a phenomenon when radiation injury of tissues with a high concentration of oxygen becomes serious, compared with the case of a low concentration of oxygen. If the behaviors of dissolved oxygen could be controlled by magnetic fields, a new type of cancer therapy might be contrived (Ueno and Harada, 1986).

**EFFECTS OF MAGNETIC FIELDS ON GAS-FLOW AND COMBUSTION**

In relation to oxygen dynamics in water, we observed oxygen dynamics in air. Combustion is an oxidation reaction which involves both a burning phenomena in the air and cell respiration in the living body. The effects of magnetic fields on combustion of hydrocarbons and alcohol with the aid of platinum catalysis have been studied to simulate in part the oxidation of organic matter in the living body, and we observed that the combustion velocities of gasoline and alcohol were influenced by magnetic fields (Ueno et al, 1985, 1986). In order to explain this phenomena, we examine the effect of gradient magnetic fields on burning flames. A candle was burned in the airgap between magnetic poles, and the candle flames were exposed to gradient magnetic fields. During magnetic field exposures, the flames were pressed down, and the shape of the flames changed like a mushroom (Ueno and Harada, 1987). The field intensity in the center of the airgap was 1.2 T.

Apart from the combustion experiments, we have observed that the flow of gases such as carbon dioxide, nitrogen, oxygen and argon are blocked or disturbed by magnetic fields. A model called a “magnetic curtain” has been introduced to explain these phenomena. It is assumed that the magnetic curtain is a wall of air caused by magnetic fields as shown in Fig. 12.

To clarify the mechanism of the magnetic curtain, trajectories of gas flow near and inside the magnetic curtain are simulated on the basis of molecular dynamics (Ueno and Iwasaka, 1990, Ueno et al, 1993). It is assumed that gas molecules such as nitrogen and oxygen molecules are particles, and these particles move in two-dimensional space. The Brownian movement of particles in air atmosphere is caused by molecular collisions. We
assumed that the dynamic movement of paramagnetic molecules such as oxygen is constrained by a magnetic force.

Let us consider the condition in which 10 particles start together at an upper starting line and go down with a velocity of mean free path. Each particle receives random acceleration through the collision. When a magnetic force is applied to the particle flow, the velocity component of the z axis is changed by the collision with oxygen particles. Fig. 13 (a) shows the trajectories of gas flow with 10 million collisions. When the region is exposed to a gradient magnetic field of 225 T/m, the gas flow is blocked, as shown in Fig. 13 (b).

We have designed an electromagnet with a pair of columnar magnetic poles in which inner sidepieces were hollowed out. A candle was burned in the hollowed space between the magnetic poles, and candle flames were exposed to magnetic fields. The flames were...
quenched a few seconds after the onset of field exposures (Ueno, 1989). The interception of oxygen by the magnetic curtain quenches the flames. In place of the candle, if a mouse or a human subject is positioned inside the magnetic curtain, as shown in Fig. 14, we have to be careful about the respiratory system being disturbed in this condition.

EFFECTS OF MAGNETIC FIELDS ON FIBRIN POLYMERIZATION AND FIBRINOLYSIS

Fibrinogen, which is the prime factor in blood coagulation, changes to fibrin monomer by the action of protease thrombin, and fibrin monomers are polymerized. Fibrin polymers are diamagnetic materials that are oriented in a magnetic field (Torbet, 1981, Yamagishi et al., 1989, Ueno et al., 1993). In the course of the polymerization process, when a magnetic field of intensity 10 to 20 T is applied, the fibrin fibers orient parallel to the magnetic fields. When no magnetic field is applied, fibrin fibers become entangled in mesh, and no orientation is observed. This is an effect of strong static magnetic fields on the blood coagulation system.

On the other hand, the fibrinolytic process also occurs in vessels in a mutually compensating and balanced state with coagulation. Plasmin, which is a serine proteinase, reacts with fibrins to digest peptide bonds by hydrolysis. Fibrin gels are dissolved when polymerized fibrins are degraded to fragments by plasmin.

A study on the positive effect of weak magnetic fields (~0.3 T) on fibrinogen degradation products level in rabbits in vivo was reported (Gorczynska, 1986).

In our study, fibrinolytic processes in magnetic fields were investigated using a fibrin plate method, whereby mean levels of FDPs (fibrin degradation products) in solutions are measured.

We investigated the effect of magnetic field exposure at 8 T on the dissolution of a fibrin-plate (Iwasa et al., 1994 a). Thrombin (80 µl, 3.1 NIH units/ml) was added to 3-ml of fibrinogen (4.8 mg/ml in 0.05M Tris-HCl buffer, pH 7.4, containing 0.10 M NaCl). This solution was divided into two dishes, and incubated at 25 °C for 60 min without magnetic fields. A hole was made in the centers of the fibrin plates with a gel puncher, and 20 µl of plasmin (4 casein units/ml) was added to each hole. Fibrin plates were incubated at 37 °C for 15 hours, either with a magnetic field at 8 T or without magnetic fields, and dissolution of both types of fibrin was observed. Mean levels of FDPs (fibrin degradation products) in solutions were obtained. Fig. 15 shows the mean levels of FDPs in samples exposed to an 8 T magnetic field and in those not exposed. Mean levels of FDPs in samples exposed to an 8 T magnetic field were on the average 15 % higher than those not exposed. The same

![Figure 15](image-url)

Figure 15. Effects of gradient magnetic fields on fibrinolysis by plasmin. Levels of fibrin degradation products are shown. Fibrin plates were incubated at 37 °C for 15 hours, with and without magnetic fields.
experiment was carried out in gradient magnetic fields (gradient fields: \( B \times dB/dz = 350 - 400 \ \text{T}^2/\text{m} \)). The mean level of FDPs released in a gradient magnetic field of up to 400 \( \text{T}^2/\text{m} \) was more than 190% of the control samples. In this case, there are large gradients along the horizontal line. Solutions containing water, plasmin and fibrin could diffuse to horizontal directions to promote the reactions of fibrinolysis. The results show that fibrin gels and water containing plasmin diffused to less intense magnetic fields, and fibrin dissolved in specific directions. It is possible to vectorially control the dissolution of a fibrin clot by magnetic forces.

We also carried out an experiment in order to understand how the fibrin oriented in a magnetic field dissolves (Ueno et al., 1993). FDPs in the dissolved holes in the fibrin prepared in either the presence or the absence of an 8 T magnetic field were assayed. Fibrin gels formed with a magnetic field were more soluble than those formed without a magnetic field. It was observed that the shapes of holes in dissolved fibrin changed to ellipsoidal patterns when fibrin plates were formed with a magnetic field at 8 T. The transversal axis of the ellipse was parallel to the magnetic fields.

As a possible mechanism of these effects, changes in concentrations of diamagnetic macromolecules in a solution in gradient magnetic fields were examined. We carried out an experiment to measure changes in the concentration of macromolecules (Iwasaka et al., 1994b). A solution of fibrinogen and thrombin in a plastic tube made of vinyl chloride, 10 mm in diameter and 310 mm long, was coagulated within 12 hours in magnetic fields up to 8 T (\( B \times dB/dz \leq 400 \ \text{T}^2/\text{m} \)). Coagulated fibrin was frozen at -20 °C, and divided into 15 fractions. Each fraction of the frozen fibrin had the same volume. After incubation with 1ml of 3.3 M urea for 12 hours at 37 °C, we measured levels of solubilized fibrin polymers as absorbance at 280 nm. Fig. 16 shows a distribution of concentrations of fibrin in gradient magnetic fields. The vertical line shows the absorbance at 280 nm corresponding to the concentrations of fibrin. The absorbance of dissolved solutions at 280 nm obtained from fibrin coagulated in 5-7 T were 20-50% higher than those in 1 T fields. The results suggested that fibrin polymers in a solution drifted in a specific direction, and concentrations of the fibrin changed, with the result that the level of solubilized fibrin polymers were inhomogeneous in a chamber. It is well known that electrophoresis of macromolecules is observed in electric fields. The phenomenon observed here indicate that “magneto-phoresis” of fibrin polymers occurred in gradient magnetic fields of 400 \( \text{T}^2/\text{m} \).

![Magnetophoresis of fibrin polymers under magnetic fields of up to 8 T.](image)
When a solution, containing water and diamagnetic macromolecules is exposed to gradient fields, magnetic forces act on the water and diamagnetic macromolecules. The distance of drifts of a macromolecule in the water in a unit time is described as follows:

\[ l = \int_0^t (\chi_s - \chi_w) V \mu_0 \Delta (H^2) - dt \cdot 2m \]

where \( t \) is time, \( V \) is volume of fibrin polymer, \( \chi_s \) is magnetic susceptibility of fibrin per volume, \( \chi_w \) is a magnetic susceptibility of water per volume, and \( m \) is mass of fibrin polymer per unit volume.

Magneto-phoresis of fibrin polymers in gradient magnetic fields occurred when magnetic energy \( -(\chi_s - \chi_w) V \mu_0 H^2 / 2 \) was larger than thermal energy \( kT \). We conclude that diamagnetic macromolecules in water drift in a specific direction due to the difference in the diamagnetic susceptibility of fibrin and water.

It is possible for large macromolecules, such as fibrins and fibrin polymers, to be affected by gradient magnetic fields. To investigate the effect of homogeneous magnetic fields on enzymatic activity of plasmin, we measured a synthetic substrate hydrolysis with plasmin (Iwasaka et al., 1994 c). As the synthetic substrate, D-Val-Leu-Lys-pNA (S-2251; molecular weight is 551.5) is a small molecule compared to fibrin, it is expected that the effects of gradient magnetic fields can be ignored. In order to reduce the effect of gradient magnetic fields, the center part of the superconducting magnet's bore, where dB/dy < 4 T/m and \( B = 8 \) T was used. The concentration of S-2251 and that of plasmin was 0.1 mM and 0.023 casein units/ml, respectively. After incubation at 37°C 200 μl of 99.9% acetic acid was added, and the absorbance was measured at 405 nm with a spectrophotometer. An 8 T magnetic field did not have a distinct effect on either the maximum velocity (Vmax) or the Michaelis constant (Km). However, when the absorbance changed non-linearly and reached 60–90% of the maximum absorbance, absorbance of the mixture exposed to 4–8 T for 60 min at 37°C was lower than control samples (maximum 11%). The results show that a long-term exposure to magnetic fields inhibited the synthetic substrate hydrolysis with plasmin.

**EMBRYONIC DEVELOPMENT OF XENOPUS LAEVIS UNDER MAGNETIC FIELDS**

We have studied a possible influence of intense magnetic fields on the early embryonic development of frogs (Ueno et al., 1984, 1990). Some of the most serious hazardous effects that could be induced by intense magnetic fields are teratogenic effects on developing embryos. Embryos of *Xenopus laevis* were exposed to magnetic fields of up to 8 T for the period from the pre-cleavage stage to neurula stage. Embryos were then cultured in Brown-Caston’s medium until the feeding-tadpole stage (Ueno, Iwasaka and Shiokawa, 1994).

We carried out an experiment, in which 100 embryos were cultured for 20 hours in magnetic fields. No apparent teratogenic effects were observed when embryos were cultured for 20 h from the stage of uncleaved fertilized egg to the neurula stage under magnetic fields of 8 T. We conclude that static magnetic fields of up to 8 T do not appreciably affect the rapid cleavage and the following cell multiplication and differentiation in *Xenopus laevis*.

We have also studied the early embryonic development of *Xenopus laevis* in a 40 nT magnetic field, or one-thousandths of the earth’s magnetic field, and obtained negative results. Thus, again under this very low magnetic field, fertilized eggs developed normally and formed tadpoles with no appreciable abnormality.

It is a miracle or paradox that animals such as *Xenopus laevis* develop normally under strong magnetic fields, although we have observed very clear biomagnetic effects such as
parting water by magnetic fields, magnetic orientation of diamagnetic polymers, and redistribution of dissolved oxygen by magnetic fields. It seems that biological cells are robust against magnetic fields.

**GENETIC EFFECTS OF MAGNETIC FIELDS ON DROSOPHILA MELANOGASTER**

The genetic effects of magnetic fields on somatic reversion and somatic recombination in *Drosophila melanogaster* have been reported (Levengood, 1966, Mittler, 1971, Koana et al. 1994, Yoshikawa et al. 1994).

We examined the somatic reversion of the white locus and the somatic recombination of *mwh* and *fly* genes in *Drosophila melanogaster* after they were exposed to a static magnetic field at 8 T for 8 hours or exposed to an alternating magnetic field (3.3 mT) at 20 Hz for 8 hours (Yoshikawa, Iwasaka and Ueno, 1994). We have developed a mutation assay system in which both types of mutations are genetically combined so as to detect mosaic spots on eye facets and on wing blades in single individuals. The reverse eye color mutation from white-ivory (*w*) to wild type (*w*) is associated with the loss of a 2.9-kilobase DNA fragment duplicated in the white locus on the X chromosome. The formation of mutant spots on the wing blades results from recombination at wing anlage cells in larvae, trans-heterozygous for the mutations *multiple wing hairs* (*mwh*) and *flare* (*flr*) on the 3rd chromosome. The static magnetic fields with 8 T were effective in producing the eye spots caused by intragenic mutation and the wing hair spots resulting from somatic recombination. In the exposure groups, eye spots occurred 1.7 times more frequently than in the control groups, and wing hair spots 1.4 times more. In contrast, the alternating magnetic fields with 3.3 mT at 20 Hz were not more efficient for induction of both somatic spots. Mutant clones induced at the beginning of larval life will be large in size while those produced in the second or third instar will be successively smaller. Hence, classification of mosaic spots into size classes and subsequent analysis of clone size distribution can be used to estimate the point in time of the induction of the mutational events. The increased number of mosaic spots in size classes 1, 2, and 3–4 of the groups that exposed to an 8 T magnetic field were characteristic when compared to the control groups. It is possible that the period of these increments may correspond to the third instar of the larval stage. It seems reasonable to conclude that an 8 T magnetic field induced reverse mutation of eye color.

A wing spot test in *Drosophila melanogaster* was also carried out by Koana et al (Koana et al, 1994). It was reported that static magnetic fields of 5 T increased chromosomal recombination 50 %.

**MAGNETIC FIELD EFFECTS IN SPIN CHEMISTRY**

Possible biomagnetic and chemical effects can be expected when biological systems are exposed to both static magnetic fields and other energy such as light and radiation (Ueno and Harada, 1986). Photochemical reactions produced by a radical-pair intermediate in solution can be exposed to show magnetic field effects that arise from an electron Zeeman interaction, electron-nuclear hyperfine interaction, or hyperfine interaction mechanism including an electron-exchange interaction in a radical-pair intermediate. That is, a common mechanism of magnetic field effects on photochemical processes is that a chemical yield of the cage- or escape-product comes to show a magnetic field dependence when a singlet-triplet intersystem crossing can be subject to magnetic perturbations. The magnetic field effects
on photochemical reactions were verified (Hata, 1976, Tanimoto et al. 1976, Shulten, 1976, Nagakura and Molin, 1992).

Chemical reactions in the skin and eyes may have potential biomagnetic effects when the tissues are irradiated by both laser and magnetic fields (Ueno and Harada, 1986). If a process of enzymatic reactions involve a radical-pair, there is a possibility that magnetic fields affect the singlet-triplet conversion rate of radical pairs. Some enzymatic reactions which involve photochemical processes are the candidates for the magnetically-sensitive enzymatic reactions.

The question of whether magnetic fields affect enzymatic activities or not is of considerable interest in biochemistry. To clarify the effect of magnetic fields on enzymatic reactions in living systems, it is important to study the enzymatic reactions that involve radicals which are generated from non-photochemical processes. We examined the possible effects of static magnetic fields on biochemical reactions catalyzed by xanthine oxidase and by catalase.

Haberditzl reported that magnetic fields of up to 6 T enhanced the activity of catalase 4.9 ~ 52% (Harberditzl, 1967). Recently, it has been reported that magnetic fields of 0.10 – 0.15 T affected B12 ethanalamine ammonia lyase (Harkins and Grissom, 1994).

Xanthine oxidase, contained in the liver, lungs, intestine and other organs, catalyzes the degradation of hypoxanthine to xanthine, and xanthine to uric acid, which is the terminal waste of purine nucleotides in mammals. During the oxidation of xanthine, the enzyme releases superoxide anion radicals as intermediates which reduce ferricytochrome c (Fe3+). Superoxide anion, as well as any type of free radical, is also paramagnetic. Reduced cytochrome c (Fe2+) has an absorbance maximum at 550 nm which can be detected by a spectrophotometer. Superoxide dismutase inhibits the reduction of cytochrome c by accelerating the dismutation of superoxide anions to hydrogen peroxide and oxygen, and the catalase converts hydrogen peroxide into water and molecular oxygen.

We observed that magnetic fields of up to 1.0 T did not alter the reduction rate of cytochrome c by superoxide anion which was produced by the reaction catalyzed by xanthine oxidase (Ueno and Harada, 1986). It indicates that neither the electron transfer from xanthine to molecular oxygen nor the transfer from superoxide anion to cytochrome c was affected by the magnetic fields of this range.

Catalase, which is an important heme protein in the body used to decompose toxic hydrogen peroxide, contains a ferric state (Fe3+) iron atom, therefore its magnetic property shows paramagnetism. The activity of catalase can be inhibited by hydrogen cyanide, potassium cyanide and others.

In the case of catalase, the influence of magnetic fields on the activity was determined by the catalytic decomposition of hydrogen peroxide. The rate of disappearance of hydrogen peroxide was followed by observing the rate of decrease in absorbance at 240 nm using a spectrophotometer. Magnetic fields of up to 1.0 T did not alter the rate of decomposition of hydrogen peroxide catalyzed by catalase (Ueno and Harada, 1986). These biochemical reactions were also exposed to gradient magnetic fields of the same intensities as used in the combustion experiments. However, no clear positive effects were observed.

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INTRODUCTION

Considerable controversy has arisen during the past decade over the question of whether public and occupational exposures to electromagnetic fields in the extremely-low-frequency (ELF) range may be linked to adverse health effects, particularly an elevation in the risk of cancer. Since the original publication by Wertheimer and Leeper (1979), which reported an apparent association between childhood cancer risk and proximity of homes to power distribution lines with a high-current configuration, there have been nearly 20 additional reports on this topic. The basic hypothesis resulting from the original study was that the contribution of power-line fields to the ambient 50/60 Hz field level in the home may be linked to cancer risk by some unknown mechanism(s). Although there were numerous technical deficiencies in the methods used in the original Wertheimer and Leeper study, more carefully designed and executed studies in recent years have added some support to the original hypothesis. For example, in a meta-analysis of 13 published studies Washburn et al. (1994) found a statistically significant elevation in the risk of childhood leukemia and nervous tissue tumors among subjects living in close proximity to electricity transmission and distribution equipment. A similar association between residential exposures and elevated cancer risk among adults has not been observed in several independent studies (National Radiological Protection Board [NRPB], 1992).

In addition to the residential cancer studies, more than 50 publications have appeared during the past decade on the subject of occupational exposure to ELF fields and cancer risk. Although the results of these studies exhibit a number of inconsistencies, there does appear to be an elevation in the risk of leukemia and nervous tissue tumors among electrical workers (Theriault, 1990; NRPB, 1992). Limited evidence also suggests an elevated risk of breast cancer among electrical workers (Matanoski et al., 1991; Demers et al., 1991; Tynes et al., 1992; Loomis et al., 1994).

A major problem in the interpretation of these epidemiological findings is the lack of convincing evidence that the underlying cause of the elevated cancer risk is exposure to...
ELF fields from power lines or in the workplace, rather than exposure to some confounding variable or set of variables that have not as yet been identified. In general, ELF field exposures based on short-term measurements have not correlated strongly with cancer risk among the exposed subjects, although a few exceptions have been reported. The issue of confounding variables has also been closely examined in several studies, but even when potential confounders such as chemical exposures have been identified, they have had little impact on the cancer risk estimates based on living in homes close to power lines or working in electrical occupations.

Another problematic issue is the fact that a biologically plausible mechanism has not been identified through which ELF fields at low exposure levels could significantly influence the development or progression of malignant tumors. As discussed below, the strength of ELF fields encountered in occupational or public settings is far too low to directly affect DNA or chromatin structure. For that reason the emphasis in laboratory studies during the past several years has been on determining whether 50/60 Hz field exposures can operate through epigenetic mechanisms to promote the development of tumors from clones of cells previously transformed either by spontaneous mutation or by exposure to carcinogenic agents such as chemicals, ionizing radiation, or oncoviruses.

The results of several recent studies that address the issue of tumor promotion by ELF fields are discussed in this article, along with other factors such as changes in gene expression and endocrine regulation that could influence carcinogenic processes. One aspect of these laboratory-based studies that merits attention is the fact that field levels required to elicit reproducible biological responses are typically larger than, and frequently orders of magnitude greater than, the weak 50/60 Hz fields to which humans are commonly exposed in the home or workplace. These exposure levels are generally in the range of 10 to 50 V/m for the electric field component and 0.1 to 0.3 μT for the magnetic field component, which induce electric fields within the body on the order of 5 μV/m or less. It is a major challenge for both laboratory research and biophysical modeling to elucidate plausible mechanisms by which weak ELF fields could significantly affect biological processes when the signal levels are comparable to, or less than, the intrinsic physical and biological noise present in living systems. Several contemporary hypotheses on the physical and chemical mechanisms through which weak ELF fields could perturb the functioning of living systems are described and critically evaluated in this article.

**PHYSICAL PROPERTIES OF ELF FIELDS**

ELF fields in tissue have a long wavelength (~1000 m at 60 Hz) and skin depth (~150 m at 60 Hz), as a result of which these fields behave as though they are composed of independent, quasistatic electric and magnetic field components (Tenforde, 1991). As a consequence, the radiating properties of ELF fields can be neglected in their interactions with tissue. Another important property of ELF fields is their extremely small energy. For example, the energy of a 60-Hz photon is 2.5 x 10^{-13} eV, which is 11 orders of magnitude smaller than the Boltzmann thermal energy, kT (= 2.7 x 10^{-2} eV at 310 K), and 14 orders of magnitude less than the energy required to break a chemical bond. Substantial laboratory evidence supports the physical expectation that ELF fields do not disrupt the chemical bonds in DNA, proteins or other biological molecules. Direct genotoxic effects of ELF fields leading to cell death, gene mutation, or neoplastic transformation would therefore not be expected, and in fact, have not been observed.

A third important feature of ELF fields applied to living organisms through air is the nonthermal nature of their interactions. The highest field in tissue that can be induced by an ELF field applied through air is about 1 V/m, which leads to a specific energy absorption
rate of $10^4$ W/kg. This rate of energy deposition is four orders of magnitude less than the body's basal metabolic rate and produces a negligible rate of temperature rise (about $3 \times 10^{-8}$ °C/s). The interactions of ELF fields applied to the body through air are therefore of a nonthermal nature.

**BIOLOGICAL EFFECTS AND MEMBRANE INTERACTIONS**

Despite the minimal perturbations of molecular structure by environmental levels of ELF fields, there is abundant evidence for responses to these fields at the tissue and cellular levels (Nair et al., 1989; Adey, 1990a; Anderson, 1991; Tenforde, 1992). For example, small functional changes in excitable tissues and neuroendocrine alterations have been reported in response to ELF fields that induce tissue voltage gradients ≤ 10 mV/m. These alterations include changes in evoked brain potentials (Graham et al., 1990), heart rate (Graham et al., 1990), and nocturnal synthesis of pineal melatonin (Wilson et al., 1981; Lerchl et al., 1990; Kato et al., 1993). In addition, a large number of cellular phenomena, including alterations in growth rate, gene expression and macromolecular synthesis, have been reported to occur in response to fields of moderate to weak intensity (Tenforde, 1992). These effects include alterations in biosynthesis of specific messenger RNA and proteins at field levels below 1 mV/m (Goodman and Henderson, 1991). Finally, there is a rapidly growing body of information that implicates the cell membrane as a primary site of ELF field interactions (Adey, 1990a; Adey, 1990b; Tenforde, 1992; Tenforde and Kaune, 1987). A wide variety of cell membrane structural and functional properties have been reported to be altered in response to ELF fields, including changes in Ca$^{2+}$ binding to anionic fixed charges at the cell surface (e.g., sialic acid residues of membrane glycoproteins), changes in the transport of ions such as Ca$^{2+}$ and the secretion of small solutes such as insulin, and alterations in ligand-receptor interactions that trigger changes in the biosynthetic and functional states of cells. The threshold tissue field levels that lead to such effects appear to vary widely depending upon the end point studied, but in nearly all cases are ≤ 0.1 V/m. In the specific case of field-induced Ca$^{2+}$ desorption from fixed-charge sites on the membrane surface, the effective field level has been reported to be as low as 1-10 µV/m (Bawin and Adey, 1976; Blackman et al., 1985).

The phospholipid bilayer that forms the structural matrix in membranes of living cells is an electrical insulator, and the membrane electrical conductivity is about five orders of magnitude less than that of the extracellular medium or the cytoplasm. As a result, the membrane of a living cell forms an excellent electrical barrier, as well as a superb chemical barrier, that mediates cellular interactions with the external environment. For this reason, it is generally believed that cellular responses to weak ELF fields are initiated by membrane interactions that serve as the primary mechanism of field transduction. Under typical exposure conditions with induced ELF fields in the extracellular medium of ≤ 1 V/m, the "leakage" field in the cell cytoplasm is less than 1 µV/m. This conclusion also holds for the circulating electric fields induced directly in the cytoplasm by magnetic induction. For example, a sinusoidal 60-Hz, 0.1-mT field induces a maximum electric field of 0.2 µV/m in the cytoplasm of a cell with a 10 µm radius. These considerations reinforce the importance of the cell membrane in ELF signal reception and transduction.

Another point to be made regarding the role of cell membranes in ELF signal transduction is the amplification of the extracellular field that occurs across the membrane. By solving Maxwell's equations for the specific case of a dielectric shell (membrane) surrounding a spherical conductor (cytoplasm), it can be easily shown that the electric field across the membrane is greater than that in the extracellular medium by a factor 1.5 R/d, where R is the cell radius and d is the membrane thickness. For a spherical cell with a radius
of 10 µm and a membrane thickness of 5 nm, the field across the membrane is therefore predicted to be 3000 times greater than that in the extracellular medium. A weak environmental field that induces a voltage gradient of 5 µV/m in the extracellular fluid thus produces a field of about 15 mV/m across the cell membrane.

**ELECTRICAL NOISE IN BIOMEMBRANES**

Several physical and biological sources of electrical noise within the cell membrane may impose a lower limit on the strength of an ELF field that can be recognized as a coherent signal (Fishman and Leuchtag, 1990; Weaver and Astumian, 1990; Adair, 1991). The four major sources of electrical noise in biological membranes include: (1) Johnson-Nyquist thermally-generated electrical noise, which produces a 3 µV transmembrane voltage shift at physiological temperatures; (2) 1/f noise associated with ion current flows through membrane channels, which typically produces a 10 µV transmembrane voltage shift; (3) "shot" noise, which results from the discrete nature of ionic charge carriers and is a minor source of membrane electrical noise; and (4) endogenous biological background fields produced by electrically active organs such as the heart, muscles and the nervous system, which can exceed the contribution of physical noise sources by an order of magnitude or more.

Because of these various sources of membrane noise, it is of interest to explore the minimum strength of induced electric fields in tissue that can achieve a signal-to-noise ratio greater than one within the cell membrane. For example, Weaver and Astumian (1990) arrived at an estimate of approximately 0.1 V/m as the smallest applied electric field that exceeds the Johnson-Nyquist noise signal in the membrane of a single cell with an elongated cylindrical geometry (such as a fibroblast, neuron, or muscle cell). Larger threshold field levels, greater than 1 V/m, were predicted for small spherical cells such as lymphocytes. Further increases in the threshold field level are expected if other sources of electrical noise within the cell membrane are taken into account.

These calculations of signal-to-noise ratio consider only the transmembrane electric potential shift introduced by an extracellular field. It has been argued rather convincingly that the field and noise sources that are most relevant to biological signal transduction are those that reside within the highly charged electrical double layer that exists at the cell surface. This double layer, frequently referred to as the Helmholtz-Stern layer, is comprised of fixed anionic charges on the outer membrane surface and diffusible cations in the surrounding medium. As described originally by Debye and Hückel, the average thickness of the diffuse electrical double layer at cell surfaces is approximately 0.8 nm in a physiological medium (Tenforde, 1970). By considering Johnson-Nyquist noise and other sources of electrical noise within the double layer, it can be concluded that the minimum electric field required in the extracellular medium to achieve a signal-to-noise ratio greater than one is about 10 mV/m.

Another important factor to be considered in calculating the threshold field level that exceeds intrinsic electrical noise in biological membranes is the junctional coupling that occurs between cells in organized tissues. For an aggregate of N cells with electrically coupled membranes, the threshold field level required to achieve a signal-to-noise ratio of one is lower than the threshold for single cells by approximately N⁻⁵/₆ as a result of two factors: (1) field amplification due to the larger size of the aggregate, and (2) reduction of membrane voltage noise as a result of the larger capacitance of the aggregate (Weaver and Astumian, 1992). For example, if an aggregate of 10⁹ electrically coupled cells is considered, the threshold field level to achieve a signal-to-noise ratio of one is predicted to be lower by 100,000 than the threshold for a single cell. Because of the junctional coupling exhibited by most biological tissues, the threshold field to achieve membrane and cellular responses may
therefore be on the order of 1 \mu V/m as contrasted to the value of about 0.1 V/m or higher predicted for single cells. A similar conclusion has been reached by Pilla (1993) from an electrical network model of cells that communicate electrically via junctional coupling. The electrical conductivity of gap junctions in cell membranes is, however, lower than that of the extracellular medium. As a result, the electrical coupling and the gain in signal-to-noise ratio for cell aggregates in tissue is probably less than that predicted from relatively simple models.

**BIOLOGICAL SIGNAL TRANSDUCTION PATHWAYS AND POSSIBLE CARCINOGENIC EFFECTS OF ELF FIELDS**

The most singly important issue in understanding the pathways by which weak ELF signals could influence membrane and cellular functions is the elucidation of mechanisms by which these fields are transduced within the cell membrane. One approach that has been taken in addressing this question is to consider the intricate biochemical pathways that have evolved as a mechanism by which a living cell communicates with its extracellular environment. The binding of a single molecule of a mitogenic substance to a specific receptor within the membrane triggers a cascade of events that involve conformational shifts in membrane-associated proteins. These events, in turn, lead to signal transduction and amplification via the production of cytoplasmic second messengers and internal effectors such as free Ca^{2+} and protein phosphorylases (kinases) that regulate DNA transcription and protein biosynthesis (Alkon and Rasmussen, 1988; Luben, 1991). The end result of a single mitogen binding event at the membrane surface is thus a cytoplasmic signal that is amplified to a level that can produce robust effects on macromolecular synthesis and cellular responses involving significant changes in functional and proliferative states.

The interaction of ELF fields with biological membranes could, in principle, lead to alterations in each component of this elegant signaling process that occurs in living cells. A useful working hypothesis is that the pericellular fields and currents induced by an applied ELF field initiate electrochemical events within the cell membrane that are important elements of the primary signal transduction and amplification process (Tenforde, 1993). These biochemically-mediated events then produce cytoplasmic second messenger responses that trigger changes in the biosynthesis of macromolecules and alterations in cellular growth, differentiation, and functional properties. During the past decade, a growing body of experimental evidence has been acquired that supports this general picture of the sequence of events leading to ELF signal transduction and amplification at the cellular level. It has been demonstrated, for example, that pulsed electromagnetic fields (PEMF) with ELF repetition frequencies inhibit the production of cAMP by bone cells in response to the binding of parathyroid hormone (PTH) to surface receptors (Luben et al., 1982). Further studies have shown that the PEMF action leads to inability of the PTH-receptor complex to activate the alpha subunit of G protein, thereby interfering with the sequence of events that activates adenylate cyclase at the cytoplasmic membrane interface (Cain et al., 1987). Other studies using human lymphocytes have shown that exposure to microwave fields with amplitude modulation at ELF frequencies leads to the inhibition of non-cAMP-dependent histone kinases (Byus et al., 1984).

The possible effects of ELF fields on the kinase-C signaling pathway are of particular interest because this pathway is known to be activated by the binding of tumor promoters such as phorbol esters. Activation of this pathway by the binding of a first messenger leads to a cascade of events that produce activated kinase-C and free cytosolic Ca^{2+} ions (Figure 1). Recent studies have demonstrated a significant 70% elevation of kinase-C activity in
Figure 1. Phosphokinase C pathway leading to an elevated intracellular calcium concentration and increased kinase activity. The binding of a mitogen to the membrane receptor triggers a membrane-associated phospholipase C (PLC) hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP$_2$), thereby producing diacylglycerol (DAG), which activates protein kinase C, and inositol 1,4,5-triphosphate (IP$_3$), which mobilizes intracellular Ca$^{2+}$. There is also evidence that the conversion by a kinase of IP$_3$ to IP$_4$ may lead to an influx of free Ca$^{2+}$ and other ions through membrane channels. These ions, together with calmodulin (CAL), lead to further activation of kinases.

Human HL-60 cells exposed to a 50-Hz pulsed magnetic field (Monti et al., 1991). The field effect was considerably damped by adding EGTA, a Ca$^{2+}$ chelator, to the medium. This observation is consistent with previous findings that kinase-C activation relies on the mobilization of Ca$^{2+}$ ions. Another relevant study is the recent finding that stimulation of Ca$^{2+}$ uptake into rat thymocytes by the plant lectin, Concanavalin A (Con-A), is significantly augmented by exposure to a sinusoidal 60-Hz magnetic field (Liburdy, 1992). In lymphoid cells the binding of Con-A to surface receptors triggers cytoplasmic signaling events involved in the kinase-C pathway, including the activation of kinase-C and an elevation of the cytosolic Ca$^{2+}$ concentration. Enhancement of the Con-A effect by a 60-Hz magnetic field was shown to be dependent upon the strength of the electric field induced in the extracellular medium.

Several *in vivo* studies have been conducted to test the hypothesis that ELF fields may act as tumor-promoting agents, possibly through the stimulation of proliferation in transformed cell populations via activation of the kinase-C signaling pathway. These studies have been conducted primarily with skin, mammary, liver, and brain tumor models in rodents. In studies on skin tumor promotion, three studies in mice have shown no promoting
effect of chronic exposure to power-frequency magnetic fields with flux densities ranging from 0.05 to 2 mT on the development of skin papillomas induced by topical application of 10 nanomole of the carcinogen, 7-12-dimethylbenz(a)anthracene (DMBA) (McLean et al., 1991; Rannug et al., 1993a; Rannug et al., 1994). In positive control groups of animals, skin tumor development was observed when the DMBA-initiated mice were treated twice weekly with topical application of the phorbol ester, 12-0-tetradecanoylphorbol-13-acetate (TPA). In one experiment the effect of an intermittently applied 50-Hz magnetic field (15 sec on/15 sec off) was tested for tumor-promoting activity in comparison with a continuously applied field (Rannug et al., 1994). A small increase in the number of skin tumors was observed in the mice exposed to intermittent versus continuous fields, but neither group of animals showed a statistically significant increase relative to control mice treated with DMBA alone. Hence there was no indication of a tumor-promoting effect with either type of magnetic field exposure.

Another in vivo study of particular interest in the context of possible ELF field effects on the kinase-C signaling pathway and tumor promotion is the recent finding by Stuchly et al. (1992) that a 60-Hz, 2-mT magnetic field exerts a copromoting effect on mouse skin carcinogenesis. In these experiments skin tumors were initiated by the topical application of 10 nanomole of DMBA, and were then promoted by the application of 4.9 nanomole of the phorbol ester TPA, once per week for a total of 23 weeks. In the group of mice that received DMBA and TPA plus exposure to the 60-Hz field for 6 h/d, 5 d/week throughout the tumor promotion phase, both the percentage of mice with tumors and the number of tumors per mouse increased more rapidly with time than in the group of mice that received DMBA plus TPA alone. For example, at week 18 the percentage of mice with tumors in these two groups were, respectively, 25% and 8% and the mean number of tumors per mouse were $1.90 \pm 0.69$ (SEM) and $0.65 \pm 0.46$. By week 23 the differences between the mice exposed to the magnetic field and the nonexposed group were no longer statistically significant. Other studies by the same group of investigators had previously shown that a 60-Hz, 2-mT magnetic field acting alone on DMBA-initiated skin cells does not have a tumor-promoting effect (McLean et al., 1991). It would be of considerable interest to conduct further experiments in which comparative measurements are made of kinase-C activity in the affected skin cells throughout the tumor promotion phase in the field-exposed and nonexposed groups of mice. Such experiments would provide a test of the hypothesis that ELF fields may exert a copromoting effect on tumor development through an influence on the kinase-C signaling pathway activated by phorbol esters.

Three studies of mammary cancer development in rodents exposed to ELF magnetic fields following tumor initiation with a chemical initiator have provided some evidence for a tumor-promoting effect of the fields (Beniashvili et al., 1991; Loscher et al., 1993; Mevissen et al., 1993). In the first of these studies, rats were injected intravenously with a 50 mg/kg dose of nitrosomethylurea (NMU), which produced mammary tumors in 54% of the rats (Beniashvili et al., 1991). This percentage increased to 86% in a group of rats initiated with NMU and exposed to a 50-Hz, 0.2-mT magnetic field for 3 h/d for 5 weeks. The latency time to tumor development was also found to decrease significantly in the rats exposed to both NMU and the magnetic field relative to rats that received only NMU. In a second study, rats that were initiated by oral doses of DMBA (20 mg total dose) were exposed for 23 weeks to a 30-mT, 50-Hz magnetic field (Mevissen et al., 1993). Although the overall tumor incidence did not differ significantly between the field-exposed and control groups, there was a statistically significant increase in the number of tumors per rat in the group exposed to the 30-mT field. A third study was conducted with the same protocol as the second study described above, except that a considerably smaller field level of 0.1 mT was used (Loscher et al., 1993). In this experiment the overall mammary tumor incidence was observed to be 50% higher in the field-exposed rats relative to the control group of animals.
Although most of the studies on chemically-initiated mammary tumor development in rats exposed to 50-Hz magnetic fields provided evidence consistent with a tumor-promoting effect of the fields, the findings of increased tumor incidence could also be related to a field-induced suppression of pineal melatonin and a resultant elevation in breast cancer risk (Tamarkin et al., 1981; Stevens et al., 1992). Further research on the effects of ELF fields on the development of chemically-induced mammary tumors in rodents, including parallel measurements of field effects on the level of pineal melatonin in the exposed animals, would provide useful insights into the pathways by which these fields may exert carcinogenic effects.

Two series of experiments have been designed to study the effects of chronic exposure to 50-Hz magnetic fields on the development of chemically-induced liver tumors in partially hepatectomized rats (Rannug et al., 1993b; Rannug et al., 1993c). In both studies liver tumor development was initiated by a 30-mg dose of diethylnitrosamine (DENA) administered intraperitoneally 24 h following partial removal of liver tissue. Tumor development was promoted with phenobarbital as a positive control. The development of transformed foci of liver cells was assayed by histochemical staining for the enzymes γ-glutamyl transpeptidase and glutathione S-transferase. The development of tumor foci in the livers of the chemically-initiated rats was not significantly influenced by a 3-month exposure to 50-Hz magnetic fields with flux densities ranging from 0.5 μT to 500 μT, thus indicating a lack of tumor-promoting effect by the fields. Similarly, no evidence was obtained for a copromoting effect in rats exposed to both phenobarbital and 50-Hz fields following tumor initiation with DENA.

A recently completed study provided evidence that chemically-induced brain tumors in rats are not promoted by exposure to 50-Hz magnetic fields (Brugere et al., submitted for publication). Brain tumors of various histological types, including gliomas and astrocytomas, were induced by intravenous injection of 50 mg/kg ethylnitrosourea (ENU) into female rats on the 19th day of pregnancy. After weaning the offspring were chronically exposed to fields of 1, 10 and 100 μT and studied for survival time. Neither male or female offspring showed a statistically significant difference in mean survival time relative to control rats that were exposed to ENU but not to 50-Hz magnetic fields.

Overall, with the possible exception of mammary tumors, the available evidence is not strong for a promoting or copromoting effect of ELF fields on tumor development in rodents. However, further studies are needed to clarify the possible influence of ELF fields on second messenger signaling and endocrine regulation in exposed animals, both of which are factors that could influence the development of tumors by promoting the proliferation of initiated cells.

**PHYSICAL MECHANISMS OF ELF FIELD INTERACTIONS**

In the search for mechanisms by which extremely weak ELF fields could exert significant biological effects, a number of innovative models have been proposed over the course of the last two decades. One class of models that has received particular attention during the past several years are resonance models that involve the combined action of an ELF field and the static geomagnetic field. For example, ion cyclotron resonance (ICR) was proposed by Liboff (1985) as a possible mechanism that could facilitate the transport of ions such as Ca** through membrane channels in the presence of the geomagnetic field and a weak ELF field tuned to the ICR frequency. Although some data on Ca** uptake by lymphocytes (Liboff et al., 1987) and diatoms (Smith et al., 1987) have been cited as lending support to this model, there are also negative experimental findings (Parkinson and Hanks, 1989; Liboff and Parkinson, 1991; Parkinson and Sulik, 1992). In addition, a number of
physical arguments can be raised against the ICR model (Tenforde, 1992), the most serious of which is the collisional damping of resonant ion motion that is expected to occur in a condensed phase (Halle, 1988).

Two other resonance models that have been proposed recently are the "quantum beats" model of Lednev (1991), in which combined static and ELF fields affect vibrational energy levels and transition probabilities of bound ions (e.g., Ca** ions bound to calmodulin), and the model of Zhadin and Fesenko (1990) in which the rotational energy levels of bound ions are pumped by combined static and ELF fields. Shuvalova et al. (1991) have presented data on the effect of combined fields on the rate of calmodulin-dependent phosphorylation of myosin that appear to support the predictions of the Lednev model. However, a study using an optical technique to study Ca** binding to calmodulin and to metallochromic dyes failed to find any effects of combined static and time-varying fields under the resonance conditions predicted by Lednev's model (Bruckner-Lea et al., 1992). Adair has pointed out that the Lednev model of resonant field effects is improbable because of the long lifetime of the excited vibrational states (about 8 sec), during which de-excitation would occur as a result of collisional damping (Adair, 1992). Similar arguments can be raised against the type of resonant field effects envisioned in the Zhadin and Fesenko model. In addition, the rate of transition of a bound ion to an excited state predicted by this model is so low that a transition would occur only once in several months at typical environmental magnetic field levels.

A large number of nonequilibrium models have been proposed in which field-induced structural and functional perturbations result from membrane interactions that exploit the existence of unstable or metastable states. Examples of such interactions are dissipative instabilities involving cooperative transitions of allosteric membrane proteins in the presence of an applied field. Critical state instabilities in membrane physical properties near the phase transition temperature can also be amplified by an applied electromagnetic field. A host of other models involve coherent field interactions that lead to the excitation of oscillating dipolar modes in membrane proteins or the production of nonlinear oscillations (solitons) that facilitate vibrational energy transfer in macromolecular structures. A number of reviews discussing the physical basis of these nonequilibrium models have been published (Adey, 1981; Taylor, 1981; Postow and Swicord, 1986; Tenforde and Kaune, 1987; Adey, 1990a).

The discovery of biogenic magnetite particles in the tissues of a large number of organisms, including several mammalian species (Kirschvink et al., 1985; Kirschvink, 1989), has led to speculation that oscillatory magnetomechanical forces and torques on these particles could provide a mechanism for the transduction of signals from ELF magnetic fields. Of particular interest is the recent demonstration of magnetite crystals in various anatomic locations within the human brain (Kirschvink, 1992a). Kirschvink et al. (1992b) have proposed a model in which oscillatory magnetic forces on magnetite particles at ELF frequencies are visualized as producing the opening and closing of pressure-sensitive ion channels in membranes. The 60-Hz field level required to overcome the effects of Brownian motion is predicted from this theoretical model to be on the order of 0.1 mT, which is within the range of fields at locations close to the surfaces of several types of household appliances and machine tools.

One difficulty with this model is the sparsity of magnetite crystals relative to the number of cells in brain tissue. For example, human brain tissue is reported to contain a few million magnetite crystals per gram, distributed in 5-10 x 10^5 discrete clusters (Kirschvink, 1992a). The number of cells in brain tissue thus exceeds the number of magnetite crystals by approximately a factor of 100. It is therefore difficult to envision how oscillating magnetomechanical interactions of an ELF field with magnetite crystals could affect a significant number of pressure-sensitive ion channels in the brain. However, the effects of such interactions on neural signaling in localized brain regions could possibly result in a
biological response, although there is no evidence at present to support this hypothesis. The possibility also exists that some subpopulations of cells may have extremely high concentrations of magnetite that experience large forces and torques in a magnetic field of moderate strength. Evidence for such cells was recently obtained in studies on magnetite in human leukemic leukocytes (Kirschvink and Kobayashi-Kirschvink, 1993). Further studies are clearly needed to reveal the biological role of magnetite and the possible mechanisms through which this mineral could play a role in ELF signal transduction.

CONCLUDING REMARKS

Evidence is growing for a central role of cell membranes in the reception, transduction and amplification of signals imposed by ELF fields. A major challenge for the future will be the elucidation of specific molecular pathways through which these fields can influence transmembrane signaling events and affect the functional and proliferative states of cells and organized tissues. Of particular importance will be studies on possible mechanisms through which ELF fields may play a role in the development of tumors. At the present time there is little evidence for a promoting or copromoting effect of ELF magnetic fields on tumor development, with the possible exception of mammary tumors in which endocrine alterations resulting from field exposure may play an important role. Further research is needed to gain an understanding of the ELF signal characteristics that are the most biologically effective, and to define the threshold field parameters above which predictable biological responses occur. Recent laboratory studies have provided a number of clues on the pathways through which ELF fields may operate at the cellular and subcellular levels. However, a great deal of research lies ahead in order to fully characterize the molecular substrates of ELF field interactions and the resultant cascade of electrical and biochemical signals that lead to cellular and tissue responses, including possible carcinogenic effects.

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THE EFFECTS OF ELF ON CHEMICAL REACTION RATES IN BIOLOGICAL SYSTEMS

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ABSTRACT

In this paper we review some of the theory and experiments associated with the effects of electric and magnetic fields at extremely low frequencies, ELF, on chemical reaction rates. The paper proposes a simple model for chemical reactions and shows that the effects of weak electric fields can change the probability that molecules of the reacting materials will encounter each other as well as shift the barrier energy for the reaction. These effects occur primarily in the vicinity of membranes or at boundaries where there are large variations in current density or the concentration of one of the chemical reactants.

INTRODUCTION

The possible effects of weak electric and magnetic fields on biological systems has attracted considerable public attention. This is particularly true after the publication of epidemiological studies which show a correlation between the proximity to power lines and an incidence of cancer. (1-4) If we are to develop a scientific base for deciding whether or not there is any reason for these concerns we are going to have to develop a chain of scientific logic which takes us from the generation of these fields through the coupling of the fields into the body and their interactions with the biological materials. This logical chain is rather long as it starts with either calculations or measurements of the fields generated by the power distribution system and then their coupling into the body. This part of the problem has been treated in a number of reviews with varying degrees of completeness. (5-8). However it is to be noted that if you want to know what the values of the electric field are at a given cell membrane at a given location in the body or at the surface of given organ or gland for a given external electric or magnetic field we have only a very approximate estimate at this time (1994). Having estimated the distribution of the fields in the body we then need to measure or calculate their effect on the biological system. We can start at the level of the forces on
ions, molecules, magnetite particles etc. and work our way up through the complexity chain through induced current flows, changes in spin orientations, shifts in energy level, changes in chemical reaction rates, transmembrane currents, cell growth rates, to changes in the functioning of biological systems and health effects. These changes occur over a wide range of times with different time constants being important for different kinds of effects. At the short end of the spectrum we have atomic and molecular transitions which occur with time constants as short as $10^{-13}$ seconds and effects on growth which show up generations later, which may be days or years. An over all review of these electrically induced effects is given in reference (9) and some of the direct magnetic field effects are covered in other chapters in this volume. In this review we will concentrate on those effects of electrical fields which can be associated with changes in chemical reactions rates. We will start with a brief discussion of how changes in chemical reaction rates effect the production of a given biological substance, followed by a discussion of how electrical fields may effect rate coefficients.

CHEMICAL REACTIONS

Chemical reactions lie at the center of the way biological systems transmit, process and store information. Chemical reactions are the key steps in the growth of cells, in putting energy into a usable form for other biological process and in encoding genetic information. These reactions can be classified in many different ways however, for the purposes of this discussion we will consider only two types of chemical reactions. The first of these is a process where two molecules or atoms in solution react to form a product. The second type of chemical reaction requires the molecule to come in contact with a bound substance. In order for either of these chemical reactions to occur the two molecules must come together with enough energy to over come any barrier that may prevent the reaction from proceeding to a lower energy state or utilize a catalyst. The energy added to an ion in solution by a low level electric field is usually negligible compared to the thermal energy. For example, the average drift velocity of a Na$^+$ ion in a field of $10^5$ V/m is estimated to be $5 \times 10^{-5}$ m/s with a thermal velocity of $4 \times 10^2$ m/s then the kinetic energy added by the drift velocity is on the order of only one part in $10^{14}$. This is truly negligible.

On the other hand, if we look at the effects of the low level electric fields on the transport of the ions, low level electric fields may have a significant role. Consider the case of chemical reaction which takes place in a homogeneous fluid if the chemical reaction has the form of (10,11)

$$ k_i = \frac{k}{(A)^n (B)^m} $$

where $(A)$ and $(B)$ are the concentrations of the two chemical reactants and $k_i$ is the rate constant for $A + B$ to C. Where $n$ and $m$ are the order of the reaction with respect to the species $A$ and $B$, respectively. In order to find the values of $n$ and $m$ one can make the concentration of one of the reactants small so that $k_i$ takes the form

$$ k_i = k (A_0)^n (B)^m $$

$$ k_i = k (B)^m $$
where we have made the concentration of \((A_o)\) large enough so that it is approximately constant, and the changes in the reaction rate can be measured by varying \(B\). Many reaction rate constants follow Arhenius equation \(k\) is given by

\[
k = zpe^{-E/R T}
\]

where \(z\) is the collision frequency for a single ion or molecule, \(p\) is the steric factor which is less than one and reflects the fact that not all collisions occur with the right orientation of the molecules to react. \(E'\) is the activation energy, \(R\) is the gas constant and \(T\) is the absolute temperature. This collision frequency is related to the total collision density \(Z\) which is the total number of collisions of the reactants per unit volume per unit time. For a diffusion controlled reaction (11)

\[
Z = \sigma(8k'T/\pi\mu)^{1/2} N_o^2 (A)(B)^m
\]

\(\mu\) is the reduced mass, and \(N_o\) is Avogadro's number, \(k'\) is Boltzmann's constant, \(\sigma\) is the combined cross section for the atoms or molecules. This collision rate is calculated from the thermal diffusion of the atoms through a solution and results in a reaction rate when \(n = m = 1\) which is given by

\[
k_i = d[A]/d\tau = p \sigma(8k'T/\pi\mu)^{1/2} e^{E'/RT} N_o^2 (A)(B)
\]

The activation energy can take a number of forms depending on the particular reaction and the environment. In a diffusion controlled reaction where the activation energy is not important the reaction rate may be approximated by

\[
k_i = 8RT/3\eta (A)(B)
\]

where \(\eta\) is the viscosity of the fluid. This expression was derived by calculating the current flow of one kind of atoms passed the other and the fraction of them which would have overlapping radii. In this case it is interesting to note the cross section of the atoms has dropped out of the expression except to the extent that it is included in the viscosity.

The number of particles available to collide with each other is modified if the current flow is modified by adding a drift or hydraulic flow velocity to the thermal velocity. If we look at the general problem from the point of view of the conservation of the number of molecules of a given kind in a small volume then

\[
\partial N_i/\partial \tau = -N_i/\tau + \nabla J/q
\]

where \(1/\tau\) is the rate at which the concentration of \(N_i\) is consumed. \(q\) is the charge on \(N_i\) and \(\nabla J\) is the gradient of the current flowing through the volume. For the cases of most interest to us we will assume are atom is charged or is an ion. Suppose we have a uniform current of ions flowing through a transparent membrane then the number of molecules per unit area, \(n_i\), passing through the membrane would be equal to the density of ions, \(N_i\), times the drift velocity, \(v_i\), or

\[
n_i = v_i N_i = J_i q_i
\]

For a uniform low level electric field, \(E\), the drift current density, \(J_i\) is given by

\[
J_i = \Sigma q_i v_i N_i = \Sigma q_i \mu_i /N_i E
\]
where \( q \) is the charge on the ion and \( \mu \) is the mobility of the charged molecule or ion. If we look at a more general case the current density is given by the sum of the drift, diffusion, and hydraulic currents so that

\[ J = \Sigma (q_i \mu_i N_i E - qD \nabla N_i) + q(\nabla P)/R' \]

where \( D \) is the diffusion constant and \( \nabla N_i \) is the concentration gradient, \( \nabla P \) is the pressure gradient, \( R' \) is the hydraulic resistance. The third term is the hydraulic flow. This leads to a complicated expression for \( N \) when \( J \) is substituted into the equation for the conservation of \( N \) as \( \tau \) may also be dependent on \( J \). Note that to get the desired values of \( J \) we need to sum over the component molecules or ions which are involved in the reaction.

In most descriptions of chemical reactions it is assumed that dominate current is due to diffusion, however, in the case where electric fields are imposed on the system the drift current needs to be taken into account. The hydraulic flow term also needs to be taken into account when we are dealing with blood flow. If we have a homogeneous region where the collision probability is independent of position then to first order \( \tau \) is independent of the current density. The added drift velocity in the direction of the field leads to an increase in the collision probability in one direction but this balanced by a reduction on the relative velocity at \( 180^\circ \). Thus modifications of the chemical reaction rate at low fields may come about through the gradient of the current density, \( \nabla J \). If the current density has an AC component then we will get an AC component in \( \nabla J, \partial N/\partial t \) and thus \( N \). The gradients in the current of one of the reactants typically become most important in the vicinity of a barrier such as a curved cell membrane where the effective resistance to the current flow varies rapidly in space as shown in Fig. 1. Thus, we would expect to see the effects of current gradients to be most important when we are looking at current flow around or between cells.

\( \tau \) may also be dependent on \( E \) at high fields levels. In this case the fields may lead to a linear increase in the disassociation rate with electric field at field magnitudes as low as 21 kV/cm (12). Additionally, there may be a modification of the number of bound water molecules associated with each ion or their distribution in space. Thus the effective radius and mobility are modified and in turn the energy barrier for the dissociation of a charged molecule or a chemical reaction.

Next, consider the case of the transport of a charged particle to the surface of a membrane where it either undergoes a chemical reaction or is bound to the membrane surface. In this case the rate of arrival of one of the component for our chemical reaction is determined by the current density. If the total current through the membrane is given by the Goldman equation (13) and there is a dominate ion in establishing the membrane voltage \( V \) such as potassium the current, \( I \), takes the form

\[ I = I_o (\exp qV/k'T - 1) \]

where \( V \) is the potential across the membrane and \( I_o \) is a constant. For DC voltage across the membrane we get an exponentially increasing current in one direction and \( I_o \) in the other. Normally \( I_o \) is small. For a low frequency AC field a power series expansion gives a DC term which is proportional to the square of the applied AC voltage across the membrane and components at the fundamental and second harmonic. This form of response has also been found by Seto and Hsieh (14) for enzyme reactions which are catalyzed at a substrate surface at low field strengths. At higher field strengths these results show a saturation of the reaction rate which is a function of the basic diffusion controlled reaction rate and space charge limitations at the interface. One would also expect limitations associated with transit times at high frequencies.
Another approach to the general problem of the effect of electric fields on chemical reaction rates is to estimate the fields required for the drift current to be equal to the diffusion current and thus the electric field on current in the medium at a distance from the barrier. If we set

\[ E = \frac{D}{\mu'} (\nabla N_i N_f) \]

If we set \( D = 1.5 \times 10^{-5} \text{cm}^2 \text{sec}^{-1} \) and \( \mu' = 5.2 \times 10^{-8} \text{m}^3 \text{V}^{-1} \text{sec}^{-1} \) then

\[ 3.4 \times 10^{-5} E = \nabla N_i / N_f \]

This means that fields of 10V/m which is known to show biological effects on cell growth rates would correspond to doubling the chemical reaction rate due to diffusion with a fractional gradient of 3.4 x 10^{-4}.

Another approach to looking at the collision frequency is to look at the distance the particle travels in a given time \( t \) and to make assumption that the number of collisions with the desired particle A with B is proportional to this distance. The mean squared distance covered by a particle undergoing a random walk is given by

\[ \langle x^2 \rangle = 2 \left( \frac{D t}{\pi} \right)^{1/2} = (2 \kappa' T t / 3 \pi^2 \eta a)^{1/2} \]

where \( D \) is the diffusion coefficient, \( t \) is time, \( \kappa' \) is Boltzmann's constant, \( \eta \) is the viscosity, and \( a \) is the radius of the molecule. If we now add to this random walk a drift velocity which is driven by an applied electric field we will increase the probability of collisions. The extent to which this probability of collision is increased is proportional to the distance traveled or

\[ \langle x^2_d \rangle = v t \]

where \( v \) is the drift velocity and \( \langle x^2_d \rangle \) is the average distance travel under the influence of the electric field, \( E \). At low field strengths \( v = \mu' E \). If we assume the same values for \( D, \mu' \) and \( E \) as above, the time for the drift distance do the electric field to equal the diffusion distance is about 2 hours. This seems to under estimate the effectiveness of the electric field, as shorter exposures on the order 20 minutes are known to show biological effects. This situation would not occur when we have a homogenous solution and the probability of collision is independent of the position.

If we now return to our fundamental equation for the reaction rate, we can vary the energy of the collisions by accelerating the particle through a potential barrier. This in turn will change the reaction rate by providing part of the energy necessary to initiate the reaction.

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**Figure 1.** Current flow around an impervious membrane.
Variations of this problem have also been treated extensively in references (15-25) for low levels of oscillating electric fields. These papers show that small fluctuations in the energy barrier, $qV$, at a membrane or in the height of the barrier inhibiting the chemical reaction lead to changes in the rate constant, $k$. Again a power series expansion gives a small change in the average values of the rate constant, $<k>$, which is proportional to the square of the potential variation.

In the forgoing examples we have assumed plane geometry and that the current is carried by only one type of charged particle. This is unlikely to describe many of the processes of interest which may involve two or more types of charged particles and complex geometries. For example if we have a spherical cell with an impervious membrane in a uniform electric field we would expect the current to flow around the cell with a non uniform current density distribution near its surface as shown in Figure 1.

In this geometry there are stagnation points with zero current flow at the top and bottom and the maximum flow occurs at the sides. This situation is further complicated by the existence of the Debye Layer where the charges are held in a relatively fixed position with respect to the membrane and surface and distance from the membrane where the ions change from fixed layer to a flowing one varies with position. Additionally the space charge layers may be large enough to change the energy of the ions so that reaction rate is modified by adding or subtracting energy to the thermal energy of the ions. This leads to an effective activation energy $E' = E + q(l - \alpha)\phi$ where $q$ is the charge and $\phi$ is the voltage across the bilayer, and $\alpha$ the transfer coefficient. The transfer coefficient takes into account the fact that the site of the chemical reaction may take place at various distances into the space charge layer depending on the site of catalytic protein. This problem has also been treated in reference (11). Other geometries involving a collection of cells lead to more complicated current distributions as a large part of any externally generated field will drive the current between cells and along channels such as blood vessels.

In addition to changes in the collision frequency the electric field may be significant in modifying the orientation of large asymmetric molecules with dipole moments and this in turn may effect the steric factor $p$ in either direction. If the molecules are more favorably aligned by the field to react, it will increase the probability of a favorable collision. Or the fields may orient the molecules in such a direction that the molecules are less likely to react. The force on a dipole is given by

$$F = \alpha' \nabla E$$

where $\nabla E$ is the electric field gradient and $\alpha'$ is the dipole moment. This force tends to align the molecule along the field lines. The degree to which this occurs is on the average proportional to the ratio $\alpha' E/k' T$. This is likely to be most important in the large fields that occur in the vicinity of a membrane, however, to the authors knowledge this effect has not been studied.

It is interesting to speculate on what the effects of chemical concentration modulation at the driving frequency of an applied AC signal or its harmonics may have on biological systems. Both the ECG and EEG have strong periodic components which have biological functions. It is reasonable to assume that externally generated chemical signals which are space and time coherent will be most important at low levels when the frequency of the external field corresponds to a natural frequency of the system. (26). It is also likely to be important when the frequency corresponds to a frequency of a self oscillating chemical reaction. (11)
CONCLUSIONS

For some simple geometries we have shown that chemical reaction rates are functions of the current flow. The effects of low levels of electric and magnetic fields at low frequencies are likely to effect chemical reaction rates by changing the encounter rate of the reacting charged particles rather than their energies. At barriers where the current flow is exponentially related to the applied potential we can expect similar effects on the chemical reaction rates. This situation is likely to apply for catalytic reactions at a membrane surface, the binding of charged ions or proteins to the membrane surface and to the transport of ions such as Ca** through a membrane. The next step in improving our understanding of the effects of low level electric fields would appear to include the improving of our understanding of how small periodic changes in chemical reaction rates effect biological systems. It is likely that the most sensitive systems will be those that involve catalytic reactions and amplification.

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INTRODUCTION

Over the past 15 years, epidemiological studies have raised concerns about possible health risks related to exposures to electromagnetic fields associated with electric power transmission, distribution and use; and to various radio and microwave field exposures in homes and schools, in the workplace, and in the environment.

From the beginning, it has been abundantly clear that energies of tissue components of these fields are extremely weak; so weak in fact, that credibility of epidemiological reports of these sensitivities requires fundamentally new concepts of the energetics of living matter. Here, further laboratory studies will be paramount.

On the other hand, further epidemiological studies appear to hold little prospect of major progress until a metric for tissue dose can be established. Laboratory studies seeking this metric now focus on the physics of atomic processes that occur within the framework of biomolecules.

Over the past decade, remarkable collaborative developments between the physical and biological sciences have moved these apparently disparate disciplines toward a single realm of science. Research on fundamental states of matter, in terms of nonequilibrium states and nonlinear electrodynamics, has gone hand in hand with a new vista incorporating these concepts at the frontiers of cell and molecular biology; and in consequence, there is now in prospect a new definition of living matter in these physical terms.

Beyond the chemistry of molecules that form the exquisite fabric of living tissues, we now discern a new frontier in biological organization, perhaps more difficult to comprehend. It is based on physical processes at the atomic level, rather than in chemical reactions between biomolecules; and as we shall see, these physical processes may powerfully regulate the products of biochemical reactions.
It will be our purpose here to trace some of the major steps that have led to this broad synthesis, which describes, at ever finer levels, an hierarchical sequence of events initiated in biomolecular interactions with environmental EM fields. We will address contributions of scientific research to some of the more pressing issues facing the electric power and communication industries, now and in the future.

MAN IN THE ELECTROMAGNETIC ENVIRONMENT

The Natural Low-Frequency Electromagnetic Environment; Changes Attributable to Use of Electric Power

All life on earth has evolved in a sea of natural low-frequency electromagnetic (EM) fields. They originate in terrestrial and extraterrestrial sources. Thunderstorm activity in equatorial zones of South America and Central Africa produces ELF fields that are ducted worldwide between the earth’s surface and the ionosphere. This ELF activity exhibits a series of peaks, known as the Schumann (1957) resonances, in the spectrum from 8 to 32 Hz. Their electric components have intensities in air around 0.01 V/m; their magnetic fields reach intensities of the order of 1-10 nanotesla (0.01-0.1 milligauss), far weaker than the earth’s static magnetic field around 50,000 nanotesla (500 milligauss). The ever-growing use of electric power over the last century has sharply modified this natural environment in urban areas (Fig. 1).

Exposure to power-frequency fields far stronger than the natural environment is now universal in civilized society. Typically, in U.S homes, ambient field levels may be in the range 0.3-3.0 milligauss (0.03-0.3 microtesla), with substantially higher fields near washing...
machines, refrigerators and air-conditioners. Electric shavers, hair dryers and electric blankets all produce local fields orders of magnitude greater than domestic ambient levels. Indeed, they may exceed environmental field levels produced by electric power transmission and distribution systems, but it should be emphasized that exposure to the former may be for short periods daily, and that they decay rapidly at short distance from the devices, whereas in housing near major power lines, exposure to the latter is likely to be for long daily epochs, typically for many years.

The Biological and Biophysical Dilemma in Reported Tissue Sensitivities to These Low-Level Environmental ELF Electromagnetic Fields

From the physicist's viewpoint, these are extremely weak fields in terms of photon energies necessary to initiate interactions with biomolecular systems. When electromagnetic radiation is described in terms of these photon "bullets", radiation with wavelengths longer than the ultraviolet region of the spectrum (350 nanometers), with photon energies less than about 12 eV (electron volts) has insufficient energy to cause ionization, involving disruption of atoms. The wavelength of 50 Hz fields is 6,000 km.

For biologists, the functional edifice of living matter has rested on heat exchange as the sole vehicle in modeling the sequence of animate processes. We are now faced with a growing realm of biological sensitivities in which tissue heating is not key to the response. We shall discuss this evidence in detail. Although there has been a persisting view in certain areas of the physical sciences that nonionizing EM fields are incapable of inducing bioeffects other than by heating (Foster and Guy, 1986; Foster and Pickard, 1987), evidence to the contrary now creates a growing international consensus (Adey, 1990, 1993; Grundler et al., 1992; Terada et al., 1994).

For both physicists and biologists, there is the central concern that these sensitivities involve stimuli below the level of intrinsic energy of thermal atomic collisions ($kT$), which has been assumed to set a threshold for all biological stimuli. The physical basis for this

![Figure 2](image_url)

**Figure 2.** A comparison of absorbed energy from imposed fields at ELF frequencies and from radiofrequency fields with ELF modulation, in studies where either bioeffects or therapeutic effects were observed. Studies 1-5 refer to therapeutic devices for muscle and joint pains (1 & 2), and to laboratory studies of brain calcium binding and modulation of peripheral vascular mechanisms (3-5). These studies all involved ELF-modulated radiofrequency fields. Studies 6-10 with ELF fields relate to therapy of wound healing (10) and regulation of calcium binding in brain tissue (7-9) and in normal and leukemic lymphocytes. (From Terada et al., 1994).
sensitivity remains incomplete, but as we shall see, theoretical and experimental evidence now points to the role of free radicals, formed briefly for nanosecond periods in the course of all chemical reactions. They offer one mechanism at the microscopic level that transcends this thermal energy barrier in the first transductive step of tissue-field interaction. Free radical sensitivity to magnetic fields may extend all the way down to zero field energy (Grundler et al., 1992; McLauchlan, 1992).

Biomolecular systems exhibit high levels of cooperativity as the basis of responses to athermal ELF fields, and to radiofrequency fields, amplitude-modulated at ELF frequencies (see Adey, 1990, 1993 for reviews). In brief, this cooperativity appears dependent in part on coherent states of populations of electric charges on strands of protein. There are independent reports of biological effects of ELF magnetic fields which show specific dependence on AC/DC magnetic field combinations.

Theoretical and experimental studies discuss low-frequency resonances for DC field-amplitude and AC field-frequency combinations (Liboff, 1985, 1992), as well as window phenomena for AC field-amplitude sensitivities. These pioneering studies have led to a predictive ionic resonance model (Lednev, 1991), based on earlier atomic spectroscopy theory. Further theoretical and experimental studies by Blanchard, Blackman et al. (1994a and b) offer an ion parametric resonance (IPR) model for parallel static and oscillating magnetic fields, with claimed effects on neurite outgrowth as a function of quasiperiodic, resonance-based predictions of the IPR model. Theoretical models of these phenomena share a common problem of competing with thermal and electrical noise inherent in living cells at normal temperatures. A possible problem for these proposed physical models may relate to the nanosecond time scales of thermal collisions, which would tend to disrupt millisecond coherence times essential for resonant interactions with an applied field at frequencies of 1-100 Hz.

MECHANISTIC CONSIDERATIONS AT CELL AND MOLECULAR LEVELS

Tissue Elements in EM Field Detection; the Role of Cell Membranes

Beyond these physical events in first detection of environmental EM fields in tissues, there appears to be a general consensus that the site of field action is at cell membranes. Strands of protein are strategically located on the surface of cells in tissue, where they act as detectors of electrical and chemical messages arriving at cell surfaces, transducing them and transmitting them to the cell interior. The structural basis for this transductive coupling by these protein strands is well known. Through them, cell membranes perform a triple role in signal detection, signal amplification, and signal transduction to the cell interior.

Calcium ions play an essential role at each step in this transmembrane signalling, and have been used as markers of EM field interactions in a variety of tissues and cell cultures. Calcium efflux studies, primarily in brain tissue, revealed sensitivities to ELF fields, and also to ELF-modulated radiofrequency fields (Bawin et al., 1975, 1976; Blackman et al., 1979, 1985 a,b, 1994; Dutta et al., 1984; Lin-Liu and Adey, 1982). In many instances, these responses were windowed with respect to field amplitude, or ELF field frequency (or ELF modulation frequency of radiofrequency fields), or both. More recent calcium influx studies have created a further consensus on the key role of calcium (Fig. 3).

These studies have focused on cells of the immune system (Lyle et al., 1991; Walleczek, 1992; Walleczek and Budinger, 1992). In two independent studies with mitogen-stimulated lymphocytes, exposure to a 3 Hz pulsed magnetic field (in the millitesla range)
Figure 3. Measures of Ca\(^{2+}\)(Mn\(^{2+}\)) uptake in human leukemic Jurkat T-cells chemically activated with a mitogen, and then exposed to a 3Hz 2mT magnetic field for 2 min. Sensitivity to the field was determined by the initial conditions of the cells. With low uptake (20,000-30,000 counts min\(^{-1}\)), there was a 5% decrease in uptake, but at high counts, (above 50,000/min) cells were unresponsive to the field. Results are expressed as means and S.E.M.; pcs/min = photon counts/sec/min. Statistical significance was assessed by the paired t-test and ANOVA. (From Walleczek, 1994).

sharply reduced calcium uptake (Conti et al., 1985a & b; Walleczek, 1992). By contrast, 60 Hz stimulation under the same conditions increased calcium influx (Walleczek and Liburdy, 1990). In two other independent studies, both using 3 Hz pulsed magnetic fields, there was a 55-60% reduction in thymidine uptake into DNA in mitogen-activated lymphocytes (Conti et al., 1985a & b; Mooney et al., 1986). These observations emphasize the importance of the state of activity of cells at initiation of EM field exposure, since cells not already stimulated with mitogens were unresponsive to the magnetic fields.

Inward Signaling at Cell Membranes; EM Fields and an Enzyme Cascade

Modulation of cell surface calcium binding by EM fields is consistent with high cooperativity, since field-induced changes in tissue calcium efflux are far greater than accounted for by the energy of the imposed field. It is thus consistent with a major amplification of the triggering signal. This amplified signal is transmitted along the strands of receptor proteins to the cell interior, where it activates enzymes located at or near cell membranes. Enzymes are protein molecules that function as catalysts, mediating chemical reactions at tissue temperatures that would only occur in their absence at far higher energy levels (e.g., temperature, pressure). They emerge unchanged from these chemical reactions, in which they may thus participate repeatedly.

Amplification of the transmembrane signal continues as it reaches the cell interior. Many signals carried across the membrane by the receptor strand first interact with a class of proteins known as G proteins, so named because they are bound to guanosine triphosphate (GTP). In a further sequence, GTP may stimulate or inhibit the powerful metabolic enzyme adenylate cyclase (ATP). One receptor protein strand may activate many molecules of G protein (Luben, 1990), leading to further amplification of the signal (Fig. 4). Magnetic fields desensitize G protein-linked receptors. Their importance in signaling within cells has been recognized in the Nobel award (Gilman and Rodbell, 1994).

From their first arrival inside the cell, transmembrane signals activate an enzyme cascade. For our purpose, we may limit consideration to a series of enzymes identified as interactive with imposed EM fields. This cascade involves metabolic, messenger and cell growth regulating enzymes.
5 0
5 0

Figure 4. Functional diagram of cell membrane, showing sequence of events associated with modulation of adenylate cyclase (AC) activity via G proteins, modeled on hypothetical interactions of pulsed magnetic fields (72 Hz, 8 gauss peak) with the osteoblast (bone cell) receptor for the parathyroid hormone (PTH) receptor. Osteoblasts exposed to pulsed magnetic fields for as little as 10 min exhibit a persistent desensitization of the effects of PTH on adenylate cyclase (Luben et al., 1982). Studies using biochemical probes of G protein coupling (Cain et al., 1987) indicate that the ability of bound hormone-receptor complex to activate G protein alpha-subunits is impaired by treatment of the bone cell with pulsed magnetic fields. This desensitization of the PTH receptor results in increased synthesis of collagen (a first step in bone formation) by the bone cell, and a decreased resorption of bone by bone-destroying osteoclasts (Cain and Luben, 1987). Both effects would favor increased bone formation in regions exposed therapeutically to pulsed magnetic fields (Bassett et al., 1982).

a. Cell metabolic activity is fueled by adenosine triphosphate (ATP). Breakdown of ATP involves the cell membrane-related enzyme adenylate cyclase. Much evidence of its sensitivity to EM field exposure has come from studies of its role in fracture healing (Luben, 1989, 1990; Luben and Cain, 1984; Luben et al., 1982). Signals from hormones binding to cell surface receptors activate adenylate cyclase. This activation is strongly modulated by a variety of low-frequency magnetic fields (Fig. 5). By removal of phosphate groups, adenylate cyclase converts ATP to cyclic-3',5'-adenosine monophosphate (cAMP), with release of considerable thermal energy. In turn, cAMP continues this enzyme cascade by transferring phosphate groups to a family of messenger enzymes, the protein kinases.

b. Messenger protein kinase enzymes, activated by these membrane-related signals, can move widely through the cell interior, signalling to many structures, including the cell nucleus. Their activity is sensitively modulated by ELF and ELF-modulated radiofrequency fields (Byus et al., 1984; Luben, 1994).
Figure 5. Adenylate cyclase activity in bone cells, stimulated with parathyroid hormone (PTH), with and without an imposed 72 Hz pulsed magnetic field widely used in therapy of ununited bone fractures (from Luben et al., 1982).

c. Cell growth and DNA synthesis involve the enzyme ornithine decarboxylase (ODC) in key regulatory steps. ODC synthesizes polyamines (putrescine, spermidine, spermine) from the amino acid ornithine. The snake-like polyamines have the highest electric charge/mass ratio of any biomolecule. ODC activity is modulated by ELF electric and magnetic fields (Byus et al., 1987; Cain et al., 1993), and by ELF-modulated radiofrequency fields (Byus et al., 1988; Litovitz et al., 1993).

In cell nuclei, polyamines are essential in DNA synthesis. Elevated levels of polyamines inside cells also triggers transcription of the proto-oncogenes c-myc and c-fos by c-ras. Thus, polyamines may participate in a cascade of events leading to communication between membrane-bound and nuclear oncogene products (Tabib and Bachrach, 1994).

Polyamines are also exported to cell surfaces (Byus et al., 1994; Tjandrawinata et al., 1994), where they exercise a quite separate regulatory role (for review, see McBain and Mayer, 1994), acting to either stimulate or inhibit the specific receptor (NMDA receptor) for the amino acid L-glutamate, which contributes to excitatory synaptic transmission at sites throughout the brain and spinal cord. Polyamine stimulatory or inhibitory actions depend on the level of a cell’s membrane potential, and on the specific polyamine molecule.

Thus, there emerges an experimental model, in which electromagnetic fields at athermal levels are first sensed by cell surface receptors, amplified, signalled to the cell interior, and then, through an enzyme cascade, determine production of chemical modulators, some of which are returned to the cell surface as their prime site of action. Signals from this cascade also initiate gene transcription in nuclear DNA (Phillips, 1993).

Outward Signaling through Cell Membranes; EM Fields as Modulators of Cell-To-Cell Communication in Regulation of Cell Growth

Cells in normal tissue may be considered an organized society, “whispering together” in a faint and private language. They communicate with their neighbors through chemical
stimuli, and through electrical fields far weaker than the huge electric barrier of the membrane potential. Gap-junctions, specialized plaques of protein placed between neighbouring cell membranes, provide electrical and chemical coupling between them (Loewenstein, 1981).

They are perforated by numerous tiny tubes (connexons) forming paths through which molecules synthesized in one cell reach the cytoplasm of its neighbour. This metabolic cooperation is essential in the regulation of normal growth (Pitts and Finbow, 1986).

By contrast, "cancer can be regarded as a rebellion in an orderly society of cells. Cancer cells neglect their neighbours and grow autonomously over surrounding normal cells. Since intercellular communication plays an important role in maintaining an orderly society, it must be disturbed during the process of carcinogenesis" (Yamasaki, 1987, 1990). Disruption of gap-junction communication can lead to unregulated cell growth (for review, see Adey, 1992). This may be reversed in cultures of cancer cells, if they make contact with normal cells (Newmark, 1987). Specific viral oncogenes cause differential effects on cell-to-cell communication, in ways that relate to suppression by normal cells of unregulated cancer cell growth, when the two cell types are grown together (Bignami et al., 1988). Introduction of an activated H-ras-1 oncogene into epithelial cells significantly reduces gap-junction communication, possibly from decreased cAMP-dependent phosphorylation (See Inward signaling above) of the principal gap-junction protein.

Experimental evidence supports a role for EM fields in modulation of this gap-junction communication. We shall consider it in the frame of currently accepted models of carcinogenesis and tumour formation.

**Multistage Carcinogenesis: Initiation, Promotion and Progression**

There is a consensus that tumor formation involves at least two steps: an early step of *initiation* and a later *promotion* effect. A single agent may cause both events, or two or more separate agents may be necessary, working together in the proper sequence. The time between exposure to an initiator and appearance of the disease (latent period) is often 20 years or more (Fig. 6).

Initiation involves damage to genetic stores of DNA in cell nuclei, but the changes are not expressed, i.e., a tumour does not result unless one or more promoting agents act repeatedly at a later time. Initiation may be a single event, as in exposure to ionizing radiation from X-rays or atomic explosions, or to certain chemicals, including tar derivatives. Initiated cells are transformed (mutated). They are cancer cells, but may remain quiescent if not stimulated by a promoter.

*Promotion* results from agents having little or no initiating action when tested alone, but markedly enhance tumour yield when applied repeatedly and intermittently following a low dose of an initiator (Slaga et al., 1978; Berridge et al., 1988; Nishizuka, 1984).

![Figure 6](image-url) Model of multistage carcinogenesis from studies in mouse skin. Initiation results from only a single exposure to a carcinogen that appears to damage nuclear DNA. Promotion involves multiple exposures at certain intervals that do not damage DNA directly. Promotion leads to conversion of benign to malignant tumors, with progression increasing the degree of malignancy. (After Weinstein, 1988).
They do not act primarily on nuclear DNA, and many are known to act by binding to receptors at cell membranes (Weinstein, 1988).

**Epigenetic (Non-Genotoxic) Carcinogenesis: Evidence for Electromagnetic Field Actions in Tumor Promotion**

New lines of research in tumor formation reflect identification of a growing number of agents, *tumor promoters*, that appear to play a causal role in human cancer, without direct action on nuclear DNA stores. Pitot and Dragan (1991) have summarized the importance of this approach as a cutting edge in current and future cancer research.

In the *genotoxic*, multistage model of carcinogenesis outlined above, which predicts multiple mechanisms and multiple defined stages of initiation, promotion and progression, transitions between successive stages can be enhanced or inhibited by different types of agents. Development of a fully malignant tumor involves complex interactions between environmental (chemicals, ionizing and non-ionizing radiation, viruses) and endocrine (genetic, hormonal) factors (Weinstein, 1988).

The *epigenetic (non-genotoxic)* model focuses on action of tumor promoting agents, many in primary interactions with membrane-associated receptors (see Section 3). The most widely used chemical promoter in experimental cancer studies is the phorbol ester TPA. With the discovery that TPA activates the membrane-bound messenger enzyme *protein kinase C* (PKC) (Castagna et al., 1982), and subsequent studies indicating that PKC is the major cellular receptor for TPA (Nishizuka, 1983, 1984), a series of bridges now unite research on tumour promotion, growth factors, *signal transduction including the action of EM fields*, and the action of specific oncogenes (Phillips et al., 1992, 1993; Adey, reviews 1990, 1992).

**Experimental Evidence from Cell and Animal Studies Supports a Model of Joint Actions of Chemical Tumor Promoters and EM Fields at Cell Membranes**

*Cell promotion model.* When normal cells (fibroblasts) and their daughter cells, previously mutated with ultraviolet light and behaving as cancer cells with uncontrolled growth, are placed together in cell culture (co-cultures), contact with the parent cells inhibits the unregulated growth. The tumor promoter TPA unbalances this contact equilibrium, allowing return of unregulated growth of mutant daughter cells and formation of tiny tumors (*foci*) in the culture dish.

Exposure of this system to a 60 Hz magnetic field (sinusoidal, 0.1 mT, 1 h exposure 4 times daily) increased by 60% the number of TPA-induced foci (*p < 0.001*) (*Fig. 7*), with an approximate doubling of the size and cell density of the foci (Cain et al., 1993). Fields alone had no effect.

In this system, cells grow to confluence in 10-14 days, and to a mature monolayer in 28 days. Thus, the intermittent exposures occurred while the cells were growing, in the presence of TPA; suggesting that 60 Hz magnetic fields act in conjunction with chemical tumor promoters to enhance development and expression of the cancerous daughter cells scattered amongst normal parent cells. This joint action of chemical promoters and EM fields is known as co-promotion.

*Cancer promotion in a mouse skin model.* A counterpart animal study to the foregoing cell culture study has used mice in an initiation-promotion experiment (McLean et al., 1991). The skin of the back was initiated with a single subthreshold dose of the carcinogen dimethylbenzanthrene (DMBA). The mice were then exposed to a 2 mT 60 Hz magnetic field for 21 weeks, to test whether the field would act as a tumor promoter. No tumors developed in either
Figure 7. Normal fibroblast cells and their daughter mutants were grown together in culture. The parent cells inhibited uncontrolled growth of the mutants. This balance was disturbed if a chemical cancer promoter (TPA) was added in nanomolar concentrations to the co-culture. Foci ("tumors in a dish") reappear. Their number, size and density were approximately doubled in the presence of a 60 Hz, 1 gauss magnetic field. (From Cain et al., 1993).

sham- or field-exposed animals. Two additional groups were then treated weekly with the tumor promoter TPA. The time to tumor appearance was shorter (but not statistically so) in the group exposed to magnetic fields and TPA. This study, which has limitations from small sample size, also suggests reduced immune surveillance by natural killer (NK) cell activity, which would otherwise prevent or retard growth of some tumor cells.

From Cells to Tissues to Organ Systems: Evidence for ELF Field Actions in Immune Responses and Brain Neuroendocrine Mechanisms

Immune surveillance protects the body against infection and the creeping tentacles of cancer, sensing the difference between self and non-self. Cellular immunity, as distinct from humoral immunity mediated by antibodies circulating in the blood, is mediated by lymphocytes in blood and tissues. It includes the natural killer (NK) cells cited above. NK cells are present in most organs, and without prior immunization, can recognize, bind to and destroy (lyse) malignant cells. Lymphocytes may also be targeted against tumor cells, again destroying them by actual contact (alloimmune cytotoxicity). In cell culture studies, this killing capacity was reduced by 60 Hz electric fields (Lyle et al., 1988), and by ELF-modulated radiofrequency fields (Lyle et al., 1983) (Fig. 8).
Cultured human tonsil lymphocytes are also sensitive to ELF-modulated radiofrequency fields, responding with a sharp but transient reduction in messenger protein kinase activity (Byus et al., 1984).

Brain neuroendocrine sensitivities to ELF fields have centered around studies of the pineal gland hormone melatonin. Melatonin is synthesized and secreted with a strong circadian rhythm, reaching a nocturnal peak around 2.0 a.m. in man and animals (Reiter, 1986). The cycle is variably sensitive to the day/night ratio of light exposure in different species. The question of its possible susceptibility to a changing electromagnetic environment has been the subject of intense study (Semm, 1983; Wilson et al., 1981, 1990). Some results of these studies remain unclear within and between species. The most consistent results from magnetic field exposures have been in the use of the Djungarian hamster (Yellon, 1994).

Acute exposures of long-day adult hamsters to a 60 Hz magnetic field (0.1 mT, 15 min) 2 h before lights off suppresses the night-time rise in melatonin in the pineal gland and in the blood. Acute exposures of short-day animals produced similar results (Yellon, 1994). By contrast, daily exposures of the same type for as long as 3 weeks had no effect.

Beyond diurnal activity rhythms, melatonin is key to a broad range of regulatory mechanisms (Reiter, 1992), including the immune system, reducing incidence of certain cancers in mice, and inhibiting growth of breast cancer cells (Hill and Blask, 1988). This inhibitory action of melatonin on human breast cancer cell growth is reported to be blocked by 60 Hz magnetic fields at a 1.2 uT threshold level (Liburdy et al., 1994). Moreover, patients with estrogen receptor-positive breast cancer have lower nocturnal plasma melatonin levels (Tamarkin et al., 1982).

Tissue Detection of Nonionizing EM Fields at Athermal Levels; the Search for the First Transductive Step in Free Radical Mechanisms

We have traced major steps in what has been learned of steps in an energetic hierarchy that couples to the cell interior cell surface signals originating in ELF fields. Our point of
departure has not considered the challenging first step in this sequence: the first detection of EM fields, often orders of magnitude weaker than the averaged thermal energy $kT$ in the sensing biomolecular substrate.

Thermodynamic considerations of tissue thermal energy do not necessarily impose a threshold limit on this primary interaction. Nonthermal states and nonlinear electrodynamics in biological systems offer a number of possibilities (Adey, 1981, 1993; Adey and Lawrence, 1984; Grundler et al., 1992; Frohlich, 1988; Kaiser, 1988; Walleczek, 1994). One answer to this important question may be found in EM field interactions with free radicals.

Free radicals are atoms or molecules with one or more unpaired electrons, which typically make free radicals highly chemically reactive (Walleczek, 1994). In chemical reactions, bonds break and reform. Most chemical bonds between atoms involve paired electrons with opposite spins, with one electron derived from each partner in the union. The spinning electron generates a tiny magnetic field. Chemical bonding occurs between atoms having opposite electron spins, and thus, magnetic fields of opposite polarity.

In a chemical reaction, the bond breaks and each partner reclaims its electron from the bond, moving away to encounter a new partner. It is now an unattached, highly reactive free radical. Reforming a bond requires a meeting between two radicals with opposite electron spins, the union producing a singlet pair. The lifetime of free radicals is typically short, in the range of microseconds to nanoseconds.

It is in this brief period that imposed magnetic fields may alter the rate and amount of product of a chemical reaction. Since the effect is only on the kinetics of chemical reactions, they are known as magnetokinetic effects (Steiner and Ulrich, 1989). They occur only in nonthermal states of biomolecular systems, defined as an insensitivity to random thermal interactions during the brief period of their existence (Walleczek, 1994). They are a consequence of a coherent quantum-mechanical step which accompanies free radical formation. McLaughlan (1992) has proposed a role for free radicals in mediating biomolecular interaction with magnetic fields at 50 and 60 Hz electric power frequencies. In his model (Fig. 9), very low static magnetic fields cause triplet pairs to break and become singlets. At higher levels around 8 mT, two-thirds of the radical pairs may not react as they would in a weaker field, "an enormous effect of a small magnetic field on a chemical reaction, and the effect begins at the lowest applied field strength...The all-important interaction has an energy very much less than the thermal energy of the system, and is effective exclusively through its influence on the kinetics; this is counter-intuitive to most scientists."

Although electron spin energies are conserved through thermal collisions, their short lifetime in relation to thermal collision frequencies has raised unresolved questions about the proposed radical-radical interactions. In a synthesis emphasizing nonthermal interactions of EM fields with cellular systems, Grundler et al. (1992) present models of the sequence of EM field transductive coupling, based on magnetic field-dependent chemical reactions, including cytochrome catalyzed enzymatic reactions that involve transient radical pairs and production of free radicals, such as reactive oxygen and nitric oxide, leading to further highly cooperative amplifications. They conclude that "imposed fields can be active even at intensities near zero." In other words, a threshold might not exist in such a system. There is growing experimental evidence supporting free radical models of magnetic field transduction in biological systems.

More than 20 enzymes are thought to incorporate radical chemistry in the conversion of substrates to products (Harksins and Grissom, 1994). As examples, i) static magnetic fields in the range 0.1-0.15 T decreased the kinetics of the enzyme ethanolamine ammonia lyase by 25 to 60%, consistent with magnetic field-induced changes in crossing rates between singlet and triplet radical states; and ii) membrane-related P-450 cytochrome enzymes are free radical-dependent and can be modulated by the putative P-450 inhibitor econazole.
Figure 9. Basic aspects of the model proposed by McLauchlan to describe interactions of environmental magnetic fields with tissue free radicals. In the hyperfine region, interactions may occur down to zero field energy, and thus substantially below the thermal collision energy of atoms and molecules in the biomolecular system (From McLauchlan, 1992).
This agent completely inhibited (even reversed) increased calcium influx into human T lymphocytes induced by a 60 Hz, 2 mT magnetic field (Walczek et al., 1994).

Nitric oxide (NO) is a powerful free radical, synthesized in cells of many tissues, and as a gaseous molecule, diffusing rapidly beyond the cell of origin. It is an important physiological modulator in brain and spinal cord, and stimulates vascular tissue, mediating penile erection. It has been implicated in the pathology of stress diseases, including coronary artery disease, and in Parkinson's and Alzheimer's diseases of the brain. NO is a modulator of regularly recurring patterns of rhythmic slow electrical waves (EEG) in brain tissue. Imposed magnetic fields (1 Hz, 0.1 mT) disrupt the regularity of these EEG wave bursts through a NO-dependent mechanism (Bawin et al., 1994). Pulsed radiofrequency fields increase NO and cyclic-GMP levels in the rat cerebellum (Miura et al., 1993).

ON THE ISSUE OF CUMULATIVE DOSE; LABORATORY EVIDENCE

In the most general terms, epidemiological data have suggested a relationship between duration of exposure to environmental EM fields, typically measured over years, and the onset of a disease process. Inherently, these long-term exposures are intermittent, whether measured on a diurnal basis or on a longer time scale. However, use of a Time-Weighted-Average (TWA) as an exposure metric has been of limited value in seeking a precise correlate with disease risk, nor have other measures of the electromagnetic environment, such as ambient field levels, established a reliable measure of dose-dependence. Though not yet offering a solution, some laboratory studies may now point the way to future research.

A recurring aspect of laboratory studies has been observations of ON- and OFF-effects. As ON-effects, cell responses to EM field exposure may be transient, even though the exposure is sustained. This was noted in human lymphocyte protein kinase responses to ELF-modulated radiofrequency fields; an initial rapid decrease in activity of 60% within 15-30 min was followed by a return to control levels within an hour, during continuing exposure (Byus et al., 1984).

In bone cells exposed to pulsed 72 Hz magnetic fields used in fracture therapy, Luben et al. (1982) noted that bone cells are desensitized to the effects of parathyroid hormone (PTH) during exposure, but regained sensitivity as an OFF-effect. Luben et al. (1994) have proposed that interference with membrane-mediated signal transduction is a plausible mechanism by which low energy magnetic fields may influence intracellular processes. Their study compared actions of 60 Hz, 0.1 mT sinusoidal magnetic fields with actions of the tumour promoter TPA. Both produced rapid transient increases in the cell membrane of the messenger enzyme protein kinase C (PKC), but reduced activity in the cell cytoplasm. Turning off the field led to a recovery of PKC, as determined by a 4-5 fold increase in total cell PKC activity over a 4 h period; during this exposure period, there was maximum desensitization of the PTH receptor.

In a different time frame, epidemiological studies have raised questions about potential health effects of large magnetic transients and heavy starting loads associated with initial operation of domestic and industrial equipment. Laboratory studies have considered the coherence time during which an EM field must be sustained at a specific ELF frequency, before switching to a different but closely related frequency, in order to initiate an enzyme response. Litovitz et al. (1993) tested ornithine decarboxylase (ODC) responses to ELF components of an EM field that switched to either 55 or 65 Hz. They reported that the
minimum coherence time for this particular response was between 1 and 10 sec to reliably elicit an enzymatic response.

It is clear that these studies are merely pointers to much needed information on an exposure metric for health effects in man. Nevertheless, they emphasize the profound complexity of tissue effects of nonionizing EM fields. Unlike ionizing radiation, where tissue dose may be calculated relatively simply from the product of tissue radiation intensity and duration of exposure, the nonionizing metric must take account of profound tissue effects attributable to intermittency of exposure, and the frequently transient character of the ensuing biological response.

**SUMMARY AND CONCLUSIONS**

Over the past 15 years, there have been emergent concerns that the great and growing use of electric power and radiofrequency communication systems throughout the world, with immeasurable benefits to all mankind, may carry a burden of adverse health effects. In this same era, research to evaluate levels of possible hazards has followed a dual course, with epidemiological and laboratory studies proceeding in parallel, but with few options for coordination.

The scope and content of this laboratory research in bioelectromagnetics has been significantly fettered in scope and content, because the only major sources of its quite meagre funding through government agencies has come from mandates in hazard research.

Despite these encumbrances, bioelectromagnetic research appears to have swept beyond immediate goals in hazard research, to approach a first understanding of the essential nature of living matter in terms of physical processes at the atomic level, far beyond the realm of chemical reactions in a biomolecular fabric.

Laboratory studies have identified cell membranes as the primary tissue site of interaction with environmental electromagnetic fields. They have determined major sequences in the coupling of cell surface signals to a cascade of high energy enzymatic mechanisms inside cells, including mechanisms regulating cell growth. These studies point to joint actions of chemical cancer promoters and EM fields at cell membranes as key steps in tumour formation. The role of free radicals in first detection of EM fields at athermal levels is supported by biophysical models and experimental data.

On the one hand, the importance of this new knowledge emphasizes emergence of bioelectromagnetics as an interdisciplinary field at the frontier of both physical and life sciences, holding prospects for major new advances in understanding functions of the human body in health and disease. On the other, without much further fundamental research, there are few prospects of developing a metric for tissue dose in EM field exposure; and without a metric, further epidemiological studies appear to hold little prospect of major progress.

The last decade has seen a progressive erosion of the normal evaluation of new knowledge by qualified experts in particular fields of medical science and medical practice through peer review and collegial exchange. In its place, there is the troubling specter of corporate lawyers importunate in their pursuit of research still in progress, in espousing a litigant's cause, rather than diligent in legal discovery aimed at establishing scientific credibility. This is exemplified in publicity afforded courtroom distortions of painstakingly acquired knowledge about interactions of the human body with environmental electromagnetic fields. More than 1900 years ago, Tacitus knew well the fate of a Roman society that had abandoned itself to the machinations of lawyers: "If noone paid for lawsuits, there would be less of them! As it is, feuds, charges, malevolence and slander are encouraged. For just as physical illness brings revenue to doctors, so a diseased legal system enriches advocates."
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PHYSICAL MECHANISMS FOR BIOLOGICAL EFFECTS OF LOW FIELD INTENSITY ELF MAGNETIC FIELDS

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This review consists of four parts. In Part One the experimental evidence for biological effects due to low intensity, extremely low frequency (ELF) magnetic fields is reviewed briefly. Part Two is a summary of reasonably well known signal-to-noise calculations showing that several of the observed cellular effects seem to result from "signals" that are below thermal noise. However some limits on the applicability of "standard" thermal noise theory to living systems are pointed out. Part Three describes theoretical approaches which assume that only the electric field, or electric current, can act at the cellular level and imply that time varying magnetic fields are effective only through the electric fields which they induce according to Faraday's law. Part Four, in contrast, describes theoretical models - and some limited experimental evidence in their support - that postulate "direct" action of the applied alternating magnetic field in synergism with the ubiquitous static field.

THE EXPERIMENTAL EVIDENCE

Although there is evidence for biological effects at the tissue and cellular level of very low intensity electric fields, they seem to be influenced by the presence of a static magnetic field. Furthermore, many of the results showing effects on cells or organ cultures by apparently low intensity electric fields were obtained by inducing such fields with time varying magnetic fields. As will be shown below, it is not clear at the present time whether the time varying magnetic field alone or the induced electric field is responsible for reported observations. For the discussion in this paper a "low intensity" magnetic field will be defined as one not much above geomagnetic strength, or weaker, i.e. below about 100 μT (= 1 Gauss). Depending upon the experimental arrangement used, the induced electric field due to a 100 μT ELF magnetic flux density (B) can be extremely small. Since the induced, circumferentially directed electric field in a homogeneous circular cylinder of radius a, with its transverse cross-section perpendicular to the B vector (varying sinusoidally in time at frequency f) is given by...
\[ |E| = \pi f B r \quad r < a \quad (1) \]

one obtains, for example, \( E_{\text{max}} \leq 57 \, \mu \text{V/m} \) when one applies a 100 \( \mu \text{T} \) 60 Hz B-field to a commonly used culture plate with 6 mm diameter wells. Several typical observations are listed in Table 1. It only serves for illustration and is far from being a complete summary of the existing experimental reports.

**SIGNAL-TO-NOISE RATIOS: MINIMUM ELECTRIC FIELD REQUIRED TO EXCEED THERMAL NOISE**

The signal-to-noise ratio problem is usually approached by applying to the cell membrane the Nyquist expression for the thermal noise voltage or current in a resistor (Weaver and Astumian, 1990). Thus

\[ \bar{V}_n^2 = 4kT R \delta f \quad (2) \]

\[ T_n^2 = 4kT (1/R) \delta f \quad (3) \]

where \( k = \text{Boltzman's constant, } T = \text{absolute temperature, } R = \text{resistance and } \delta f = \text{bandwidth}. \) Since the membrane is represented as a resistor in parallel with a capacitor, the steady state voltage across both must be the same and the thermal energy of the resistor must be equal to the energy stored in the capacitor

\[ \frac{1}{2} C \bar{V}_n^2 = \frac{1}{2} kT \quad (4) \]

| Table 1. Reports on *in vitro* biological effects of time varying magnetic fields that could induce only very low intensity electric fields |
|-------------------------------|-----------------|---------|
| **Author and effect**          | **Induced E in V/m (peak)** | **B in \(\mu\text{T}\) (peak)** |
| P. Semm, R.C. Beason (1990): Recordings from ophthalmic nerve of Bobolink, 0.5 Hz +DC. 1 sec exposure [assuming 1 ms rise time, \((\delta B/\delta t) = (2) \times 10^{-4}\)] | \(< (2) 10^{-6}\) | 0.2 |
| Goodman-Henderson (1991): HL 60 Cells. Increased level of RNA transcripts, 60 Hz, 20 min. exposure. | \(10^{-5}\) | 8 |
| D.B. Lyle et al. (1991): T-Lymphocytes, Ca-Uptake, 13.6 Hz +DC. | \(< (0.8) 10^{-5}\) | 20 |
| M.G. Yost, R.P. Liburdy (1992): Rat thymic Lymphocytes, Inhibition of Ca-uptake, 16 Hz +DC, 60 min. exposure. | \((2.4) 10^{-5}\) | 42 |
| S. Mehta et al. (1993): Human Lymphocyte growth, 15 Hz, 60 Hz, 0 DC, 24 hrs. | \(< (1.5) 10^{-5}\) | 86 |
| M. Kato et al. (1993): Melatonin level in rats, Rotating 50 Hz B-field. | \(= (3) 10^{-6}\) | \(\approx 1\) |
| R.P. Liburdy et al. (1993): Modulation of oncostatic action of melatonin on breast cancer cells, 60 Hz B-field, 0 DC. | \(< (4.5) 10^{-6}\) | \(\approx 1.2\) |
and one obtains

\[ \mathcal{P}_{\|}^2 = \frac{kT}{C} \quad \text{or} \quad \delta f = \frac{1}{4RC} = \frac{\sigma_2}{4\varepsilon_2} \quad (5) \]

where \(\sigma_2\) and \(\varepsilon_2\) are, respectively, the conductivity and dielectric permittivity of the membrane. The amplitude of the applied electric field \(E_0\) that is necessary to produce a transmembrane potential equal to \(\sqrt{\mathcal{P}_{\|}^2}\) is then calculated. Assuming a perfectly homogeneous spherical cell of inside radius \(a\), with a perfectly homogeneous cell membrane of thickness \(\Delta\), one obtains for the radially directed electric field inside the cell membrane

\[ E_r = \left( D - \frac{2F}{\rho^2} \right) \cos \theta \]

\[ D = \frac{F}{a^3} \frac{2Y + \sigma}{Y - \sigma} \]

\[ F = \frac{3E_0 \sigma_2 a^3 (Y - \sigma)}{2Y (b^3 - a^3) + Y\sigma (5b^3 + 4a^3) + 2\sigma^2 (b^2 - a^2)} \]

\[ b = a + \Delta \quad (6) \]

where \(Y = \sigma_2 + j\omega\varepsilon_2\) is the generalized conductivity of the membrane (\(\sigma_2 = \) conductivity and \(\varepsilon_2 = \varepsilon_2\varepsilon_0 = \) dielectric permittivity). The conductivities of the intercellular medium and of the cytoplasm are assumed to be both equal to \(s\). The colatitudinal angle \(\psi\) is measured from an axis parallel to the applied electric field \(E_0\). When \(D \ll a\) and \(s \gg |Y|\) equ. (6) reduces to

\[ E_r = \frac{3\alpha}{2\Delta} E_0 \cos \theta \quad (7) \]

A common procedure is to compare the peak value of the transmembrane voltage, \(E_r \Delta\), given by equ.(7), i.e. for \(\psi = 0\), with the noise voltage given by equ. (2) or (5) to find the value of the applied electric field \(E_0\), min that is necessary to give a transmembrane potential just equal to \(\mathcal{P}_{\|}\) or \(I_{\|}\). However it is more appropriate to either average the voltage \((E_r D)\) over the surface of the sphere for comparison with \(\mathcal{P}_{\|}\), or to compare the noise current given by equ. (3) with the signal current obtained by integrating the signal current density \(Y E_r\) over one hemisphere. In either case one must evaluate \(\int_0^\pi d\psi\) and the result is

\[ E_{0,min} \approx \frac{2}{3a^2} \sqrt{\frac{\Delta kT}{\pi\varepsilon}} \quad (8) \]

This result should also be obtained by equating the energy contained in the electric field within the membrane to the thermal noise energy

\[ \frac{1}{2} \epsilon \int E(\theta)^2 d\psi = \frac{1}{2} kT \]

\[ E(\theta)^2 = E_0^2 + E_0^2 \quad (9) \]
where \( E_0 \) is the average value of the field components in the membrane at \( r = b \) and \( r = a^+ \) shown in Table 2. However a slightly different result (smaller by 13\%) is the consequence of using the approximation \( E_0 \ll E_r \) and using not entirely precise approximations for \( C \) or \( R \) in evaluating \( T^r \) and \( T^p \). This alternate expression for \( E_{0,\text{max}} \) is

\[
E_{0,\text{max}} \approx \frac{1}{a^2} \sqrt{\frac{A \Delta T}{3 \pi \varepsilon}} \tag{10}
\]

Using equ. (10) for values of \( a = 10 \, \mu\text{m}, \Delta = 6 \, \text{nm} \) and \( \varepsilon_r \approx 6 \), one obtains

\[
E_{0,\text{max}} \approx 2.3 \, \text{V/m}
\]

while equating the peak value (for \( \cos \theta = 1 \)) of the transmembrane voltage with \( V_\text{m} \) given by equ. (5) would give this value divided by \( \sqrt{3} \) or 1.3 V/m. Neglecting the non-uniform distribution of the electric field along the cell surface and assuming long cells of length = 150 \( \mu\text{m} \) and \( a = 25 \, \mu\text{m} \), Weaver and Astumian (1990) obtain \( E_{0,\text{max}} \approx 8 \times 10^{-2} \, \text{V/m} \). They also point out that if the cell membrane somehow can decrease the bandwidth from that given by equ. (5) (471 Hz when \( \sigma_r = 10^4 \, \text{S/m} \) and \( \varepsilon_r = 6 \)) to 10 Hz, the value of \( E_{0,\text{max}} \) would be reduced by \( 10.471 \) which would give (for the values of \( a, \Delta \) and \( \varepsilon_r \) selected above)

\[
E_{0,\text{max}} \approx 0.34 \, \text{V/m}
\]

Furthermore, since the applied signal is coherent over time \( t \) while the thermal noise is not, the signal-to-noise ratio would improve by a factor \( \sqrt{t} \). However, using Weaver’s and Astumian’s long cell with \( E_{0,\text{max}} = 0.08 \, \text{V/m} \), a 20 minute exposure time (as in the Goodman-Henderson experiments, 1991) would give \( E_{0,\text{max}} \approx 3 \times 10^{-4} \, \text{V/m} \) which is still 30 times larger than the \( 10^{-5} \, \text{V/m} \) reported as effective in several experiments. Of course, if the cell has a mechanism for frequency selection, \( \delta f \) might be as low as 1 Hz, but that would

### Table 2. Electric fields inside and at the surface of thin spherical membrane for field \( E_0 \) applied at \( r >> a \) in a conducting medium

<table>
<thead>
<tr>
<th>( r )</th>
<th>( E )</th>
<th>( \theta E_0 \cos \theta )</th>
<th>( - \theta E_0 \sin \theta )</th>
<th>( z E_0 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( r &gt;&gt; b )</td>
<td>( E = r \frac{3}{2} E_0 \frac{\sigma_r}{\sigma \Delta} \cos \theta )</td>
<td>( - \theta \frac{3}{2} E_0 \sin \theta )</td>
<td>( \frac{3}{2} E_0 )</td>
<td></td>
</tr>
<tr>
<td>( r = b^+ )</td>
<td>( E = r \frac{3}{2} E_0 \frac{\sigma_r}{\sigma \Delta} \cos \theta )</td>
<td>( - \theta \frac{3}{2} E_0 \sin \theta )</td>
<td>( 0 )</td>
<td></td>
</tr>
<tr>
<td>( r = b^- )</td>
<td>( E = r \frac{3}{2} E_0 \frac{\sigma_r}{\Delta} \cos \theta )</td>
<td>( - \theta \frac{3}{2} E_0 \sin \theta )</td>
<td>( 0 )</td>
<td></td>
</tr>
<tr>
<td>( r = a^- )</td>
<td>( E = r \frac{3}{2} E_0 \frac{\sigma_r}{\Delta} \cos \theta )</td>
<td>( - \theta \frac{3}{2} E_0 \sin \theta )</td>
<td>( 0 )</td>
<td></td>
</tr>
<tr>
<td>( r = a^+ )</td>
<td>( E = r \frac{3}{2} E_0 \frac{\sigma_r}{\Delta} \cos \theta )</td>
<td>( - \theta \frac{3}{2} E_0 \sin \theta )</td>
<td>( 0 )</td>
<td></td>
</tr>
</tbody>
</table>

Inner radius = \( a \), outer radius = \( b \), \( \Delta = b - a \), membrane conductivity = \( \sigma_r \), membrane dielectric permittivity = \( \varepsilon_r \), \( Y = \sigma_r + j \omega \varepsilon_r \), conductivity of exterior \( \sigma \), interior \( \sigma \) and \( \varepsilon \) are, respectively, unit vectors in the radial, colatitudinal and vertical directions. These formulas do not take into account the counter-ion layer present on biological cell membranes.
still give $E_0 \approx 10^{-4}$ V/m for the long cell which is not typical of the lymphocytes used in several experiments. (B cells, for example, are nearly spherical with $a \approx 10 \mu m$).

It has also been suggested by Litovitz et al. (1994) that spatial coherence of the applied field, as compared with spatially incoherent noise, may contribute to an improvement of the signal-to-noise ratio (S/N). It is postulated that each of a large number of receptors (Lauffenburger and Linderman, 1993) on a single cell “sees” a different noise field while being subjected to a spatially coherent applied field. Each of the affected receptors would then send the same small chemical “signal” to the cell interior where all the component signals would be added to produce at total response above noise. However equations (2), (3), (9) or (10) take this already into account, at least partially, since the noise energy $(1/2) kT$ was evaluated for the entire cell membrane. We considered the membrane as a single system and evaluated a total resistance or capacitance to compute $\mathcal{P}_0$. From equ. (9) it is apparent that if different parts of the membrane were to be considered as separate, relatively isolated systems of smaller volume than the entire membrane, the S/N ratio would decrease! The $E_0$ required to overcome thermal noise would apparently vary inversely as the square root of the system volume. If patches of the cell membrane of area $\delta A$ were to be better described as separate systems (all of thickness $\Delta$), one would obtain for each patch

$$E_{0, \text{min}} \approx E_{0, \text{min, total}} \sqrt{\frac{A}{\delta A}}$$

if the applied field $E_0$ gave the same transmembrane voltage over the entire cell; but in view of the $\cos \theta$ in equ. (7) any independent patch at the equator would require an infinitely large $E_0$. Considering the fact that the cell membrane is a very poor electrical conductor and is also very inhomogeneous, it may not be unreasonable to model it as several RC combinations connected in parallel, each with its own noise source of energy $kT/2$, and giving a correspondingly lower S/N ratio. These considerations do not imply that the mechanism of “summation by multiple receptors” to improve the total S/N ratio is impossible, but do show that the S/N ratio at each small receptor would be much worse than calculated for the entire membrane.

Since equ. (9) shows that the S/N improves if the volume of the system is increased, it is also worthwhile to consider the outside of the cell surface, and in particular the thin layer where counter-ion motion takes place under the influence of the component, $E_0$, of the applied electric field that is tangential to the surface. For the spherical cell considered above, values of the radial electric field $E_r$ and the tangential electric field $E_{\theta \parallel}$ are listed on Table 2. Both inside the membrane and immediately adjacent to it on the outside (at $r = b^{-}$ or $r = b^{+}$ in Table 2)

$$E_{0, \parallel} = \frac{3}{2} E_0 \sin \theta$$

while the radial electric field immediately outside the membrane is

$$E_{r, \perp} \approx \frac{3 Y a}{2 \sigma \Delta} E_0 \cos \theta$$

For typical values such as $\sigma = 1 \text{ S/m, } |Y| \approx 10^{-7} \text{ S/m, } a = 10^{-4} \text{ m, } \Delta \approx 10^{-8} \text{ m, one obtains } E_{\text{th,b}+} << E_0$, therefore $E_0$ can be used for $E(\theta)$ in equ. (9). The result for the minimum tangential electric field in a counter-ion layer of thickness $\Delta$ necessary to exceed thermal noise is
\[
\tan E_{0, \text{min}} = \frac{1}{\varepsilon_1} \sqrt{\frac{2\pi}{a \cdot \eta d}}
\]

(14)

where \(\varepsilon_1\) is the effective dielectric permittivity of the cell due to its counter-ion layer which can be as high as \(10^7\varepsilon_0\) at ELF. Using this value and \(d = 10^{-9}\) m. one obtains

\[
\tan E_{0, \text{min}} \approx 5.1 \text{ V/m}
\]

which is of the same order of magnitude as the applied field required to generate a transmembrane voltage larger than thermal noise. The appropriate value for \(d\) is not really known and could be larger, but this would still not decrease \(\tan E_{0, \text{min}}\) very much. All the possibilities for improvement by frequency selectivity \((\delta f \ll 1/RC)\) and integration over time discussed above also apply here. Nevertheless, while the action of the tangential electric field may be at least as important as the transmembrane potential in affecting, for example, chemical receptors on the cell surface, or cell-to-cell communication (Polk, 1992), the S/N ratio based on the assumption of a thermal noise limit is probably not much different in the two cases.

Mathematical models may, or may not be good representations of physical reality. It is important to realize that the cell membrane, as well as the cell interior and exterior environment, is extremely inhomogeneous and therefore field expressions (7), (12) or (13) are at best very crude approximations. Even more important is the assumption of a thermal noise limit embodied in equs. (2) to (5). The \((1/2)kT\) thermal energy per degree of freedom, i.e. per variable necessary to describe the state of the system, requires that the "device" (which may be a cell or cell membrane) does not have a steady input of power. Such an input - which may be chemical in nature - could produce ordered ion motion that is not small in comparison with the pre-existing random motion. Chemically initiated spatial and temporal oscillations of calcium ion flux, for example, have been observed (Berridge and Gallione, 1988; Jacob, 1990) and been modeled in terms of non-linear dynamics (Eichwald and Kaiser, 1993; Myer and Stryer, 1988). Applied oscillating fields could very well couple to such oscillations. "As soon as one introduces an active device, such as a biased diode or a transistor, the whole argument in terms of thermodynamic equilibrium collapses so that one must examine the device in terms of internal mechanisms rather than as a black box" (Bell, 1985). This statement, written in relation to analysis of electronic devices, applies equally to living cells. The thermal noise equations assume completely random motion of charges and would not be valid if many or most charges are subject to organizing forces in some preferred direction. All of this must be considered in the search for mechanisms that can be responsible for biological effects of electric and magnetic fields.

**PROPOSED MECHANISMS FOR ELECTRIC FIELD COUPLING AT THE CELLULAR LEVEL**

Biological effects of electric fields are well known and at higher field intensity levels - above thermal noise - they are also reasonably well, although not completely, understood. The discussion here is concerned only with the "low level" extremely low frequency (ELF) effects, for which examples were given on Table 1, and which are identified as "subtle 'long term' effects" in Table 3 that summarizes the field amplitudes responsible for different types of biological action.

Reviews are available where some possible physical mechanisms for the production of biological effects by low intensity electric fields are discussed in some detail (Grundtler et al., 1992; Polk, 1992). Mechanisms that have been proposed are listed below in Table 4.
Table 3. Amplitudes required for ELF and pulsed electric field effects

<table>
<thead>
<tr>
<th>Effect</th>
<th>V/m Inside Tissue or Fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electroporation</td>
<td>$10^5$</td>
</tr>
<tr>
<td>Depends on E ($\Delta t$)</td>
<td>$10^4$</td>
</tr>
<tr>
<td>Cell Rotation in Insulating Fluid</td>
<td>$10^3$</td>
</tr>
<tr>
<td>Nerve/Muscle Stimulation</td>
<td>$10$</td>
</tr>
<tr>
<td>Subtle “Long Term” (t &gt; 10 min) Effects</td>
<td>$10^{-3}$ to $10^{-4}$</td>
</tr>
</tbody>
</table>

All of these mechanisms must be based on interaction of electric fields $E$ with charges $q$ (which will be mostly ions in the biological environment) or electric dipoles $p$. The pertinent equations are

$$F = qE$$  \hspace{1cm} (15)

for the force on a single charge, and

$$F = (p \cdot V)E$$ \hspace{1cm} (16)

$$T = p \times E$$ \hspace{1cm} (17)

for, respectively, the force and torque on electric dipoles. As shown by equ. (16), translatory motion of dipoles (as in “dielectrophoresis” - see Pohl, 1978) requires a field gradient, while a uniform field is sufficient to set an electric charge into motion. Within a fluid medium the motion of charges and dipoles at velocity $v$ will be opposed by viscous forces given by Stokes' law for linear motion when the (spherical) particle (of radius $a$) is large in comparison with any “granularity” in the fluid

$$F = 6\pi a \eta v$$ \hspace{1cm} (18)

where $\eta$ is the fluid viscosity. Possible attachment of charged particles to structures (e.g. protein or nucleotide molecules) giving electric forces will quickly lead to non-linear differential equations for the resulting motion. In addition, conservation of charge will

Table 4. Proposed physical mechanisms for interaction of low intensity ELF electric fields with living organisms

<table>
<thead>
<tr>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Improvement of S/N ratio for time varying magnetic field by interconnection through “passive” gap junctions (Polk, 1992; Pilla et al., 1991).</td>
</tr>
<tr>
<td>2. Effect of non-linear gap junctions (Gailey, 1993).</td>
</tr>
<tr>
<td>3. Response of non-linear and “chaotic” systems to small inputs (Grundler et al., 1992; Ruelle, 1991; Frohlich, 1986; Baeriswyl et al., 1987).</td>
</tr>
<tr>
<td>4. Effects due to electric polarization forces (K. McLeod, 1993a).</td>
</tr>
<tr>
<td>5. Interaction with endogenous Ca oscillations (Grundler et al., 1992; Wacl cez, 1993).</td>
</tr>
</tbody>
</table>
always have to be satisfied, expressed by the relation between current density and volume charge density $\rho$

$$\nabla \cdot \mathbf{J} = -\frac{\partial \rho}{\partial t} \quad (19)$$

where the total current density $\mathbf{J}$ is not only due to the applied electric field, but will in general also be due to non-uniform charge concentrations brought about by chemical processes and leading to a diffusion current density $\mathbf{J}_d$ given by

$$\mathbf{J}_d = -q D V C \quad (20)$$

where $C$ is the ion concentration per unit volume and $D$ is the diffusion constant given by

$$D = u k T \quad (21)$$

with $u$ being the hydrodynamic mobility $= (\text{velocity/force}) = (\text{electric mobility}/q)$.

A schematic diagram of the non-linear processes characteristic for many cells is illustrated by Fig. 1 (Kaiser, 1994; Eichwald and Kaiser, 1993). In such non-linear systems transitions occur between different stable states, e.g. from oscillation to no oscillation, or from oscillation at one frequency to oscillation at a different frequency. In the presence of noise the phenomenon of “stochastic resonance” may then become important (McNamara and Wiesenfeld, 1989; Moss, 1991; Benzi et al., 1982) when an exogenous periodic driving force, such as a weak ELF electric or magnetic field, is applied. If a bistable or multistable nonlinear system is driven by random fluctuations (“noise”) as well as by a coherent signal, the output, i.e. the transition between states, involves interaction between noise and input signal. As a consequence the ratio of the output signal (which, in general, can contain different frequencies than the input signal) to the output noise can become much larger than the input signal-to-noise (SNR). In fact, as illustrated by Fig. 2, the output SNR for a fixed input signal first increases with increasing input noise before it eventually decreases. As pointed out by Kaiser (1993), stochastic resonance may very well provide a satisfactory explanation of observed biological effects in response to weak applied electric or magnetic fields, and an experimental example has recently been described (Douglass et al., 1993). Since biological entities - cells as well as entire organs or animals - represent extremely complex interconnected nonlinear systems, it is likely that exact field-to-chemical reaction or field-to-biological process transduction mechanisms will not be the same in different systems or under different circumstances. Therefore several of the mechanisms which have been suggested in the past (Table 4) and possibly others, may represent the first step in the weak coherent excitation needed for stochastic resonance. However, the fundamental physical laws summarized by equ. (15) to (21) will always be involved, albeit in their combination leading most likely
to non-linear differential equations with extreme sensitivity to initial or boundary conditions.

Although involving time varying magnetic fields $B$, another set of circumstances should be mentioned which would allow cells or protein molecules to differentiate between an electric field that is induced by a time varying magnetic field according to Faraday’s law

$$\int E \cdot dl = - \frac{\partial}{\partial t} \int B \cdot ds$$

(22)

and the electric fields of random orientation due to thermal noise or even larger biological electric “noise” generated by nerve and muscle action. Since these noise fields are due to relatively slow charge motion, they are effectively quasi-electrostatic fields $E_s$ for which

$$\nabla \times E_s = 0$$

(23)

On the other hand for the electric fields induced according to Faraday’s law the curl, or circulation per unit area, is always non-zero

$$\nabla \times E = - \frac{\partial}{\partial t} B$$

(24)

Since most protein molecules, and notably cell surface receptors, as well as DNA and RNA molecules, are of spiral shapes, the latter electric fields ($E$) may be particularly effective in influencing charges or dipoles located on such molecules. Further consideration of the laws governing electric fields characterized by non-zero curl in “chiral media” (Lakhtakia and Varadan, 1989) is likely to be useful. In any case, the paths of currents in biological systems will be very different depending upon whether the imposed ELF electric fields are due to a primary source that generates an electric field or are induced by a time varying magnetic field (Polk, 1992; Stuchly, 1994).
PROPOSED MECHANISMS FOR ELF MAGNETIC FIELD COUPLING AT THE CELLULAR LEVEL

Epidemiological findings that suggest the possibility of health effects as a result of power frequency field exposure (for example, Feychtling and Ahlbom, 1993) implicate weak magnetic rather than electric fields. Several of the biological effects of ELF fields listed on Table 1, as well as others, exhibit frequency “windows” - that is effects at some frequencies, but not at others. In general the observed effects do not become stronger as the applied frequency is increased (Liboff et al., 1983) which might be expected in view of equ. (1) if they were linearly related to a magnetically induced electric field. Equ. (1) follows from equ. (22).

To test whether some cellular effects are due to magnetic or induced electric fields, a few experiments were performed with exposure dishes that are divided by barriers into several concentric rings (Misakian and Kaune, 1990). When subjected to an axially oriented, uniform ELF magnetic field, cells in different rings are then exposed to the same magnetic, but different electric fields given by equ. (1). Such experiments have shown that at least some biological consequences of ELF magnetic field exposure were not due to the induced electric field (Liburdy et al., 1993; Blackman et al., 1994).

In this context it is useful to point out that the generation of an electric field by a time varying ELF magnetic field is an extremely inefficient way of converting magnetic to electric energy. If a magnetic flux density $B_m$ within a cylindrical volume of radius $a$ contains an energy $(1/2) [B_m^2/\mu] [\text{volume}]$ and a magnetic flux density $B_v$ induces an electric field $E$ so that $(1/2)E^2$ integrated over the same volume is equal to the energy corresponding to $B_v$, it is easily shown, using equation (1), that the ratio $B_v/B_m$ is given by

$$\frac{B_v}{B_m} = \frac{2}{a \pi f \sqrt{\mu_0 \varepsilon}}$$

where $\mu_0$ is the permeability of free space. For a radius, $a$, of 3 cm one obtains at 60 Hz values of $10^6$ or $10^7$ depending upon whether one assumes for the dielectric permittivity, $\varepsilon$, that of free space ($\varepsilon_0$) or that of muscle tissue at ELF ($\varepsilon \approx 10^6 \varepsilon_0$). If biological experimentation shows that effects are produced by very weak time varying magnetic fields, it is therefore clearly important to consider all possible mechanisms for direct magnetic field-tissue interaction and not to assume a priori that biological effects can only be due to the induced electric fields. Mechanisms proposed thus far are listed on Table 5.

<table>
<thead>
<tr>
<th>Table 5. Mechanisms proposed for “direct” effects of ELF magnetic field effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>3. Nuclear magnetic resonance (Blackman et al., 1989; Polk, 1992).</td>
</tr>
</tbody>
</table>
Cyclotron Resonance

Several experiments involving ELF magnetic field exposure of cells (Yost and Liburdy, 1992; Blackman et al., 1994), marine diatoms (Smith et al., 1987) and rodents (Thomas et al., 1986) showed a distinct frequency dependence which depended upon the amplitude of a DC magnetic field that was applied simultaneously and in parallel orientation with the AC field. Although the attempt to repeat the diatom experiment in another laboratory was not successful (Parkinson, 1992), there is still a need to explain the other results which did appear to give a DC dependent “resonance”. Liboff (1985) and McLeod and Liboff (1987) proposed cyclotron resonance in transmembrane channels. Cyclotron resonance of a freely moving charge is due to the Lorentz force $F$ exerted on a charge $q$ which moves at a velocity $v$ through a DC magnetic field $B_0$.

$$ F = q v \times B_0 \quad (26) $$

If a sinusoidally alternating magnetic field $B_1$ is applied in parallel with $B_0$, the electric field induced according to Faraday’s law will cause the charge to gain energy (from the electric field) as it moves in circular orbits of increasing radius, provided the frequency of $B_1$ is given by

$$ f_c = \frac{qB_0}{2\pi m} \quad (27) $$

or an integral multiple thereof with $m$ being the mass of the ion. If the charged particle is located within a viscous medium, ion-ion and ion-neutral collisions will prevent progression to larger orbits and, as shown by Durney et al. (1988), resonance effects will not occur unless the effective collision frequency is smaller than the frequency of the applied field. Since ions in living organisms are almost always located within a fluid, viscous medium that gives high collision frequencies, the simple cyclotron mechanism does not provide a satisfactory explanation of the observed phenomena. In addition, ions in living organisms are likely to be hydrated so that $m$ in eqn. (27) will have to include the mass of the attached water molecules. Eqn. (27) will then give different values depending upon the size of the hydration sheath.

Ion Parametric Resonance

The “parametric resonance” model, as proposed by Lednev (1991, 1993), postulates Zeeman splitting by a DC magnetic field $B_0$ of infrared ($\approx 10^{12}$ Hz) vibrational modes of an ion. The mode separation will be at the ion cyclotron frequency, given by eqn. (27), and will be in the power frequency range for several physiologically important ions when $B_0$ is of the order of magnitude of the geomagnetic field. DC amplitude-resonance frequency combinations for some ions are shown on Table 6.

Following earlier work by Podgoretskii and Krustalev (1964), Lednev predicts that application of an alternating magnetic field $B_1 \cos(\Omega_c t)$ should modify the time average $\tilde{p}$ of the transition probability $p$ from an excited vibrational state to the groundstate as given by

$$ \tilde{p} = K_1 + (-1)^n K_2 J_n \left( \frac{nB_1}{B_0} \right) \quad (28) $$

The constants $K_1$ and $K_2$ are related by $K_1 =$ and $K_2 =$ which depend upon the original vibration amplitudes and $\Omega_c = 2\pi f_c$. $J_n$ is the $n$'th order Bessel function and $n$ is an integral
fraction of the resonance frequency $f_c$. Thus the $J_n$ term would indicate a dependence of $\tilde{\rho}$ upon the ratio of AC to DC field amplitudes, either at the frequency $f_c$ (when $n = 1$) or at its subharmonics ($n > 1$). Expression (28) is the time average of the transition probability evaluated over a complete period of the applied field; the instantaneous value of $p$ is given by

$$p = K_1 + K_2 \cos \left[ \Omega t + \left( \frac{n B_b}{B_0} \right) \sin \left( \Omega \frac{t}{n} \right) \right]$$

The principal objections raised by Adair (1992) to the validity of the theory are the requirement that the vibrating ion not be hydrated if $m$ in equ. (27) is to be known, and that the residence time of the ion in the excited vibrational state must be at least as long as the period of the applied oscillating field. Lednev suggested that the calcium ion within the calmodulin complex may be sufficiently shielded from the aqueous environment to guarantee adequate lifetime. Within the calmodulin complex the Ca-ion would also not be hydrated. Lednev furthermore assumed that the change in Ca** transition probability would somehow affect the ability of the ion to participate in biologically important chemical reactions.

Recently Blackman et al. (1994) subjected “PC-12” cells to combinations of AC/DC magnetic fields and found that the number of cultured cells with neurite outgrowths decreased (in comparison with unexposed cells) as a function of the AC/DC amplitude. However that behavior was restricted to narrow frequency bands determined by the amplitude of the applied DC magnetic field, suggesting a “resonance” behavior. They also were able to fit their data to a Bessel function as indicated by Fig. 3. However they assumed a dependence

$$\tilde{\rho} = K_1 + K_2 (-1)^n J_n \left( 2nB_b/B_0 \right)$$

where the factor of (2) in the argument of the Bessel function accounts for a dependence on the $B_b/B_0$ ratio which differs substantially from that predicted by Lednev’s equ. (28) as illustrated by Fig. 4. Blanchard and Blackman (1994) presented a modification of the original Lednev theory which leads to equ. (30). It can be shown however (Polk and Wu, 1994; Engstrom, 1994, 1995) that the factor of 2 is incompatible with the basic assumption of Zeeman splitting. As indicated on Fig. 3 the data presented by Blackman et al. (1994) also fit the $J_n(4B_b/B_0)$ function over the range of $(B_b/B_0)$ shown in their paper. Since the order $n$ of $J_n$ in equ. (28) must be a positive integer, the $J_0(4B_b/B_0)$ function likewise does not serve to support the theory as expressed by equ. (28). However the $J_0$ result could conceivably be a consequence of equ. (29) in the presence of collision damping or of several closely spaced vibrational modes since the second term in that equation can be expressed as a series of Bessel functions including $J_0(nB_b/B_0)$. Blackman et al. (1994) indicate by fairly elaborate curve fitting to match their data with equ. (30) that the results support resonances or “windows” corresponding to ions of vanadium, magnesium, manganese and hydrogen. In view of the uncertainties involved, their conclusion appears to be premature. The authors
**Figure 3.** Fit of neurite outgrowth data to $J_1(2s)$ and $J_0(4s)$ functions where $s = (B_1/B_0)$. Based on experimental data (— — —) shown in Blackman et al. (1994).

**Figure 4.** Functions $-J_1(s)$ and $-J_1(2s)$ appearing in equations (28) and (30): Lednev vs. Blanchard-Blackman dependence on the $s = (B_1/B_0)$ ratio at the first (principal) resonance.
recognize that "additional work is needed to test the agreement between experimental results and theoretical predictions over an extended range of $B_{\text{AC}}$" and that "exposure conditions should also consider single ion resonances, beginning with hydrogen at $n = 1$, to establish what ions in key biological test systems respond consistently with the IPR model". In this context a quantum mechanics based analysis of the model, including numerical calculations, by Engstrom (1994, 1995) may help to resolve many remaining questions concerning the validity of the model. The results obtained by Blackman et al. nevertheless confirm the importance of considering in ELF exposure experiments the relative orientations, as well as magnitudes of DC and AC magnetic fields, the applied AC frequency and the charge to mass ratio of either ions or charge groups that are biologically important in the particular system under investigation.

**Nuclear Magnetic Resonance**

Many, but not all, atomic nuclei rotate about their axis. The "spin" angular momentum quantum nuclear $I$ can have integral or half-integral values between $-12.5$ and $+12.5$ (Weast et al., 1986). Since nuclei also carry an electric charge, rotation about their axis produces a magnetic moment $\mu$ and in the presence of a static magnetic field $B_0$ a torque

$$T = \mu \times B_0 \tag{31}$$

will tend to orient the spin axis along preferred directions. Transitions between discrete orientations correspond to changes in energy

$$\Delta W = \frac{\mu B_0}{I} \tag{32}$$

Transfer from one discrete energy level to the next higher one requires irradiation by a signal of the nuclear precession frequency $f_n$ such that

$$\Delta W = f_n h \tag{33}$$

where $h$ is Planck's constant. From equ. (32) and (33) it follows that

$$f_n = \frac{\mu B_0}{hf} \tag{34}$$

In an earlier review (Polk, 1992) it was pointed out that NMR as a possible mechanism for biological effects of low intensity alternating magnetic fields, in the presence of the earth's static field, has both very attractive and very unattractive features. First, the characteristic precession frequencies, $f_n$, are below 1,000 Hz for many biologically important elements at earth strength fields. Thus, with $B_0 = 50 \, \mu T$, $f_n$ is equal to 827 Hz for $^7\text{Li}$, 653 Hz for $^{23}\text{Na}$, and 99.3 Hz for $^{39}\text{K}$. Secondly, stability of the resonance lines (or "high Q" of the resonance peaks), reflected in the relatively long relaxation times, should make it possible to observe resonance excitation by weak alternating magnetic fields of the appropriate frequencies.

Unattractive features, which make NMR unlikely as an interaction mechanism are, first, the small probability of spin alignment in a weak applied field. Thus the ratio $R$, of the maximum energy of $\mu$ in a field $B_0$ to the thermal energy is

$$R = \frac{\mu B_0}{kT} \tag{35}$$
For the hydrogen nucleus, which is employed in medical diagnosis at fields of the order of 1 T, R = 3.3 (10⁻⁶) while R = 3.8 (10⁻¹⁰) for ³Li at 100 µT. Whether this difference by four orders of magnitude makes biological effects of NMR at such levels of magnetic field completely impossible is still debatable. Effects could only occur if the resonance phenomenon would influence extremely critical processes - such as enzyme mediated reactions - where the effect of a very minute change can be greatly amplified. It is also questionable how a change in spin state could significantly influence chemical structure - although the reverse is true, as indicated by the spin-lattice and spin-spin relaxation phenomena (Ando and Webb, 1983) that make NMR useful for medical diagnosis. The spin-spin relaxation phenomenon, in particular, is an indication that changes in the spin state of one nucleus do affect its surroundings. However strong interaction with the nuclear environment, such as by ¹⁴N nuclei which have an electric quadrupole moment (Haken and Wolf, 1984), would lead to significant line broadening (Paudler, 1971) and thereby to the loss of observable frequency sensitivity at ELF.

NMR has been suggested as an explanation of relatively low field intensity effects, notably changes in dielectrophoretic yield and dielectric permittivity, that were observed at a few kHz (Aarholm et al., 1988). NMR has also been mentioned as a possible explanation of field effects on the efflux of radio-active calcium ions (⁴⁰Ca⁺) from chick brain tissues (Blackman et al., 1988). The NMR phenomenon is attractive in the latter context, because the experiments showed sensitivity to steady and alternating magnetic fields which were mutually perpendicular, as required for NMR excitation. However, it should be noted that the effect would only occur with radioactive Ca, and not with the non-radioactive ⁴⁰Ca isotope, which accounts for 97 percent of all Ca occurring in nature. ⁴⁰Ca has zero nuclear spin and therefore no magnetic moment. (Some other biologically important elements with zero spin are ¹²C, ¹⁶O, ⁵⁶Fe, ²⁴Mg and ³¹S.) One way of differentiating between an NMR effect and any type of whole ion-resonance involving Ca, would be to repeat experiments, which require a radioactive isotope, with both ⁴²Ca and ⁴⁵Ca. These isotopes have the same spin (−7/2) and practically the same μ (−1.3172 and −1.316 in nuclear magneton units) and therefore the same NMR frequencies fₐ. However the ionic masses are sufficiently different (43/45) to give a 4.5 percent change in the cyclotron resonance frequency fₑ given by equ. (27).

**Magnetic Field Effects on Free Radical Reactions**

Free radicals are important in many biological processes (Freifelder, 1982). For example, free radicals are formed as intermediate products when light is incident upon the visual pigment Rhodopsin. Chemical reactions that involve free radicals are strongly influenced by static magnetic fields. This has been known for some time (Hoff et al., 1977; Blankenship et al., 1977; Werner et al., 1978). However, more recent work (Hamilton et al., 1988; McLauchlan, 1989) suggests that DC fields as low as 10 Gauss may affect such chemical processes. By implication, as will be shown below, alternating fields of the same order of magnitude, acting in concert with steady fields should also influence reaction yields.

Several observations in this area are based on Pauli's exclusion principle, which states that the electronic states of an atom can only be occupied in such a way that no two electrons have exactly the same set of quantum numbers. Thus if there are, for example, two valence electrons in the same orbital, characterized by the same set of orbital quantum numbers, their individual spin quantum numbers must be +1/2 and −1/2; i.e. their spins must be in opposite directions. If two electrons in a chemical bond are paired in this manner and if this bond is broken, for example, by incident light, resulting in two free radicals, subsequent recombination is only possible if the two electrons preserve this oppositely directed spin. Interaction with the local magnetic field - due to nuclear magnetic moments or nearby other spinning
and orbiting electrons - can, depending upon details of the particular molecular structure, either favor or destroy opposite spins. In the latter case, of now equally oriented spins, recombination of the radicals becomes impossible. Conversely, the radical pair may have been formed from excited atomic states with the unpaired electrons coming from different atomic shells. In that case they may already have equal spin preventing chemical combination of intermediate products.

As long as the electrons have opposite spin, the products have "singlet" character, i.e. the total quantum number $J$, which characterizes the electron states, is equal to the orbital quantum number $L$, since the spin quantum number $S = (1/2) - (1/2) = 0$ and $J = S + L$. However, as the products diffuse, some fraction will acquire "triplet" character (i.e. the electron spins may become parallel; then $S = \pm (1/2 + 1/2) = \pm 1$ and $J$ can have 3 values, $L + 1$, $L - 1$ and $L$, in view of the quantum rules for combination of angular momenta. If the products were initially in the triplet state, diffusion will have the opposite effect, i.e. partial conversion from triplet to singlet character.

The singlet and triplet states have, in general, different energies, as indicated at $B = 0$ on Figure 5. Any magnetic field, including that of nearby magnetic nuclei, will cause triplet states, $T_{+1}$ and $T_{-1}$, which have electron spin in the direction of the field, to gain or lose energy. Therefore the energy levels of these states will separate with increasing $B$, as indicated on Figure 5. Interconversion between the singlet and triplet states can occur either by external energy input, or between $T_0$ and $S$ through a distance dependent "electron exchange interaction" at any level of $B$. However, at some critical level of $B = B_c$, interconversion between $T_{+1}$ and $S$ is also possible without external energy input. Hamilton et al. (1988) have shown experimentally that this level is approximately 1 mT in their pyrene-dimethylaniline system.

Since interconversion between singlet and triplet states in the direction of greater singlet product will make possible chemical combination, application of the correct value $B_c$ will obviously affect the rate of chemical reaction. However it is possible that an ambient flux density (from external and internal sources) may have a value $B_\alpha$, which is either slightly larger or smaller than the required $B_c$. In that case addition of an alternating field $B_A \cos \omega t$
(with a period $2\pi/\omega$ longer than necessary for $T_1 \to S$) will periodically establish optimum conditions for conversion. On the basis of theoretical and experimental results (Hamilton et al., 1988) it is therefore at least possible that combination of DC and AC magnetic fields of the order of 0.5 mT (=1/2 of the measured $B_c$) could affect chemical reactions in biological systems that involve free radicals as intermediate products. However there appears to be no experimental evidence that alternating fields of a few $\mu$T would be effective. A more detailed review of this topic has recently been prepared by Walleczek (1995).

**Interaction with Endogenous Ferrimagnetic Particles**

Kirschvink et al. (1992) have demonstrated that magnetite biomineralization occurs in the human brain. Kirschvink and Kirschvink (1995) have also found that one line of human leukemic white blood cells is nearly 100 times more magnetic than brain tissues giving cellular magnetic moments of $\sim 10^{12}$ Am$^2$. Kirschvink (1992) then suggested that the interaction energy of even weak ELF magnetic fields with magnetic particles of magnetic domain size would be larger than the thermal energy of $(1/2)kT$ per degree of freedom within a system in thermal equilibrium. Using a model of magnetic particles floating in a fluid medium, Adair (1992) proposed “that a 60 Hz magnetic field weaker than 5 $\mu$T cannot generate significant biological effects at the cell level through action on magnetic elements”. However such a conclusion seems to be at least premature, because not enough is known about the relation of the magnetite particles to other components of the cell interior, or of the cell membrane, to formulate a mathematical model that is adequate for precise numerical predictions.

Any kind of anchoring of the “magnetosomes” to the cell structure would require description of the dynamics by a non-linear differential equation and even a linearized equation would not be one with constant coefficients unless the applied alternating magnetic field is exactly perpendicular to the geomagnetic field and the magnetosome deflection is very small. In addition, the effective viscosity of the fluid within the cell interior (in the vicinity of the magnetosome), which is a critical parameter in the Adair model, cannot be specified very well. But even if this clearly oversimplified model is used, but combined action by multiple magnetosomes within the cell is allowed, it appears that a signal-to-noise ratio well above one might be possible with a 60 Hz magnetic field of 2 $\mu$T (Polk, 1994). Neither estimate can be considered adequate at the present time. However it is likely that any effect of multiple magnetosomes within a cell would make use of their coherent motion, thus there would be no significant thermal noise limitation if the energy supplied by an applied field to a single magnetosome would be much smaller than $kT$. For single domain particles of Fe$_3$O$_4$ the magnetic moment is given by (Frankel, 1986)

$$\mu = \text{(volume)}(4.8)10^5 \text{ Am}^2$$

and the energy supplying torque to a spherical particle of 60 nm diameter in a 2 $\mu$T field would already be 0.1 $kT$ at 37°C. Therefore it is important to perform further experimental work on the behavior of such particles within biological cells, or of similar particles that might be present in cell culture medium as suggested by Kirschvink (private communication, 1994).

**CONCLUSIONS**

Biological effects of high intensity ELF electric fields, such as muscle stimulation and - at still higher intensities - cell damage by electroporation and heating, are reasonably
well (although not completely) understood. However power frequency effects of magnetically induced electric fields of less than 10⁻³ V/m or alternating magnetic fields of less than about 100 µT cannot be explained very well at the present time. The biological processes involved, notably signal transduction at the cell membrane and subsequent biochemical processes involving "second messengers" are beginning to be identified (Adcy, 1990; Luben, 1993). These processes involve vast amplification of the incident "signal" with metabolically supplied energy. The physical mechanisms of conversion from an electric or magnetic field to biochemical process are however unknown at the present time. As a consequence we do not know which aspect of the applied or environment field is important. Properties such as polarization, duration and/or intermittency of the signal, frequency and harmonic content may turn out to be as important as amplitude. The biological, chemical and mechanical condition of the organism (e.g. stage in cell cycle, stimulation by a mitogen or cell density) will also influence the effectiveness of the applied field. A key question, which is still only resolved for a few experimentally observed effects, is whether specific biological results are caused directly by the magnetic field or by the electric field that is induced in tissue by a time varying magnetic field. Particularly for fields larger than about 100 µT direct "magnetoochemical" effects have been suggested and the presence of magnetite particles in some tissues may also play a biological role. Experimental findings suggest that effects of induced electric fields are particularly likely when their magnitudes at the tissue or cell level are relatively "large", of the order of 10⁻³ V/m or larger, (McLeod et al., 1993b; Liburdy, 1992; Lyle et al., 1988), and when multiple cells are connected by gap junctions. Induced electric fields may also affect the motion of "counterions" on the cell surface and possibly the structural fluctuations of nucleic acid molecules. While most proteins have a small net electrostatic charge, nucleic acids are polyelectrolytes with large net charge and are surrounded by counterions (McCammion and Harvey, 1987). However in view of the inefficiency of electric field induction by an ELF magnetic field, illustrated by equation (25), the mechanisms for "direct" interaction of time varying magnetic fields with biological processes need serious consideration. This is particularly necessary when the applied ELF magnetic fields that produce unambiguous biological effects are of such amplitude and orientation (in relation to the culture medium or animal) as to produce very small induced electric fields. Experimental results have suggested that the relative magnitude and direction of a static magnetic field (such as that of the earth) can determine the biological effectiveness of a simultaneously present alternating field in some biological systems under controlled laboratory conditions.

REFERENCES


Biological Effects of Low Field Intensity ELF Magnetic Fields

ELECTROMAGNETIC FIELDS AND
CELLULAR SYSTEMS

Signal Transduction, Cell Growth and Proliferation

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INTRODUCTION

This paper reviews some of the in vitro research conducted in our laboratory to identify cellular responses to ELF fields. Studies we have conducted support an interaction site involving ligand-receptor events such as antibody binding to its cell surface receptor since we have observed changes in receptor-initiated calcium influx in cells exposed to magnetic fields. These changes suggest that signal transduction (ST) which is initiated by ligand-receptor binding plays a role in this interaction. Others have also reported that ELF fields influence enzyme activation, gene expression, protein synthesis and cell proliferation, all of which are triggered by earlier ST events at the cell membrane.

The concept of ELF fields altering early ST cell membrane events and thereby influencing intracellular cell function via the ST cascade is a plausible biological framework currently being investigated for understanding ELF effects on cells. For example, the consequence of an increase due to ELF fields in mitogenesis, the final endpoint of the ST cascade, is an overall increase in the probability of mutagenesis and, consequently, cancer according to the Ames epigenetic model of carcinogenesis. Consistent with this epigenetic mechanism and the ST pathway to carcinogenesis is recent evidence that ELF fields can alter breast cancer cell proliferation and can act as a co-promoter in vitro.

In addition to investigating the biological nature of ELF field interactions, we have conducted biophysical studies to determine whether the electric (E) or the magnetic (B) field, or if combinations of static B and time-varying B fields represent the exposure metric for the cell. This dosimetry question relates directly to understanding fundamental interaction mechanisms and to the development of a rationale for ELF dose-threshold guidelines. An E field-mediated interaction has interesting consequences for microdosimetry at the cellular level and is mechanistically consistent with an interaction at the cell surface since the E field does not penetrate the cell membrane. Recently studies from our laboratory and others have
suggested that an ELF B field by itself or in combination with a static B field may also elicit cellular effects. Thus, in addition to E-field mediated effects, other interaction mechanisms as yet not fully understood may operate at the cellular level. In addition to the question of an exposure field metric, the biological state of the target cell is important in ELF interactions. Biological factors such as cell type, cell cycle, cell activation, age of donor animal, passage number of cell line, presence of specific growth/mitogenic factors, temperature, shape and cell density packing during exposures have been shown to play a role in mediating ELF interactions with cells.

FIELD COUPLING AND THE SIGNAL TRANSDUCTION CASCADE

The ST cascade is an important, plausible, common theme in cellular interaction studies and represents the most plausible biological framework for understanding cellular responses to ELF fields. This interaction model is shown in Figure 1 in schematic form. ELF fields influence the ST cascade at the level of the cell membrane and trigger changes in calcium influx and/or receptor binding. Subsequent events distal to ligand-receptor binding such as gene expression and protein synthesis which lead to cell proliferation are ultimately influenced as the initial changes in calcium signaling are propagated down the ST cascade. Several recent articles dealing with ELF interactions with cellular systems and signal transduction are recommended for additional reading.1–9

THE CELL MEMBRANE AS AN INITIAL FIELD COUPLING SITE

Signal transduction involves cell surface interactions and it is the basis for cell communication with its environment. ST is a general process in which a ligand molecule binds to its receptor site on the cell surface and triggers a cascade of biochemical events in the cell membrane that lead to enzyme activation, gene induction, protein synthesis and ultimately mitogenesis and cell proliferation.9,10 Signal transduction is required for cell function, growth and differentiation. Two major signaling systems involve receptor tyrosyl kinases and receptor phospholipid breakdown in the cell membrane. The insulin receptor protein, for example, is a tyrosyl kinase that is activated by insulin binding. In the second system, which is widely distributed and found in all cells that are calcium dependent, a ligand binds to its receptor and triggers phospholipid breakdown in the cell membrane leading to the generation of "second messengers" that control a myriad of metabolic, cell growth, and differentiation events. Many different receptors share this ST pathway for economy. For example, when Concanavalin A (Con-A) or other mitogen binds to the T-cell receptor (TCR) of T-lymphocytes it triggers phospholipid breakdown leading to inositol(1,4,5)trisphosphate (IP₃) which in turn elevates intracellular calcium. Importantly, this elevation of calcium ion
Table 1. Mitogen-Activated signal transduction in the T-lymphocyte

<table>
<thead>
<tr>
<th>RESPONSE TO CONCANAVALIN A</th>
<th>FIRST DETECTED</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Ca+2], Calcium Influx</td>
<td>0 - 5 Minutes</td>
</tr>
<tr>
<td>[pH], IP3 Increase</td>
<td></td>
</tr>
<tr>
<td>PKC Activation</td>
<td></td>
</tr>
<tr>
<td>c-FOS mRNA Increase</td>
<td>5 - 50 Minutes</td>
</tr>
<tr>
<td>c-MYC mRNA Increase</td>
<td></td>
</tr>
<tr>
<td>Stimulation of Glycolysis</td>
<td></td>
</tr>
<tr>
<td>Increase Metabolite Uptake (e.g. Uridine)</td>
<td></td>
</tr>
<tr>
<td>Increased Inositol Incorporation into IP3</td>
<td></td>
</tr>
<tr>
<td>Increased General Protein Synthesis</td>
<td>&gt; 300 Minutes</td>
</tr>
<tr>
<td>Increased General RNA Synthesis</td>
<td></td>
</tr>
<tr>
<td>Increased General DNA Synthesis</td>
<td></td>
</tr>
</tbody>
</table>

concentration is a second messenger event; calcium ions bind to proteins such as calmodulin and kinases which sustain the ST cascade within the cell that ultimately leads to DNA, RNA, and protein synthesis, cell proliferation and clonal expansion of the T-cell.

In our laboratory calcium influx has been followed in two ways: using calcium-45 to follow influx and using FURA-2 to monitor in real-time changes in free intracellular calcium in cells. Extracellular calcium enters the lymphocyte through a channel that is open when Con-A binds to the T-cell receptor on the cell surface. The first event is Con-A receptor binding to the extracellular domain of the TCR which activates a tyrosine kinase in the cytoplasmic domain. This occurs instantaneously and it represents the first coupling event in the ST cascade. Tyrosine phosphorylation of the G-protein complex leads to activation of phospholipase C which cleaves phosphoinositol in the cell membrane to yield IP$_3$ and diacylglycerol (DAG). IP$_3$ opens the calcium channel and also acts as an endogenous calcium ionophore to release calcium from stores in the endoplasmic reticulum and mitochondria. DAG activates protein kinase C which among other things regulates hydrogen ion transport and intracellular pH. Thus within seconds after receptor ligatation there are near instantaneous increases in intracellular calcium and intracellular pH. We have followed both of these parameters in real-time in lymphocytes exposed to ELF fields. Next, early response ST genes such as c-MYC are activated within sixty minutes and the cell ultimately progresses into mitogenesis at times > 300 minutes. This time course is presented in Table 1.

Amplification is a key feature of ST that is relevant to ELF field interactions. The ST cascade achieves enormous amplification and we have postulated that an ELF-mediated modification of an early event at the cell membrane involving calcium ion influx could lead to, and explain, significant changes in subsequent ST events such as gene expression and cell growth.\cite{2-6} Signal propagation involves at least two, and usually many steps that amplify the effect of the initial binding event at the cell surface. For example, the binding of one ligand molecule to its receptor at the cell surface leads to activation of multiple transducer proteins or to the influx of a large number of calcium ions which will activate intracellular enzyme molecules.

Evidence for 60 Hz magnetic fields increasing calcium-45 influx during signal transduction in the lymphocyte has been reported from our laboratory.\cite{11, 12} A brief 60 minute exposure of rat thymic lymphocytes to a 220 Gauss magnetic field ($\text{E}_{\text{induced}} = 1.0 \text{ mV/cm}$) at 37°C was performed in the presence or absence of Con A. Our magnetic field exposure system and the special multi-ring annular ring petri dishes we designed are described below in greater detail. Non-activated cells (no mitogen) were unresponsive to the magnetic field; calcium-45 influx was not altered. When Con A was present, the magnetic field led to an
Calcium Uptake in Thymocytes: Effect of a 60 Hz Magnetic Field on Con-A Activated Cells

![Graph showing calcium uptake](image)

- **% Con-A Activation**: 100, 30, 220, 275, 75
- **% MF Increase**: 100, 275, 80, 40, 350

**Exposure Conditions**: 220G (rms), 1 mV/cm (max), 15uA/cm^2 (max), 37°C, 60 minutes

* p < 0.03, n=3 (unpaired)
* p < 0.01, n=5 (paired)

Figure 2. Cells assessed for calcium uptake in the absence or presence of Con-A, and in the presence of Con-A and a magnetic field. Exposures at 1.0 mV/cm, 220 Gauss, 16 uA/cm^2, 20 ug/ml Con-A, 37 ± 0.05°C, 60 minutes.

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**A. Exposure Geometry**

Current carrying Pt-Electrodes embedded in Agar

Fluorescence Excitation

Fluorescence Emission

Optical Slit

Fluorescence Cuvette

**B. Electric Field in Cuvette**

Agar Diffusion Barrier

Current carrying Pt-Electrodes embedded in Agar

Electric Field Lines

Fluorescence Cuvette

Ohm's Law

V = IR

E = V/Electrode Spacing

- Electric field is uniform throughout center of Cuvette

Figure 3. Schematic diagram of one of our fluorescence cuvette exposure devices.
increase in calcium-45 influx of 50 - 200%. This variation depended on the level of calcium influx achieved by Con A; weakly activated cells were associated with maximal response to the field. This relationship is shown in Figure 2.

We have investigated the effect of ELF fields on calcium influx using a different approach involving real time fluorescence spectroscopy. Real-time changes in intracellular calcium ([Ca^{2+}]_{i}) in cells exposed to 60 Hz fields was reported from our laboratory.\(^3\) The advantage of this approach is the ability to observe field effects as they occur in real time. Thymic lymphocytes were loaded with the calcium sensitive dye FURA-2AM and the cells exposed to a 60 Hz electric field (1.7 mV/cm) in an electrode cuvette similar to that shown in Figure 3 while fluorescence was monitored; we have made some electrodes using stainless steel and platinum foil and we have used some different electrode shapes. A cuvette of this general design with an optical slit permits cells to be exposed to a uniform 60 Hz electric field while simultaneous monitoring intracellular free calcium. Figure 4 shows that levels of intracellular calcium in resting cells (no mitogen) were not apparently influenced by the field and this is consistent with the calcium-45 influx data for resting cells, mentioned above. When the mitogen Con-A was added to the cells during field exposure levels of intracellular calcium began rising and diverged to reach higher values than that for unexposed, Con-A treated cells. These studies suggest that the initial rise in calcium, the early phase due to intracellular calcium release over approximately 100s, was not altered by the field, and that the steady-state levels of calcium influx were enhanced thereafter. These results are in general agreement with the calcium-45 influx studies discussed above in which Con-A treated cells displayed an increase in calcium-45 influx during field exposure at \(E_{\text{induced}} = 1.0 \, \text{mV/cm}\).

The biological significance of these real-time fluorescence studies has some relevance to the question of an interaction site. Our observation that the initial rise in intracellular calcium during Con-A treatment was not influenced by the field indicates that release of calcium from intracellular stores inside of the cell during ST was not disturbed by the field. It is known that the initial rise in intracellular calcium during ST is due to release of calcium from the mitochondria and the endoplasmic reticulum. Our finding that only the plateau phase was increased during field exposure indicates that influx of calcium through a ligand-mediated channel in the cell plasma membrane was influenced by the field. This follows since the plateau phase is sustained by the influx of extracellular calcium across the

**Figure 4.** Real-time measurements of \([Ca^{2+}]_{i}\) in rat thymocytes during exposure to 60 Hz electric fields. The field effect is associated with an increase in intracellular calcium during mitogen activation.\(^3\) 1.7 mV/cm; 37 ± 0.05°C.
plasma membrane through a specialized ligand-gated channel. Therefore our real-time study suggests that the cell membrane and calcium influx was involved in the field interaction.

The above work from our laboratory leads to several important hypotheses about ELF field coupling to cells: (1) the cell membrane is involved in cellular responses to ELF fields, (2) ELF fields affect receptor-ligand ST events such as ion transport at the cell surface, (3) ELF fields can profoundly potentiate suboptimally activated cells during ST.

GENE ACTIVATION

The research discussed above suggests that calcium influx is enhanced during ST in the presence of ELF fields. We postulated that such ELF field effects on calcium cycling can play a significant role in triggering mid-stage ST events such as c-MYC mRNA induction via the ST cascade. This means that ELF field effects on early events such as calcium signaling are propagated down the ST cascade to alter subsequent mid-stage events. Above it was mentioned that increases in calcium influx are early events during ST and occur within the first several minutes whereas the induction of mRNA occurs at least 60 minutes into the ST cascade.

Within the ST framework gene activation is triggered after an initial event at the cell surface such the binding of a hormone or growth factor to its receptor. Since membrane effects are observed experimentally in our studies of ion transport during signal transduction, discussed above, we reasoned that ELF fields could subsequently activate genes through the ST pathway. This is an epigenetic interaction mechanism that is consistent with a wide range of laboratory evidence arguing against a direct effect of sinusoidal power-frequency ELF fields on DNA.

Our approach to establish a linkage between ELF field effects on early and mid-stage ST events was to measure both parameters simultaneously in the same cell preparation exposed to 60 Hz magnetic fields.

To do this we correlated alterations in calcium influx and in c-MYC mRNA induction measured in the same exposed cell population. The hypothesis, mentioned above, was that increases in calcium influx induced by the ELF field would subsequently lead to increases in cMYC mRNA transcription via the signal transduction cascade. This represents an attractive and highly plausible biological framework for understanding how a magnetic field effect at the cell surface, i.e. calcium ion influx can influence subsequent events such as RNA, DNA, and protein synthesis.

We followed c-MYC mRNA in these studies because the oncogene MYC plays an important role in ST. MYC belongs to a set of cellular messengers commonly referred to as “immediate early response” genes since their expression is activated by a variety of mitogenic stimuli, independent of de novo protein synthesis, early during the G_i to G_j transition of cells from a resting to a growing state. The protein products of immediate response genes such as MYC are thought to facilitate progression of the cell through the cell cycle and synthesize DNA in S phase. MYC polypeptides play roles in the transcriptional and post-transcriptional control of other cellular genes and in DNA replication. Strong evidence indicates that they mediate their function as site-specific DNA-binding proteins; cells keep MYC under very tight regulation.

In our experiments rat thymocytes were placed in a special glass annular ring cylinder and exposed to an incident 220 Gauss 60 Hz magnetic field (E\_induced = 1.7 mV/cm) for 60 minutes, 37°C, as described. The cell sample was harvested and split into two aliquots and simultaneously assessed for calcium influx and for cMYC mRNA.

Figure 5 depicts the results from three independent experiments in which calcium influx was measured in thymic lymphocytes in the absence of Con-A, in the presence of
Figure 5. Calcium influx and cMYC mRNA transcripts are elevated in the same population of mitogen-activated rat thymocytes following 60 Hz magnetic field exposures: calcium influx data. Results from three independent experiments in which samples were split for simultaneous assessment of calcium influx and cMYC mRNA.\textsuperscript{15} 1.7 mV/cm, 220 Gauss, 27 mA/cm\textsuperscript{2}, 1.0 \mu g/ml Con-A, 37 ± 0.05°C, 60 minutes. Mean ± S.D.

Con-A (1 \mu g/ml), and in the presence of Con-A plus a magnetic field. Non-activated thymocytes (no Con-A) exhibited a baseline calcium influx of 10,400 ± 386 cpm/10\textsuperscript{5}cells. A dose of Con-A at 1 \mu g/ml did not result in a significant increase in calcium influx. However, when cells were exposed to a 22 mT magnetic field in the presence of Con-A, an approximate 1.5 fold increase in calcium influx was observed. This is consistent with our previous observations mentioned above of enhanced calcium influx for mitogen-activated thymocytes exposed to a 22 mT, 60 Hz magnetic field.\textsuperscript{2,3,11,12} It is important to note that mitogen-activated calcium influx in lymphocytes is dependent on animal age, mitogen dose, and immune status of the animal.\textsuperscript{2} In these studies each of the independent experiments employed thymocytes pooled from three, age-matched animals, and this pooled population responded suboptimally to a dose of 1 \mu g/ml of Con-A. This permitted detection of an increase in calcium influx of Con-A treated cells due to the field. Cells maximally activated by Con-A are at their maximal dynamic limit and further calcium influx in response to an applied magnetic field is not possible.

Figure 6 shows Northern blot analyses that were repeated three times on the same RNA sample from one experiment to demonstrate the precision and reproducibility of this technique prior to optical density calibration and normalization to message for the housekeeping gene glyceraldehyde-6-phosphate dehydrogenase (GAPDH), which is not altered during ST. Northerns were quantitated using a CCD-camera and the digitized images of bands were integrated on a pixel-by-pixel basis so that [total band gray-scale intensity x mm\textsuperscript{2}] was computed and this quantity was then linearized by conversion to [OD x mm\textsuperscript{2}]. Panel A shows the images obtained for each Northern. Panel B represents the regions of interest for the three bands in each Northern. Panel C shows the image after flat-fielding and thresholding. Panel D shows surface plots of pixel intensity for the bands in panels C. We compared the mean pixel intensity for these bands using a multivariate analysis of variance statistical program that analyzed for differences across bands in these experiments. Mean pixel intensity (arbitrary units ± S.D.) was -Con-A = 332 ± 128; +Con-A = 398 ± 48; +Con-A plus 60 Hz magnetic fields = 864 ± 181. A statistically significant difference was not detected between -Con-A and +Con-A (p > 0.05). A statistically significant difference was detected between +Con-A and +Con-A plus 60 Hz at the p = 0.0114 level. Thus, we were able to obtain statistically significant results in the absence of O.D. calibration and normalization to glyceraldehyde-6-phosphate dehydrogenase (GAPDH), discussed below. Normalization
Figure 6. CCD-camera imaging of c-MYC mRNA Northern: Comparison of three independent blots of the same RNA sample from one experiment. Panel A: flat-fielded images of c-MYC bands. Panel B: Regions of interest for the bands. Panel C: Thresholded images of the Northern blots. Panel D: surface plots of pixel intensity for bands in each image.

is important since it corrects for any generalized, nonspecific changes in total mRNA in the cells.

In order to determine whether the above effect is specific for the c-MYC mRNA species or due to a general increase in mRNA abundance, we analyzed levels of RNA encoding a "housekeeping" protein GAPDH, on the same blot. RNA samples from the above experiment, plus RNA samples from two additional, independent experiments were analyzed. For each Northern blot this standardization procedure involved normalizing the quantitated band intensity (O.D. x mm$^2$) for c-MYC by the associated band intensity for GAPDH. We observed that GAPDH mRNA does not vary across treatment groups corresponding to -Con-A, +Con-A, and +Con-A plus ELF field$^{15}$. This indicates that GAPDH is not affected by the mitogen Con-A or the ELF field and this is not surprising since GAPDH is not activated during ST. For this reason we used it as an appropriate control for normalizing changes in c-MYC mRNA.

Using GAPDH to normalize our c-MYC mRNA data we computed the relative ratios of c-MYC mRNA abundance to GAPDH abundance across the three experiments with the c-MYC/GAPDH ratio for non-activated cells set to one. Figure 7 shows the ratio of c-MYC mRNA/GAPDH mRNA for cells in the three independent experiments of Figure 5. Levels of c-MYC mRNA expression in non-activated cells were not observed to be statistically different than cells treated with the suboptimal dose of 1 $\mu$g/ml Con-A. In contrast, cells treated with Con-A plus magnetic fields exhibited an approximate 3.0-fold increase in
Calcium influx and cMYC mRNA transcripts are elevated in the same population of mitogen-activated rat thymocytes following 60 Hz magnetic field exposures: c-MYC mRNA Data. Results from three independent experiments in which samples were split for simultaneous assessment of calcium influx and cMYC mRNA. Relative transcript levels for c-MYC mRNA were standardized to the housekeeping enzyme GAPDH by quantitation using cooled-CCD imaging analysis. See Figure 5.

Relative transcript levels for c-MYC mRNA were standardized to the housekeeping enzyme GAPDH by quantitation using cooled-CCD imaging analysis. See Figure 5.

This data provides evidence that ELF effects on these two ST events are linked, and, based on calcium's role in the ST cascade, this provided strong evidence for an interaction mechanism in which ELF fields trigger calcium influx at the level of the cell membrane and this leads to subsequent changes in events in ST pathway. This ST interaction model explains how ELF field effects have the potential to alter subsequent cellular events in this cascade.

Other laboratories have investigated the effects of fields on cMYC mRNA prior to our studies. Goodman and Shirley-Henderson reported that cMYC mRNA was enhanced by magnetic fields\textsuperscript{13} and by 60 Hz sinusoidal electric fields (0.3 x 10\textsuperscript{-4} V/m).\textsuperscript{19} Recently Phillips and colleagues reported that a brief 1 Gauss magnetic field exposure of a T-lymphoblastoid cell line, CEM-CM3, also increased mRNA transcripts.\textsuperscript{20} Their report was the first to employ the nuclear run-off assay technique to assess alterations in specific gene transcription for MYC, JUN, FOS and protein kinase C.

**CELL PROLIFERATION**

The final endpoint in the ST process is cell proliferation which results in mitogenesis. Since cell proliferation is the ultimate endpoint for the signal transduction pathway, it is important for studies to be conducted to evaluate the effect of electromagnetic fields on cell proliferation and growth, particularly of cancer cell lines. This we have done for the case of human breast cancer cells. This is an important question since ELF fields have been postulated as a significant risk factor in human breast cancer epidemiology.\textsuperscript{21-24}

Our studies were recently published and suggest that human breast cancer cell growth in vitro can be altered by a 60 Hz magnetic field.\textsuperscript{25} In these studies, melatonin, a hormone with natural oncostatic activity, was employed to suppress breast cancer cell growth and this response was investigated during field exposures. This is analogous to the studies discussed above in which a mitogen such as Con-A is used to trigger the ST cascade and cells are exposed to 60 Hz fields. This concept is a critical feature in cellular studies since resting or quiescent cells, as discussed above, do not show sensitivity to an imposed ELF electric or magnetic...
field. In general cells appear to be most responsive to fields when they are engaged in signal transduction mediated by cell membrane events such as receptor binding and calcium cycling.

We conducted experiments employing a well-characterized human breast cancer cell line, MCF-7, and we tested whether the growth of MCF-7 cells is suppressed by melatonin in the presence of a 2 or 12 mGauss 60 Hz sinusoidal magnetic field. Melatonin is a hormone and natural growth inhibitor of estrogen positive breast cancer cells which is normally released into the blood stream at night. Melatonin is of interest since (a) it displays natural oncostatic action towards breast cancer cells, (b) melatonin release into the blood stream in animals has been reported to be depressed by 60 Hz magnetic fields, and (c) 60 Hz magnetic field exposures are postulated to be a risk factor in human breast cancer, mentioned above.

IN VITRO CELL CULTURE EXPOSURE SYSTEM

Prior to performing in vitro experiments on cell growth we had to innovate an approach to exposing cells in culture to very uniform ELF magnetic fields while maintained inside of commercially available incubators. All in vitro experiments in which cells are grown in commercial incubators and subsequently exposed to ELF fields have to be done very carefully taking into account several important considerations. In the past in vitro cell culture experiments have neglected, in general, the fact that all commercial incubators generate magnetic and electric fields due to the operation of the heating and gas exchange electronics associated with the incubator; and considerable variation can exist across manufacturers. Since the endogenous magnetic and electric fields incubators can vary greatly, both in spatial distribution and in time, complete field mapping is essential to fully characterize the interior of the incubator.

In addition to the question of ensuring that our cells were exposed to a reasonably uniform ELF magnetic field in our incubators (≤ 10%), we also addressed the important question of ensuring that our cells during routine propagation in culture had a known electromagnetic history. Thus we routinely propagate all of our cells using the system described below to ensure that the cells have a known prior ELF exposure history before conducting field exposure experiments.

The approach we use is to shield the interior space of the incubator using 80% nickel mu-metal. One of our mu-metal chambers inside a cell culture incubator is shown in Figure 8 (Co-Nectic AA Foil, Magnetic Shield Corporation, Perfection Mica Co., Bensenville, IL). This mu-metal chamber is ventilated at the upper and lower corners with 2.5 cm diameter holes that have 2.5 cm extension tubes to minimize entry of stray magnetic fields. The chamber has a hinged door so that it can be opened and closed in a manner identical to the commercial incubator door. Our use of a closed, ventilated mu-metal shielding is the best solution for eliminating all of the endogenous magnetic and electric fields generated by the operation of the cell culture chamber. There exist time varying magnetic field generated during the transient operation of the gas solenoids of the incubator and during the transient heating pulses of the outer water-jacket of the incubator; in addition some incubators have electrically-heated door panels. All of these fields have 60 Hz and/or ELF components and their time profiles can vary significantly depending how the frequently the incubator is opened. Another significant problem is that these fields can display significant spatial variation within the incubator. Most if not all incubators have their electronic equipment mounted on the top of the incubator so that cells placed on the top shelves are exposed to more intense endogenous fields that cells placed on the very bottom of the incubator. By using a mu-metal shielded chamber we avoid all of these problems.
To expose cells to a uniform ELF magnetic fields we used two identical, double-wound, four-coil, Merritt exposure systems one of which is also shown in Figure 8. The figure shows the four-coil Merritt design in which there are four double-wound coils wrapped around a plastic frame. These coils generate a large and relatively uniform area for exposing an array of cell culture plates if Merritt’s turns ratio of 26/11/11/26 is followed in winding these four coils (17.0 Ω, 6.57 mH). With the center axis of the coil system used as a reference line and the center point on this axis designated the origin, the four coils had the following spacing with respect to the origin: 16.7 cm, 4.23 cm, -4.23 cm, and -16.7 cm. Commercially available standard speaker cable with two parallel wire tracks was used for the double-wrap cable. Built into the circuit energizing these coils was a switch used to reverse the direction of the current in one of the wires in the speaker cable comprising the double-wound coils. When current is applied in the anti-parallel configuration (passive) the magnetic fields from the double-wound coils cancel, and when current is applied in the parallel configuration (active) a magnetic field is established. This feature can be used to perform a control exposure in one incubator to determine if coil heating and coil vibration is a factor; relatively high field exposures greater than approximately 1 Gauss may result in heating and vibration but this is not the case for fields in the 12 - 15mGauss range used in our studies. Each coil system was driven by identical signal generators available from Dynascap Corp., Chicago, IL (B&K Precision Model 3020). Each of the four coils comprising the exposure system was shielded by wrapping the wire bundles in two layers of heavy-duty aluminum foil, with a break of several inches, to eliminate the electric field components generated by current running the wire wrappings. The magnetic field generated by this coil orientation is perpendicular to the
plane of the cell culture plates. Culture plates are positioned on a perforated, Plexiglas platform that corresponds to the middle plane of the coil. Field uniformity is conservatively ≤ 10% over the central area where cells are placed. Note that an external temperature probe is fed through one of the ventilation holes of the chamber to monitor temperature continuously during exposures.

**BREAST CANCER CELL GROWTH STUDIES**

We tested the hypothesis that 60 Hz magnetic fields block melatonin’s growth inhibitory (oncostatic) action on MCF-7 cell proliferation. We have observed that normal MCF-7 cell growth is not altered by these magnetic fields in the absence of melatonin. Dr. D. Blask has reported the melatonin inhibits the proliferation of MCF-7 cells when it is present in cell culture media at concentrations corresponding to the physiological range of $10^{-9}$ to $10^{-11}$M. We first performed experiments to confirm melatonin’s inhibition of MCF-7 cell growth. Growth curves for MCF-7 cells in the absence or in the presence of $10^{-11}$M melatonin are presented Figure 9. In these experiments cells were placed in an incubator with the exposure coils energized in the antiparallel configuration (passive) so that a environmental-level background 60 Hz magnetic field of 2.0 mG was present. This magnetic field simulates a background field commonly found away from electrical appliances in work places or homes. Cell proliferation revealed typical exponential growth over the 7-day period. When melatonin was present, an approximate 25% inhibition of growth was detected at day 7. These results confirmed Blask’s original observation of melatonin’s inhibition of MCF-7 cell growth mentioned above. However, it is important to note that MCF-7 cells from different laboratories can display heterogeneity in their response to melatonin, and some subclones can exhibit growth inhibition of up to 70% in the presence of melatonin. Some MCF-7 subclones may exhibit no sensitivity to melatonin - this may depend on passage number, serum factors, or other unknown parameters.

Simultaneously with the 2 mG 60 Hz magnetic field exposures above we conducted exposures to 12 mG in a matched incubator. We note that 12 mG will be used in the text that follows with the understanding that mean field values for our experiments fall within the range from 12 to approximately 15 mG(rms). This field level range represents an environmental level that is at or near the high end of magnetic field strengths people can experience from exposures to common household or work place appliances, much higher field levels
however, can be encountered very near electronic devices commonly found in the kitchen. A growth curve for MCF-7 cells in the presence or absence of melatonin in a 12 mG magnetic field is shown in Figure 10. As can be seen an exponential growth curve for the MCF-7 cells in absence of melatonin and 12 mG (solid line) was obtained that is superimposable with that obtained for cells in the presence of melatonin and 12 mG field (dashed line). This data reveals that the 12 mG magnetic field in the presence of melatonin at $10^{-11}$M blocked melatonin's growth inhibition. Compare this result at 12 mG with the approximately 25% growth inhibition observed in the 2 mG field shown in Figure 9. These two experiments were performed simultaneously with matched incubators and cells from the same passage.

The above experiments have been repeated a number of times for melatonin at $10^{-9}$M to confirm these results for this physiological concentration of melatonin. Presented in Figure 11 is a summary graph for seven experiments, using cells from different passages, in which 2 mG and 12 mG exposures were performed simultaneously in matched incubators. We observe that melatonin resulted in an approximate 15% growth inhibition in a 2 mG magnetic field and that this was blocked by a 12 mG.

Our findings on MCF-7 cells suggest that (a) this in vitro effect is a cellular level response to magnetic fields involving cell proliferation and growth, (b) it involves an interaction that requires the presence of melatonin which is a natural oncostatic agent, and

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**Figure 10.** Melatonin's action on MCF-7 cell growth in a 12.0 mG 60 Hz magnetic field.

**Figure 11.** Summary data of 60 Hz magnetic fields on Melatonin's action on MCF-7 cell proliferation.
(c) a dose threshold appears to exist between 2 and 12 mG. These findings represent some laboratory evidence for a cellular level response to ELF magnetic fields that is dependent on the presence of melatonin a naturally occurring hormone that has relevance to human breast cancer.

Several models have been proposed to elucidate the link between ELF magnetic field exposure, melatonin, and breast cancer incidence. These models suggest that the most important aspect of this link is a decrease in melatonin secretion in response to an in vivo magnetic field exposure concomitant with enhancement in the production of prolactin and estrogen; the latter events are thought to increase the growth of susceptible breast epithelial cells. Our laboratory results, in contrast, deal with in vitro exposure of breast cancer cells and, therefore, relate to cellular events that occur distal to an in vivo effect on the pineal gland which regulates melatonin's secretion into the bloodstream. Keeping in mind the caveat that our results relate to an in vitro cellular effect, our findings suggest the possibility of an interaction between ELF magnetic fields, breast cancer cells, and melatonin may exist at the cellular level. This means that in addition to an ELF magnetic field effect on melatonin release into the bloodstream, there might exist an ELF field effect on melatonin's function at the level of the target cell, e.g., human breast cancer tissue and its proliferation. This needs to be tested in animal model systems to be verified.

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EFFECTS OF ELECTROMAGNETIC FIELDS
ON K⁺(Rb⁺) UPTAKE BY HeLa CELLS

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INTRODUCTION

The effects of static or time-varying magnetic fields on membrane transport of alkali
cations have not been reported more frequently than studies on Ca²⁺ fluxes. Gualtierotti et
al. [1964] observed decrease in Na⁺ transport in frog skin on exposure to a static magnetic
field of less than 1 T. Collis and Segal [1988] reported influences of exposure to a weak
pulsed magnetic field (PMF) on bi-directional ²⁴Na⁺ fluxes through rabbit colon epithelium,
finding that the effects on influx and efflux of the ion differed depending on the direction
of the magnetic field. In contrast, Hinsenkamp et al. [1985] and Farndale et al. [1987] failed to
detect any significant effects of exposure to a PMF on the activities of the Na⁺-pump and
Na⁺/K⁺/Cl⁻-cotransport of human red cells. Stevenson and Tobey [1985] found that exposure
to a PMF inhibited ⁴²K⁺ uptake slightly, but not significantly. As these results are discrepant,
further studies on the effects of various magnetic fields on membrane transport of monova-
lent cations are necessary.

In the present study, we tested the effects on active and passive influxes of Rb⁺ (as a
substitute for K⁺) into HeLa cells of strong homogeneous magnetic fields. We also tested the
effects on these influxes of strong, time-varying magnetic fields, which have not previous
been investigated extensively, but which could be useful for assessing the physiological
effects at a cellular level of repeated exposures of the human body to a strong magnetic field
over a short period.
ELECTROMAGNETIC FIELDS

Magnetic fields were produced by an electromagnet designed and set up by Hitachi Metal Indus. Co. (Tokyo, Japan). The apparatus had two vertically arranged coils, each of which was attached with a pole piece of round polar face of small (50 mm) or large (100 mm) diameter. The distance between the two polar faces was changeable. A thyristor rectifier (RCX-100H2, Sansha Electric MFG. Co., Tokyo, Japan), normally used for xenon lamp ballast, was chosen as a suitable power source of the electromagnet and produced coil currents of 17 to 100 A (Figure 1A). In approximate proportion to a current, the magnetic flux density of 0.3 to 1.7 T was produced with the large polar face, as measured with a digital gaussmeter (501, Nihondenjisokki, Tokyo, Japan), when the distance between the two polar faces was 20 mm. At maximum, about 2 T was produced with the small polar face under the same conditions. The magnetic flux density regulated at 1.5 T at the center of the large polar face was found to be distributed homogeneously within 45 mm from the center (Figure 1B). A similar distribution of a magnetic flux density of 1.8 T was observed within 18 mm from the center of the small polar face. Therefore, culture dishes were placed within these distances for testing the effects of magnetic fields on cell functions. The electromagnet was fitted with a water cooling system, so the temperature on the surface of the polar face did not exceed 50°C.

Time-varying magnetic fields were produced by automatic switching of the power source with an electronic device, which enabled us to change the magnetic flux density from 0.07 to 1.77 and 1.54 T, respectively, in about 1 s and in the reverse direction in 3 s when the pole pieces with the small and large faces were used. For simplicity, we took the durations of the switching on and off times, i.e., "on-time" and "off-time", to be equal. These durations were chosen to be 3 s or longer, since it took less than 3 s for the eddy current to return to zero after the switching off, as judged from the results in Figure 2. Because of this sufficiently long interval, the eddy current occurring on switching off in a preceding cycle of changes in the magnetic flux density did not overlap the

Figure 1. Characteristics of the electromagnet. A. Relation between the magnetic flux density and current of the power source. B. Distribution of the magnetic flux density on the polar faces.
current induced by switching on in the following cycle. Panels A and C of Figure 2 show examples of the quasi-rectangular forms of changes in the magnetic flux density with "on-time" and "off-time" periods of 5 s, when the pole pieces of the small (50 mm diameter) and large faces (100 mm diameter) respectively, were used. The magnetic flux density changed from 0.07 to a maximum of 1.77 T in A and to that of 1.54 T in C. Panels B and D show the spike-like changes in dB/dt and mean values of the eddy current densities corresponding to the changes in the magnetic fields in panels A and C. The eddy currents induced by switching on were greater than those induced by switching off. The cycles of changes in the magnetic flux density were estimated to be 6.5 and 8.4 s from the results in panels B and D. Since the density is proportional to the horizontal distance r from the center of the culture surface and a change in the magnetic flux density dB/dt, the density of the eddy current i was calculated by the following equation:

\[ i = \gamma (r/2) dB/dt, \quad (1) \]

where \( \gamma \) is the conductivity (1.59±0.82 S/m) of the culture medium. The mean value of the current density in a culture dish was obtainable by the equation.

\[ i = \int_0^R \int_0^r \frac{r dr}{\pi} \frac{r dr}{\pi} \gamma (R/r) dB/dt, \quad (2) \]

where \( R \) is the radius of the culture surface (16.9 mm).
FREQUENCY SPECTRA OF $B$ AND $dB/dt$

We analyzed the amplitudes of fundamental waves of the magnetic flux density $B$ and its differential $dB/dt$ as functions of the angular frequency $\omega$. Since the magnetic field used for these analyses changed between nearly 0 and 1.6 T at a cycle $T$ of 6 sec,

$$f_0=1/T=1/6 \text{ [Hz]}$$

and

$$\omega_0=2\pi f_0 = 2\pi/T=\pi/3 \text{ [rad/sec]}.$$

Let

$$B(t)=a_0+a_1 \cos(\omega_0 t+\theta_1)+a_2 \cos(2\omega_0 t+\theta_2)+a_3 \cos(3\omega_0 t+\theta_3)+\cdots, \quad (3)$$

where $\theta_0$ indicates the phase of a certain component at time $t=0$ ($t=0$). Then, the amplitudes of the components are regarded as

$$a_0=F(0)/T, \quad a_1=2F(\omega_0)/T, \quad a_2=2F(2\omega_0)/T, \quad a_3=2F(3\omega_0)/T.$$

$F(\omega)$ corresponds to the frequency of change in the magnetic field $f(t)$ at any time. We can also represent $dB/dt$ by a similar expression

$$dB(t)/dt=b_0+b_1 \cos(\omega_0 t+\theta_1)+b_2 \cos(2\omega_0 t+\theta_2)+b_3 \cos(3\omega_0 t+\theta_3)+\cdots, \quad (4)$$

where $b_0$ corresponds to $a_0$ in equation (3).

These amplitudes for the magnetic flux density $B$ and its differential $dB/dt$ were calculated as functions of the angular frequency $\omega$ and shown in Table 1 and Figure 3A, and Table 2 and Figure 4A, respectively. Figures 3A and 4A represent different patterns of changes in the amplitudes between $B$ and $dB/dt$ with increase in $\omega$. The amplitude of $B$ is relatively large at $\omega=0$ and $\omega_0$, but decreases abruptly with increase in $\omega$ after $2\omega_0$. In contrast, the amplitude of $dB/dt$ is extremely small at $\omega=0$ but becomes maximal at $\omega_0$ and then gradually decreases with increase in $\omega$. However, the amplitude cannot be ignored even at higher values of $\omega$.

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<td>$7\omega_0$</td>
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$\omega_0 = 2\pi/T (T=6 \text{ sec})$. 

Table 1. Amplitudes of the components of $B$, i.e., $2F(\omega)/T$, calculated from the curve of $B$ as a function of time.
Table 2. Amplitudes of the components of dB/dt, i.e., \(2F(\omega)/T\), calculated from the curve of dB/dt as a function of time

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</table>

\(\omega_0 = 2\pi T / (T = 6 \text{ sec})\).

We also show \(B\) and \(dB/dt\) as functions of the frequency \(f\) in Figs. 3B and 4B. Similar patterns of change in the amplitudes to those shown in Figs. 3A and 4A are still observed. The amplitude of the components of \(B\) is relatively larger at \(f = 0\) and 1/6 Hz and decreases abruptly after \(f = 2/6\) Hz, whereas the amplitude of the components of \(dB/dt\) tends gradually to decrease with increase in \(f\). These results revealed that \(B\) mainly consisted of components of the lowest frequencies, whereas \(dB/dt\) as well as the eddy currents had more complicated frequency spectra.

CELL CULTURE

HeLa cells (strain S3) purchased from ICN Biomedicals Inc. (Costa Mesa, CA) were maintained by serial culture in glass culture flasks containing 10 ml of culture medium consisting of modified Eagle’s minimum essential medium (mMEM) enriched with amino acids and vitamins [Miyamoto et al., 1976] and supplemented with 10% (v/v) calf serum. Growing cells were washed with Dulbecco’s phosphate-buffered saline (PBS), detached from the culture flasks and dispersed by digestion with 0.5% trypsin (1:250, Difco Laboratories, Detroit, MI), and suspended in the same culture medium at a density of 5x10⁴ cells/ml. Aliquots of 2 ml of the cell suspension were inoculated into plastic culture dishes (35 mm diameter, Corning Glass Works, Corning, NY) and incubated for 48 h at 37 °C in an incubator under humid air containing 5% CO₂.

CATION FLUX ASSAYS

The culture medium was discarded and the cultures were once washed with mMEM containing 5 mM RbCl (super-pure grade, Merck, Darmstadt, Germany) instead of 5 mM KCl (Wako Pure Chemical, Osaka, Japan) and incubated in the same medium containing RbCl. This medium also contained 25 mM N-2-hydroxy-ethylpiperazine-N’-2-ethanesulfonic acid (HEPES, Sigma Chem. Co., St. Louis, MO) to maintain the pH at 7.2. These procedures were carried out in a special incubator in which the temperature of the air could rapidly be changed to that required for exposure to the magnetic fields. Rb⁺ was used in place of K⁺ because the membrane transport of HeLa cells recognize Rb⁺ and K⁺ equally [Miyamoto et al., 1978]. Rb⁺ influx was assayed for 15 min immediately after medium
change, unless otherwise stated, because both active and passive Rb⁺ influxes take place at the initial rates for at least 20 min [Miyamoto et al., 1986; Ikehara et al., 1982 and 1993].

K⁺ efflux was assayed as time-dependent decrease in the cellular K⁺ content after replacement of K⁺ by Rb⁺ (see Figure 11). The rate constant of K⁺ efflux, i.e., the rate constant of decrease in cellular K⁺, was estimated from the slope of the linear part of the semi-logarithmic plot of the cellular K⁺ content vs. the time after replacement of K⁺-medium by Rb⁺-medium. K⁺ efflux, i.e., the rate of cellular K⁺ loss, was calculated by multiplying the rate constant by the cellular K⁺ content at the beginning of the assay. Therefore, Rb⁺ influx and K⁺ efflux could be assayed concurrently. Cation fluxes are expressed in nmol/mg protein/min.

**CHEMICAL ASSAYS**

When these flux experiments were finished, the cultures were washed six times with cold 0.15 M LiCl in 15 s. Volumes of 3 ml of cold deionized and distilled water were added to the culture dishes, and then the cells were detached from the culture dishes and dispersed in the dishes with a silicone-rubber policeman. Aliquots of 2 ml of the cell suspension were sampled and mixed with 2 ml portions of 30 mM LiCl. These mixtures were kept at least overnight at room temperature and then their cellular contents of Na⁺, K⁺ and Rb⁺ were
determined with a flamephotometer (170-30, Hitachi Ltd., Tokyo, Japan) with 15 mM LiCl as an internal standard. Cellular contents of cations are expressed in nmol/mg protein.

Other samples of 1 ml of cell suspension were mixed with 1 ml of 1 N NaOH for protein assay by the method of Lowry et al. (1951) with bovine serum albumin (fraction V) as a standard.

For assay of the cellular ATP content, 2 ml portions of cell suspension in cold water prepared by the same procedure to those described above were incubated for 5 min in a boiling water bath to extract ATP, and ATP was measured by the luciferin-luciferase reaction in a luminescence photometer (Monolight™ 500, Analytical Luminescence Laboratory, San Diego, CA) as described previously [Ikehara et al., 1990]. The cellular ATP content is expressed in nmol/mg protein.

CULTURE TEMPERATURE REGULATION

We designed special incubators to keep the temperature of cell cultures strictly constant during assay of cation fluxes on exposure to an electromagnetic field. One of the incubators fit for the large polar face is shown in Figure 5. This incubator made of copper plate (1.5 mm thick) was of 129 mm diameter and 20 mm thickness, so that it could be placed in the 20 mm gap between two polar faces. The upper portion of the incubator consisted of four chambers of 37 mm diameter and 13 mm depth to contain four plastic culture dishes. This portion was covered with a plastic lid (1 mm thickness) with four round pads of styrene foam (35 mm diameter and 3 mm thickness) to cover the culture dishes for adiabatic purposes. Four thermistor sensors (1 mm diameter) connected to a thermometer (D117,
Figure 5. Schematic presentation of a special incubator to maintain the temperature of cultures. B, water bath; C, cell culture; D, culture dish; L, plastic lid; S, styrene foam; T, thermometer sensor. [Yamaguchi et al., 1992]

Takara Ind. Co., Tokyo, Japan) were inserted to the bottoms of the culture dishes through pin-holes in the lid and the pads for monitoring the temperatures of cultures continuously during assay of cation fluxes. The lower portion of the incubator consisted of a water bath, through which water flowed in the directions indicated by arrows and distributed heat evenly. So, differences in temperatures between any two dishes did not exceed 0.2°C and heating of the culture dishes by the hot polar faces was prevented. Normally, the temperature of the dishes was adjusted to be 37°C, but it could be changed from 0 to 50°C. One of the incubators was placed in the electromagnet and the other was kept outside the magnetic field as a control. We also made the similar incubators with a smaller diameter of 80 mm to contain only one dish to fit the small polar face. These incubators were normally used in combination with the air incubator described before for adjusting the temperature during medium replacement. This was because it took about 5 min to increase the temperature, from, for example, 37 to 40°C in these incubators, and this delay would influence Rb⁺ influxes determined in 15 min.

MICROFLUOROMETRY

The membrane potential of the cells was determined by a modification of the method of Wright et al. [1981]. We applied micro-fluorophotometry to cells loaded with a fluorescent indicator, 3,3’-dipropylthiadicarbocyanine iodide (diS-C3-(5)) (Molecular Probes, Eugene, OR), keeping the intracellular K⁺ concentration at 139 mM. The cyanine dye was added to the culture medium at 1 μM immediately after 2 h exposure to the time-varying magnetic field. For determination of fluorescence, the excitation wavelength was fixed at 540 nm and emission was determined in a wide wavelength range from 580 to 680 nm to collect sufficient energy of fluorescence with a modified fluorescence microscope (Optiphot, Nikon, Tokyo, Japan) equipped with a photon counter (PC-545A, NF Circuit Design Block Co., Yokohama, Japan). The membrane potential was calculated as the K⁺ equilibrium potential in the
Effects of Electromagnetic Fields on $K^+(Rb^+)$ Uptake by HeLa Cells

Figure 6. Major pathways of $K^+$ uptake in the membrane of HeLa cells.

presence of a $K^+$ ionophore (4 μM valinomycin), and the potential was related to the emission intensity. When 150 mM $K^+$ was needed, an impermeable anion gluconate was used instead of Cl$^-$ as a counter anion to prevent rupture of the cell membrane due to strong cell swelling.

Microphotographs of cells loaded with the fluorescent dye were taken with the same fluorescence microscope in the same emission wavelength range at a magnification of 200x (see Figure 13).

We also monitored changes in the electrical charge on the cell surface with the fluorescent pH indicator 4-heptadecyl-7-hydroxycoumarin by a modification of the method of Pal et al. [1983]. Immediately after adding this indicator to the culture medium at 6 μM, we exposed the cultures to the magnetic field for 2 h, and then determined total fluorescence energy in an emission wavelength range from 420 to 680 nm with the fluorescence microscope at excitation wavelengths of 340 and 380 nm. The ratios of the emissions at these wavelengths, $E_{340}/E_{380}$, were calculated and the ratios of exposed and control cells were compared.

HOMOGENEOUS FIELD EFFECTS ON Rb$^+$ INFLUX

Figure 6 is a schematic presentation of major pathways of $K^+$ influx through the membrane of HeLa cells, consisting of an Na-pump [Ikehara et al., 1984a; Ikehara et al., 1984b], a Na$^+/K^+/2Cl^-$ cotransport pathway [Miyamoto et al., 1986; Ikehara et al., 1990 and 1993] and a Ca$^{2+}$-dependent $K^+$ channel of the relatively small conductance [Ikehara et al., 1991; Takahashi et al., 1993]. About 50-70% of the total Rb$^+$ uptake was inhibited by 10 mM ouabain and about 70-80% of ouabain-insensitive Rb$^+$ uptake was inhibited by 0.1 mM furosemide, indicating that about 50-70% of the Rb$^+$ uptake was mediated by the Na-pump, and about 20-40% by the cotransport pathway. The fraction of the total $K^+$ influx mediated by $K^+$ channel was not determined, since $K^+$ permeability could be measured exactly only by the patch-clamp technique.

Figure 7 shows the effects of various magnetic flux densities of homogeneous magnetic fields from 0 (the level of terrestrial magnetism) to 1.6 T on active and passive Rb$^+$ influxes of HeLa cells at two different temperatures. No significant effects of the magnetic fields of different magnetic flux densities on total Rb$^+$ influx were detected without 100 μM ouabain but passive influx was observed in the presence of the inhibitor (ouabain-insensitive influx) at a normal temperature of 37.4° C (panel A, t-test at $p>0.05$). Therefore, the difference between the total and ouabain-insensitive Rb$^+$ influxes (ouabain-sensitive, active
Figure 7. Effects on Rb⁺ influxes into HeLa cells of homogeneous magnetic fields of various magnetic flux densities from 0 to 1.6 T. A. (37.4±0.2)°C. B. (42.0±0.1)°C.

Figure 8. Effects of a homogeneous 2 T magnetic field on Rb⁺ influxes at different temperatures. A. Total, ouabain-insensitive and ouabain-sensitive Rb⁺ influxes. B. Arrhenius plots of data in A.
influx mediated by the Na-pump) was unaffected. Data in this panel are mean values of results in six separate experiments expressed in arbitrary units. As a homogeneous magnetic field of even 2 T did not influence Rb+ influx in the absence or presence of ouabain at 37°C (see Figure 8), homogeneous magnetic fields did not markedly influence the active and passive Rb+ influxes when the magnetic flux density was less than 2 T. We also tested the effects of homogeneous magnetic fields of similar magnetic flux densities on ouabain-insensitive Rb+ influx in the presence of 0.1 mM furosemide at 37°C, but again observed no significant effects of the magnetic fields (data not shown). These results reveal that magnetic fields of less than 2 T do not significantly influence Rb+ influx mediated by the Na+/K+/2Cl⁻-cotransport or the leakage pathway(s) at normal temperature.

Similarly, no significant effects on Rb+ influx of homogeneous magnetic fields of the same magnetic flux densities were detected at an abnormally high temperature of 42°C (Figure 7B; t-test at p>0.05). Data in this panel are mean values of results in two separate experiments.

Kinouchi et al. [1988] have reported theoretical estimations of the Lorentz force for suppressing diffusion of charged particles such as Na⁺, K⁺, Ca²⁺ and Cl⁻, and plasma proteins. They pointed out that the threshold field strength for suppression is so high, (more than 10⁴ T) that the Lorentz force does not affect the diffusion of these particles at a magnetic flux density of a few T. Their estimation is consistent with our finding of no significant effects on passive Rb+ influx at less than 2 T.

Figure 8A shows the effects on active and passive Rb+ influxes of HeLa cells of a homogeneous 2 T magnetic field over a wide range of culture temperatures of 10 to 45°C. Rb+ influxes without ouabain increased linearly with increase in temperature from 10 to 37°C, and decreased at temperatures above 40°C in both control cells (exposed only to the terrestrial magnetic field) and cells exposed to the magnetic field (upper panel). No significant differences between the influxes into control cells and those exposed to the magnetic field were observed at any temperature tested (t-test, p>0.05). Ouabain-insensitive influx of about half the total influx increased with temperature from 20 to 37°C, but there was no significant difference between the values in cultures in control and tested conditions. Ouabain-sensitive Rb+ influx also increased with increase in temperature from 10 to 40°C, but unlike the ouabain-insensitive influx, it did not decrease markedly at 45°C (lower panel). Again, there was no significant difference between ouabain-sensitive Rb+ influx in control cells and those exposed to the magnetic field in the temperature range tested. The marked decrease in Rb+ influx without ouabain above 40°C was mainly due to decrease in passive flux at abnormally high temperatures.

These results show no significant effects on Rb+ influx of a strong homogeneous magnetic field of up to 2 T, implying the absence of effect of steady magnetic fields with a magnetic flux density of less than 2 T on Rb+ influx.

The data in Figure 8A are represented by Arrhenius plots in Figure 8B. The plots for ouabain-insensitive Rb+ influxes of control cells and those exposed to a magnetic field show linear distributions of experimental points from 10 to 37°C along the regression lines obtained by the least squares method, but above 37°C and especially above 40°C the points markedly deviated downward from the lines (upper panel). From the slopes of these regression lines, we estimated the activation energies for ouabain-insensitive (passive) Rb+ influxes to be 60.5 and 51.7 kJ/mol in control cells and cells exposed to the homogeneous 2 T magnetic field, respectively. Statistic analysis verified that the differences between the variances of the data (F-test), the regression coefficients (t-test) and the intersections of the regression lines with the ordinate (t-test) in control cells and those exposed to the magnetic field were all insignificant (p>0.05). We also compared the regression lines for ouabain-sensitive Rb+ influxes (lower panel) and found that none of the three parameters were signifi-
Table 3. Effects on Rb\(^+\) influx of strong, time-varying magnetic fields of different frequencies. [Yamaguchi et al., 1992]

<table>
<thead>
<tr>
<th>Frequency (Hz)</th>
<th>On-Off-time (sec)</th>
<th>Rb(^+) Influx (nmol/mg protein/min)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>19.6±2.2</td>
<td>7</td>
</tr>
<tr>
<td>1/20</td>
<td>10</td>
<td>16.7±2.1*</td>
<td></td>
</tr>
<tr>
<td>1/10</td>
<td>5</td>
<td>16.6±3.2</td>
<td></td>
</tr>
<tr>
<td>1/6</td>
<td>3</td>
<td>14.5±3.5**</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Passive</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.5±1.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.5±1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.8±1.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.8±1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Active</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.1±1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.3±1.8*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.9±2.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.9±3.6**</td>
<td></td>
</tr>
</tbody>
</table>

Values are means±SD, n, number of samples. * and **, Significantly different from values for control cells at p<0.05 and p<0.01, respectively.

Significantly different in control and test cells. The activation energies estimated from the regression lines were 54.9 and 51.4 kJ/mol for control and test cells, respectively.

Tenforde and Liburdy [1988] reported that a membrane consisting of phospholipid bilayers is markedly influenced by external magnetic fields near the phase transition point. They observed increase in efflux of a chemical agent from liposomes at a temperature (40.5°C) close to the phase transition point of the phospholipid on exposure to static magnetic fields, and found that the effect of the magnetic field was saturated at 0.1 T. Liburdy and Vanek, Jr. [1985] demonstrated significant increase in permeability to Na\(^+\) of red cell membranes on exposure to microwaves in the narrow temperature range of 17.7 to 19.5°C. However, a phase transition point has not been observed in the membranes of living cells of other than red cells, possibly due to the more complex components of cell membranes, including various proteins, than of artificial membranes. Consistent with this idea, the temperature-dependent characteristics of cell membranes are reported to be more complicated than those of membranes consisting of pure organic substances [Aceto et al., 1970]. The results in Figure 8 do not support the possibility of membrane phase transition in HeLa cells between 10 and 37°C.

TIME-VARYING FIELD EFFECTS ON CATION FLUXES

Table 3 shows the effects of the frequency of time-varying magnetic fields of the type presented in Figure 2A. The effects on Rb\(^+\) influx of magnetic fields with different durations of “on-time” and “off-time” up to 5 min were tested. However, when these durations were longer than 30 s. i.e., the frequency was less than 1/60 Hz, there was no significant influence of exposure on any fraction of Rb\(^+\) influx (data not shown). With decrease in the durations to 10, 5 and 3 s, corresponding to frequencies of 1/20, 1/10 and 1/6, respectively, total Rb\(^+\) influx without ouabain tended to decrease. There were significant differences between the influxes into control cells and those exposed to magnetic fields at frequencies of 1/20 (p<0.05) and 1/6 (p<0.01) by Dunnett’s t-test. Passive Rb\(^+\) influx in the presence of ouabain also tended to decrease with increase in the frequency, but the change was not significant. Ouabain-sensitive, active Rb\(^+\) influx was significantly inhibited by exposures to magnetic fields at these frequencies (p<0.05). Only active Rb\(^+\) influx at 1/10 Hz was not significantly inhibited in this experiment, probably because of the large coefficient of variance. In another experiment, in which the coefficient of variance at this frequency was smaller (data not shown), decrease in Rb\(^+\) influx was demonstrated to be significant (p<0.05). A frequency of 1/6 Hz was used in following experiments, unless otherwise stated.
For further investigation of the effects of time-varying magnetic fields, the time of Rb\(^+\) accumulation in the cells was prolonged to 2 h and the accumulations in control cells and those exposed to a magnetic field were compared (Figure 9). The accumulations of Rb\(^+\) in control and exposed cells without ouabain were not markedly different in the first 15 min. This insignificant difference was not consistent with the significant effect in Table 3, because we used the type of magnetic field shown by Figure 2C (type 2C), which would induce a weaker eddy current than that induced by the magnetic field of type 2A used for the results in Table 3. Since we needed more samples for the present experiment, we used the special incubator containing four culture dishes with pole pieces with a face of 100 mm diameter. The accumulation was significantly inhibited after 30 min, as judged by Dunnett's t-test of one-way layout after analysis of variance (ANOVA test, \(p<0.05\)). The inhibitory effect of the magnetic field became more significant with time until at least 120 min. In contrast, Rb\(^+\) accumulation in the presence of ouabain was not significantly influenced by exposure to the magnetic field. Hence, the inhibition of total Rb\(^+\) accumulation was due to inhibition of ouabain-sensitive Rb\(^+\) accumulation. These results showing that active, but not passive Rb\(^+\) influx is inhibited are essentially consistent with those in Table 3.

As we found that the Na\(^+-\)pump was inhibited by exposure to the magnetic field, we next tested the effect of exposure to the magnetic field on the cellular ATP content (Figure 10). Results showed that the ATP contents of cells whose Rb\(^+\) accumulations are shown in Figure 9 were not significantly influenced by exposure to the magnetic field for periods of up to 120 min, irrespective of the presence of ouabain.

The effects of exposure to the magnetic field on K\(^+\) efflux were also tested. The K\(^+\) contents of cells exposed for various periods after replacement of K\(^+\) by Rb\(^+\) in the medium were plotted on a semi-logarithmic scale and rate constants were determined by the procedures described before (Figure 11). The plots for both exposed and control cells were linear with
time, but the slope of the regression line for exposed cells was steeper than that for control cells, and the rate constants for control and exposed cells were determined to be 0.0071 and 0.0105/min, which were significantly different by the t-test (p<0.01). Since the cellular K\(^+\) content at the start of exposure was 932 nmol/mg of protein, the K\(^+\) effluxes from control and exposed cells were calculated to be 6.66 and 9.79 nmol/mg protein/min. These results demonstrate stimulation of K\(^+\) efflux by exposure to a magnetic field and suggest exposure-induced decrease in the sum of the intracellular K\(^+\) and Rb\(^+\) contents; i.e., the K\(^+\) content of cells in the normal medium.

**TIME-VARYING FIELD EFFECTS ON CELLULAR ELECTRICAL PROPERTIES**

We tested the effects of exposure to a time-varying magnetic field on the electrical properties of cells, using a fluorescent probe of the membrane potential, diS-C3-(5). Results showed that the fluorescence emission of cells loaded with the dye and exposed to the magnetic field for 2 h was significantly weaker than that of control cells (Table 4), suggesting a marked decrease in the emission of dye molecules attached to the cell surface or in the cells. Inhibition of the Na-pump (Figure 9), decrease in the cellular K\(^+\) content (Figures 9 and 11) and increase in the cellular Na\(^+\) content (data not shown) on exposure to the time-varying magnetic field also support the possibility of the occurrence of depolarization. Therefore, the exposure might cause extreme depolarization of the cell membrane.

To test this possibility, we examined the ratios of the emissions of cells incubated in media with various K\(^+\) concentrations in the presence of valinomycin to the emission of cells in normal medium without valinomycin to determine whether the ratio was proportional to the logarithm of the K\(^+\) concentration (Figure 12). Result clearly demonstrated a linear relation of the emission ratio of control cells with the logarithm of the K\(^+\) concentration, i.e.,

**Table 4.** Effect of exposure to a strong, time-varying magnetic field on emission of HeLa cells loaded with the fluorescent indicator of membrane potential diS-C3-(5). [Yamaguchi et al., 1992]

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Emission Intensity (arbitrary units)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n)</td>
</tr>
<tr>
<td>I</td>
<td>1±0.187 (38)</td>
</tr>
<tr>
<td>II</td>
<td>1±0.477 (53)</td>
</tr>
</tbody>
</table>

Values are means ± SD. n, number of cells determined. *, significantly different at p<0.001.
the membrane potential. Thus the dye acted as an indicator of the potential in control cells. In contrast, the emission ratio of exposed cells did not respond to a wide change in the K⁺ concentration in the medium, and the intensity of emission was again very weak. This suggests that the decrease in the emission intensity of exposed cells was not due to membrane depolarization, but probably to an exposure-induced change in electrical charge on the cell surface, since the dye molecule has a net negative charge.

Microphotographs of cells loaded with a fluorescent dye taken with the fluorescence microscope are shown in Figure 13. In control cells (panel A) the fluorescent dye has a more homogeneous distribution than in exposed cells (panel B), in which the distribution of dye is uneven and has a granular appearance. This uneven staining with the charged dye suggests change in the distribution of electrical charge on the cell surface on exposure.

**Figure 12.** Effect of exposure to a strong, time-varying magnetic field on emission of cells loaded with the fluorescent indicator diS-C3-(5) and incubated in media with various K⁺ concentrations in the presence of valinomycin. [Yamaguchi et al., 1992]

**Figure 13.** Microphotographs of HeLa cells loaded with the fluorescent indicator diS-C3-(5) taken with a fluorescence microscope at a magnification of 200 (objective lens 40x). A, Control cells. B, Exposed cells.
Next we tested the effects of the time-varying magnetic field on the electrical charge on the cell surface with the fluorescent pH indicator 4-heptadecyl-7-hydroxy coumarin. The ratio of emissions E340/E380 decreased with increase in pH of the culture medium from 6 to 8 (Figure 14). This result implies that the positive charge on the cell surface increases with increase in pH of the medium. This decrease in the emission ratio with increase in pH of the medium was also observed in cells exposed to the magnetic field. However, the exposed cells showed significantly higher values of the emission ratio at all pH values tested (p<0.01 by Dunnett’s t-test after ANOVA test).

The wavelengths of 340 and 380 nm are near the peaks in the spectra of the undissociated and dissociated forms of the fluorescent reagent, respectively (Pal et al., 1983). Therefore, the increase in the emission ratio E340/E380 with decrease in pH of the medium represents a relative increase in the undissociated form of the reagent with decrease in pH in both control and exposed cells. Exposure to the magnetic field seemed to inhibit the pH-dependent dissociation of H⁺ from the reagent attached to the cell membrane by increasing the negative charge on the cell surface. These results on the emission ratio are consistent with those shown in Table 4 and Figure 13. Thus we concluded that exposure to the magnetic field induced a relative increase in the negative charge on the cell surface.

An AC current has been demonstrated to decrease the activity of Na⁺,K⁺-ATPase in suspension [Blank and Soo, 1989]. On the other hand, there is a report that exposure to an ELF electric field increases the negative charge and exposure to an ELF magnetic field decreases the hydrophobicity of the surface of Physarum polycephalum [Marron et al., 1988]. Also, exposure of cultured U937 cells to a pulsed magnetic field of repeated bursts of 25 Hz has been shown to increase the negative charge on the cell surface [Smith et al., 1991]. Electro-osmotic flow of medium occurs when an electric field is applied in parallel to the cell surface, and negatively charged macromolecules are moved on the cells surface and accumulated on one side of the cell, as demonstrated by the accumulation of concanavalin A receptors on the cathodal side of muscle cells [McLaughlin and Poo, 1981].
We observed significant inhibition of the Na-pump by the strong, time-varying magnetic field and increase in the negative charge on the surface of HeLa cells. As an electrical force acting on a charge on the cell surface is proportional to the imposed current density, the magnitude of the eddy current would play an important role. In fact, we showed stronger effect of the magnetic field at higher magnetic flux density (compare the results in Table 3 and Figure 9). The frequency of change in the field is also important, as shown in Table 3. Inhibition of the Na-pump by exposure to the time-varying magnetic field would have a close relation to the eddy current or change in the electrical properties of the cell surface caused by the current.

REVERSIBILITY OF TIME-VARYING FIELD EFFECTS

Finally, we tested the reversibility of the effects of exposure to the strong, time-varying magnetic field on Rb⁺ influx and change in the cellular electrical properties.

Column A of Figure 15 shows Rb⁺ influx into exposed cells in 15 min in Rb⁺ medium after exposure in normal K⁺-medium for 75 min (total exposure time, 90 min). Column B shows that of cells incubated in Rb⁺-medium after exposure for 90 min in K⁺-medium, then incubation 30 min in K⁺-medium outside the magnetic field. Column C shows Rb⁺ influx of control cells. Values are means and SD of Rb⁺ influxes in seven independent experiments, four dishes being used in each experimental group. Mean Rb⁺ influx of exposed cells (column A) was significantly smaller than that of control cells, as judged by Dunnett's t-test after the ANOVA test (p<0.01), whereas the mean Rb⁺ influx shown in column B was not (p>0.05). Similar results were obtained in other experiments. Therefore, the inhibition of active Rb⁺ influx caused by exposure to the magnetic field was reversible.

Figure 16 shows the reversibility of the effect of the magnetic field on fluorescence emission of cells loaded with diS-C3-(5). Column A shows the emission intensity of cells exposed for 90 min, and columns B and C show those of cells exposed for 90 min and then placed outside the magnetic field for 30 and 60 min, respectively. Column D shows the fluorescence emission of control cells. The emission intensities shown by columns A and B were significantly lower than that of control cells according to Dunnett's t-test (p<0.01), but that shown by column C was not (p>0.05). Therefore, the effect of 90 min exposure was reversed after 60 min incubation outside the magnetic field.

The results in Figures 15 and 16 demonstrate the reversibility of the effects of the strong, time-varying magnetic field on both Rb⁺ influx and fluorescence emission of diS-C3-(5). These results support the idea that inhibition of the Na-pump by exposure to the magnetic field is closely connected with change in the electrical properties of the cell surface. However, further investigations are needed to determine whether the change in the electrical properties is directly connected to inhibition of Na-pump activity, because, the effects of

![Figure 16. Reversibility of the effect of exposure for 90 min to a strong, time-varying magnetic field on fluorescence emission of cells loaded with diS-C3-(5). A, Exposed. B and C, Exposed and then placed outside the magnetic field for 30 and 60 min, respectively. D, Control.](image-url)
exposure may be related to more substantial change in membrane structure than simple change in the surface properties, since more than 30 min was required for complete recovery.

CONCLUSIONS

Exposure to strong homogeneous magnetic fields with various magnetic flux densities of less than 1.6 T had no significant effect on either active or passive Rb* influxes into HeLa cells at normal or high temperatures. Exposure to a similar magnetic field of 2 T at different temperatures of 10 to 45°C did not cause any change in active or passive Rb* influx, and no evidence was obtained for the presence of a phase transition point of the cell membrane between 10 and 37°C.

In contrast, exposure to a strong, time-varying magnetic field of quasi-rectangular wave form caused significant inhibition of active Rb* influx when the frequency of change in the magnetic field was more than 1/20 Hz. Conversely, K+ efflux was stimulated, but passive Rb* influx was unaffected. Analyses of the amplitudes of the frequency components of the time-varying magnetic field B and its differential dB/dt revealed that B mainly consisted of components with the lowest angular frequencies, whereas dB/dt contained components of various frequencies. The inhibition of active Rb* influx was not due to change in the cellular ATP content.

Results obtained by micro-fluorometry with fluorescent probes of the membrane potential (diS-C3-(5)) and pH (4-heptadecyl-7-hydroxy-coumarin) showed change in the electrical properties of the cell surface on exposure to the time-varying magnetic field. Results suggested an uneven distribution of electrical charge and an increase in negative charge on the cell surface. The inhibition of active Rb* influx and the change in electrical properties of the cell membrane were reversible. Further studies are needed to determine whether change in electrical properties is the direct cause of inhibition of the Na*-pump.

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Effects of Electromagnetic Fields on $K^+(Rb^+)$ Uptake by HeLa Cells


EFFECTS OF EXPOSURE TO A 50 HZ MAGNETIC FIELD ON MELATONIN IN RATS

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1. INTRODUCTION

There has been increasing concern over possible human health risks associated with exposure to alternating current (AC) magnetic fields induced by transmission and distribution systems. To accurately assess the possible health risk, it is necessary to conduct a wide range of studies with appropriate exposure systems both for animal and human subjects. Because AC transmission lines are three phase, the magnetic field at ground level tends to be elliptically polarized (Deno, 1976) and is represented fairly well by a rotating vector. In the laboratory, this rotating vector can be simulated by the sum of horizontal and vertical magnetic fields 90° out of phase. It is very important to allow the simultaneous exposure of as many animals as possible. An exposure facility must be designed to fill these requirements.

Melatonin, which is synthesized in the pineal gland, has been assumed to have linkage to the etiology of cancer in at least the following three ways; 1) melatonin itself is oncostatic, 2) melatonin enhances certain facets of immune function and 3) melatonin functions as an inhibitor of the hypothalamic-pituitary-gonadal axis, hence it may reduce the availability of hormones that are required for the growth of certain hormone-dependent breast, ovarian and prostate cancers (Wilson and Anderson, 1990).

Welker et al. (1983) reported that experimental inversion of the horizontal component of the natural magnetic field during nighttime led to a significant decrease of pineal serotonin-N-acetyltransferase (NAT) activity and melatonin content in rats. Since then several papers have been published investigating the effects on pineal activity of geomagnetic fields inversion (e.g., Olcese and Reuss, 1986; Olcese et al., 1985; Stehle et al., 1988). Lerch et al. (1990) reported that pineal NAT activity of rats was depressed by intermittent exposure to magnetic fields. Yellon (1991) reported that 15-min exposure to a 60-Hz horizontal magnetic field, at a strength of 0.1 mT, suppressed the nighttime melatonin rise of Djungarian hamsters.
No papers, however, have been published concerning the effects of long-term exposure to alternating current magnetic fields on pineal gland activity. Therefore, in this experiment we investigated the effect of exposure to 50-Hz circularly and linearly polarized magnetic fields for 6 weeks on melatonin concentration in rats, using a newly developed exposure facility.

2. EXPOSURE SYSTEM

2.1 System Design and Calculation

Many systems have been proposed for exposure to magnetic fields (Miller et al. 1989; Wolpaw et al. 1989; Baum et al. 1991; Yasui & Otaka, 1993). A magnetic field can be generated by using a pair of Helmholtz coils. This provides a uniform field in an area perpendicular to and around the center of the axis between paired coils. Due to difficulty in establishing uniformity of magnetic fields for as large a volume as possible within limited experimental space, our proposed system consists of five equally spaced square coils, forming the surface of a cube. Initially, we referred to the three-coil design of Cohen’s (1992) exposure system used in the New York Project. Later, we extended our system to five sets of coils which would establish a relatively large uniformity within a limited experimental space. Figure 1 shows our proposed loop paired coils for a one-axis magnetic field. The configuration of five rectangular loops of dimensions d x d and spacing of d/4 between loops was proposed with different numbers of turns in pairs of loops, for generating a magnetic field. By superposition of fields obtained from the coil pairs C1 and C2, and single coil C3, total magnetic flux density B(x,y,z) at point P(x,y,z) is calculated by using the results given by Misakian (1984). After connecting five coils in series and adjusting the currents in the coils, a considerably uniform region can be achieved.

We assume that the current ratio is a:b:c:b:a when the five coils are connected in series and that the uniform field volume is about 0.6d x 0.6d x 0.6d cube in the center of d x d x d cubic volume. To obtain the current under this condition, the magnetic fields for Br(x,y,z) at three points inside the volume are chosen to be nearly the same values. Because of the cubic symmetry of our proposed configuration, the calculation led to the conclusion that the current ratio is 11:2:5:2:11, which is the same ratio of turns of coils. Using this current ratio and assuming d =1m, the uniform field volume is about an 0.6 m cube in the center.

This one-axis system is a suitable exposure system for animal experiments. If there is a requirement for both horizontal (Bh) and vertical (Bv) magnetic fields, two groups of coils can be set to provide vertical or horizontal orientations, respectively. Circularly polarized magnetic fields can be provided by two pairs of orthogonally placed coils.

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**Figure 1.** Coordinate system and configuration of the proposed loop paired coils for generating a one-axis magnetic field. (Shigemitsu et al. Bioelectromagnetics (1993) vol 14: 107-116).
2.2 System Construction

Figure 2 shows the exposure system. The system is formed as a 1.86 m cube. Because of limitation of space, two identical systems for exposure and sham-exposure were placed in the same room.

The floor plan of this room is shown in Figure 3, which gives approximate dimensions and shows the placement of the two systems. The x, y, z-directions were set in relation to the wall. The x is west-east while the y is south-north. The two identical systems are 3.7 m apart from center to center for x and 2.6 m apart for y. Design parameters for magnetic field generation are as follows:

**Horizontal.** Five 1.7 m square wooden frames at intervals of 0.425 m, with coils having 66 turns of insulated wire (cross section 2.0 mm²) in the outer paired coils, 12 turns...
in the middle and 30 turns in the center coil. This group is the entire 1.7 m cube. For these coils, the resistance was 8.0 Ω and the inductance was 73.6 mH.

**Vertical.** Five 1.8 m square wooden frames at intervals of 0.45 m, with coils having the same numbers of turns and the same insulated wire as the horizontal coils. This group is the entire 1.8 m cube, for in these coils the resistance was 13.5 Ω and the inductance was 79.7 mH.

**Rotating.** The cube for the horizontal magnetic field coils fits inside the cube for the vertical magnetic field coils with planes of the two sets of coils perpendicular to each other. This arrangement gives the horizontal magnetic field in the x-direction and the vertical magnetic field in the z-direction. An elliptically polarized magnetic field can be obtained if each coil is energized separately from the power supply.

Each system has three 1m square wooden shelves forming a 1m cube. The wooden shelves are employed as cage supports. These cage shelves are isolated from the coils which are supported by another wooden frame. This isolation eliminates mechanical vibration of the cages. Animal-housing space is within the 1m cube. Water supply and food pellets are placed on the top of each cage through a stainless steel grating. The standard wooden chip is used for bedding. Nine standard cages (30 cmW x 25 cmL x 17 cmH) can be placed on each shelf. However, a total of 24 cages were placed for the actual experiments because no cage was set on the center of the shelves so as to improve homogeneity of light distribution. To equalize illumination within the cages during experiments, animal cages were interchanged among the three shelves.

### 2.3 Magnetic Field Distribution

The magnetic field strength and distribution within the animal-housing space was measured by meters we developed (Shigemitsu et al. 1991). Figure 4 shows the results for uniformity of the magnetic fields. It shows the variation of the normalized magnetic flux density as a function of x, horizontal distance from the center of the coil at four different planes inside the 1m cube. Fig. 4A is the results of applying the vertical fields. Fig. 4B is the results of applying the horizontal ones. The overall uniformity of the magnetic field is more than 98 % within the animal-housing space of the facility.

### 2.4 Magnetic Field Circuitry

The power supply is located in the next room. The single phase 50 Hz, 200 V power is supplied to two inverter units having 1.5 kVA capacity which independently provide power to both coils. This energizing system can also provide 60 Hz. The system was designed to produce circularly polarized magnetic fields up to 0.3 mT without heating and vibration. Due to low heat generation in the coil, a temperature rise inside the animal cage could not be detected. This system adjusts the phase difference with an accuracy of 5°. The horizontal and vertical magnetic fields can be energized simultaneously. The exposure room door is locked to insure safety for laboratory personnel.

### 2.5 Stray Field

Because the exposure and sham-exposure systems are placed in the same room, the magnetic field of the exposure system will spill over into the sham-system. Using the MFM-14A meter, measurements of the stray field were made at four positions on the middle shelf of the 1 m² area in the sham system when the exposure system was turned on. The ratio
Effects of Exposure to a 50 Hz Magnetic Field on Melatonin in Rats

Figure 4. The magnetic field distribution along the x-axis and the y-axis on four different planes inside the 1 m cube of the animal-housing space. The values of the vertical axis are normalized to the values at the center. A: Results of applying the vertical magnetic field. B: Results of applying the horizontal field. (Shigemitsu, et al. Bioelectromagnetics (1993) vol. 14: 107-116).

of the exposure level to the sham level was overall 50:1. The ratio has a somewhat smaller gradient, about 2 %, along the diagonal direction in the sham system. The stray field in the sham system is somewhat distorted to ellipsoidal polarization compared with the circularly polarized magnetic field in the exposure system.
2.6 Background Level of Magnetic Field

The background level of the 50 Hz magnetic field in the exposure room was measured by using an MFM - 14 A meter. The average level was 0.014 μT. This value ranges from 0.034 μT to 0.01 μT depending on whether air-conditioning units are in operation. The value of the horizontal component of the static magnetic field was in the range of 25-27 μT and its orientation was parallel to the y-direction (Gauss Meter HM 201, Mishima Industry). The vertical component was in the range of 38-42 μT. There was no difference in static magnetic field between exposure and sham-exposure locations.

2.7 Harmonic Distortion

By placing the MFM-14 meter on the middle shelf of the exposure system, the harmonic distortion of the magnetic field was measured. The output of this meter was analyzed on a signal analyzer (SC-2100C Iwatsu). The total harmonic distortion was very small, about 0.03% (Shigemitsu et al. 1993; Kato et al. 1993).

2.8 Noise

The noise level was measured by using a sound-level meter (Octave band filter NX-01A, sound level meter NA-61, Rion). By placing a microphone in the center of the room and in both exposure and sham - exposure systems, the measurements were made with the air conditioner turned on or off and the circularly polarized magnetic field turned on at 0.4 mTrms \( (B_i=B_z) \). Results show that the air conditioner is the main contributor to noise. The sound level was about 70 dB, which is the general room noise. There was no difference in the sound level with the exposure system turned on or off.

2.9 Light Intensity

Four standard fluorescent lights were installed on the ceiling 3.5 m above the floor and another two were set up beside each system 1 m apart and 1 m from the floor. Light scattering panels were set up on the top of the two systems. The overall light intensity was 20.4 - 84.4 lux with 24 animal cages inside the animal-housing space.

2.10 Temperature and Humidity

The temperature and relative humidity inside the experimental room were continuously recorded. The temperature was maintained at 21 ± 2°C. Relative humidity varied from 40 to 60% depending on changes in the air-conditioning cycle and the season.

3. MELATONIN CONCENTRATION

By using this newly developed exposure facility and Wistar-King male rats as subjects, three experiments were conducted to determine whether 6 weeks of exposure to circularly polarized, horizontally and vertically oriented magnetic fields suppresses melatonin content in plasma and the pineal gland (Kato et al. 1993,1994). Plasma and pineal melatonin were assayed by radioimmuno-assay (RIA).

In the first experiment rats were exposed continuously to a circularly polarized magnetic field at 1, 5, 50 or 250 μT (spatial vector rms).
Figure 5. Levels of plasma melatonin at 12:00 and 24:00 h at different strengths of the magnetic field across time. Sample numbers of the rats, density in microteslas, and date of experimentation are indicated at the bottom of the figure. For example, 91/8 means the main part of the 6-week exposure was carried out during August 1991; C=control. The ordinate shows melatonin levels in picograms per milliliter. The levels determined at 0.02 μT, 0.01 μT, and 1 μT, 90/12 were from the rats housed in the sham-exposure facility in which the waveform of the field was ellipsoidal. (Kato, et al. Bioelectromagnetics (1993) vol. 14: 97-106).

Figure 5 summarizes the “no-fields” data and the data obtained by applying different strengths of the magnetic fields across time. No statistical difference was observed among the nighttime values for the control, 0.02 μT, or 0.01 μT (both stray fields), while a significant difference was detected between the control and 0.1 μT exposed animals at 12:00 h. There was a significant decrease in melatonin content at 12:00 h for fields stronger than 0.1 μT and at 24:00 h for fields stronger than 1 μT, compared with the control. There were no significant differences among the values at 24:00 h for exposures to fields stronger than 1 μT. At 12:00 h a significant decrease was observed at 50 μT.

Figure 6. Concentrations of melatonin in plasma and the pineal gland at 12:00h and 24:00h at horizontally oriented (Horizontal) and vertically oriented (Vertical) 1 μT magnetic fields. C is control values collected in control experiments. (modified from Kato, et al. Neuroscience Letters (1994) vol. 168: 205-208).
In the second experiment, rats were exposed to horizontally oriented, 1 μT magnetic fields ($B_x = 1 \mu T$, $B_y = 0$) and in the third experiment, magnetic field was vertically oriented, 1 μT ($B_h = 0$, $B_v = 1 \mu T$). Results from both experiments are shown in Figure 6, in which melatonin suppression at 24:00h after 6 weeks of exposure was not observed.

4. DISCUSSION

When one thinks about possible mechanisms of field exposure in animals, including humans, electric current induced in the body under electric and/or magnetic fields is a likely factor influencing body organs. Eddy currents induced by magnetic fields flow in loops which are greatest near the periphery of the body. The pineal gland of the rat is located just beneath the dura and skull, while in other mammals such as the cat, sheep, monkey, baboon and human, the pineal gland is located deep inside the brain. Considering these anatomical differences, we suggest that the pineal gland of the rat may be exposed to stronger eddy currents than in other mammals. Since the biological effects may not be simply explained by current density alone (K. Isaka, personal communication), other factors such as direction of the current, phase angles of the field, waveform, speed of onset of the field (Rogers et al., 1991), and location of the organs should be taken into consideration.

Olcese et al. (1985) compared the effects of magnetic field exposure on melatonin content between rats with severed optic nerves and intact animals, and Reuss and Olcese (1986) compared the effects between rats kept in a completely dark room and those kept under dim red light. Under both experimental conditions there was no effect of magnetic field exposure on melatonin level in the rats whose optic nerve system was not activated by light stimulus. Hence they concluded that light stimulation to some extent is essential for the magnetosensitivity. It has long been known that extremely low frequency (ELF) and transient magnetic fields of moderate flux densities generate visual phenomena called magnetophosphenes. Lövsund et al. (1979) reported that magnetophosphenes are generated in the retina and are in the same channels that normally propagate signals induced by light. Therefore, it is conceivable that signals set up in the retina by dim red light and magnetic fields, of which magnetic field alone is subthreshold, eventually reach the pineal cells to lower the synthesis of melatonin.

In our first experiment of circularly polarized field exposure at 1 μT suppressed melatonin concentration, while in the following experiments of linearly polarized, vertical or horizontal, magnetic field exposure it was demonstrated such suppressing effects do not take place. Because all other obvious experimental conditions, such as geomagnetic fields, age and strain of the rats, exposure period, etc. were quite similar in all of our experiments, differences in results obtained with rotating field exposure vs vertical or horizontal field can reasonably be attributed to differences in magnetic field characteristics.

Whether the primary effects of magnetic field exposure on melatonin production arise from induced currents in the pineal gland, from alterations in the tonal aspects of neuronal signaling, or from as yet unrecognized mechanisms, is a question that remains to be resolved in future studies.

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INVESTIGATION OF EXPOSURE TO EXTREMELY LOW FREQUENCY (ELF) MAGNETIC AND ELECTRIC FIELDS

Status of Laboratory Animal Studies

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ABSTRACT

There is now convincing evidence from a large number of laboratories, that exposure to extremely low frequency (ELF) magnetic and electric fields produces biological responses in animals. Many of the observed effects appear to be directly or indirectly associated with the neural or neuroendocrine systems. Such effects include increased neuronal excitability, chemical and hormonal changes in the nervous system, altered behavioral responses, some of which are related to sensing the presence of the field, and changes in endogenous biological rhythms. Additional indices of general physiological status appear relatively unaffected by exposure, although effects have occasionally been described in bone growth and fracture repair, reproduction and development, and immune system function. A major focus of ongoing research in the laboratory is to determine whether the epidemiological-based suggested association between EMF exposure and risk of cancer can be supported in studies using animal models. Three major challenges exist for ongoing laboratory research: 1) knowledge about the mechanisms underlying observed bioeffects is incomplete, 2) we do not as yet understand what physical aspects of exposure produce biological responses, and 3) health consequences resulting from ELF exposure are unknown. Although no animal studies clearly demonstrate deleterious effects of ELF fields, several are suggestive of potential health impacts. From the perspective of laboratory animal studies, this presentation will discuss biological responses to ELF magnetic and/or electric field exposures.

INTRODUCTION

Over the past several decades, the likelihood for humans and animals to be exposed to ELF magnetic (H) and electric (E) fields has increased substantially. Furthermore, the...
level of exposure has increased by orders of magnitude over the natural background of generally very low E and H fields. Such elevated fields span all frequency ranges; however, specific interest is currently focused on the potential biological impact of increased power frequency (50 and 60 Hz) fields. Detailed reviews on the bioeffects of ELF fields are available\textsuperscript{1,2,3}.

In the past twenty-five years, research programs throughout the world have made significant progress in describing interactions between H and E fields found in the environment and living organisms. Also addressed have been questions regarding biological effects from such fields, both real and potential, and whether such effects are permanent or transient, detrimental or beneficial. In H and E field studies that have been reported, exposure levels have been utilized from 0.1 to 30 millitessla (mT) and from a few volts/meter (V/m) to more than 100 kV/m. Similarly, a broad range of biological endpoints have been examined for evidence of possible ELF field exposure effects.

It should be noted that most indices of general physiological status appear to be relatively unaffected by exposure to ELF fields. It is also generally recognized that exposure to such fields does produce responses in specific biological systems, however, health implications for humans and animals have yet to be determined. Where experimental effects are demonstrated, the mechanisms of interaction between the field and the organisms remain largely unknown. For instance, it is not known whether observed effects result from fields acting at the surface of the body, E fields induced in the interior of the body, or from H fields penetrating the body.

Even though the effects of H and E fields in humans are of primary importance, many areas of biological interest are more effectively investigated using other animal species. Results from experimental laboratory research in rats, mice, birds, swine, and nonhuman primates form the basis for this paper, with emphasis on those areas which appear to be most sensitive to ELF fields and which may provide information of most relevance to human ELF exposure.

NERVOUS SYSTEM

Many of the biological effects which have been reported in animals or humans exposed to ELF H or E fields appear to be associated with the nervous system. Such system responsiveness to fields might be anticipated since the nervous system plays a basic role in the interaction of animals with their environment. Indeed, other biological systems may be influenced indirectly by ELF exposure through neural/neuroendocrine (hormonal) functions. Reported nervous system effects from ELF exposures include changes in behavioral and activity response; chemical changes in nerve cells; changes in the excitability of nerves; altered neurotransmitter and neurohormone levels; and disruption of biological rhythms.

Behavior: Among the most sensitive measures of perturbation in a biological system are tests which determine modifications in the behavioral patterns of animals. Behavioral studies in several species provide evidence of E field perception, as well as indications that behavior can be altered by exposure. The threshold of detection of 60-Hz E fields has been reported to be between 4 and 10 kV/m in rats\textsuperscript{4}. E-field thresholds in other animal species, including mice\textsuperscript{5}, pigs\textsuperscript{6}, and birds\textsuperscript{7}, have been reported in the 25- to 35-kV/m range. Perception of H fields has not been observed in the submillitelsa range\textsuperscript{6}, however, visual perception in the form of phosphenes has been demonstrated with H fields above 20 mT in humans\textsuperscript{9,10}.

Avoidance behavior of animals has been investigated at several E-field strengths. With some exceptions, animals generally avoid exposure to E fields over 50-75 kV/m\textsuperscript{11}.  

Under those levels, changes in physical activity responses have been reported although the changes are usually transitory [see review 12]. Effects of H fields on behavior are curious in that many of the investigations performed at low field intensities showed behavioral alterations (primarily activity changes) [see review 13]. In contrast, studies conducted at higher H-field intensities demonstrated no evidence of effects on animal behavior.

Neurochemistry. A number of studies have investigated the central nervous system (CNS) for changes in various chemicals in the brain upon exposure to ELF fields. In general, these studies report small, albeit highly variable changes in certain neurotransmitters14. The data provide limited evidence that exposure to E fields in the ELF range may cause slight changes in nervous system function. The number of experiments is not large, and there are significant questions about the strength and validity of several of the studies. Recent experiments suggest significant changes in norepinephrine content in specific brain regions of the hamster exposed to 60-Hz magnetic fields15.

Neurophysiology. In the area of neurophysiology a confusing array of studies claims both effects and noneffects of ELF-field exposure. A case in point is the commonly used measure of general CNS activity, the electroencephalogram (EEG) where significant alterations have been reported in some studies but not in others14. In an assessment of a more specific electrical "fingerprints" of the brain, evoked responses, no effects caused by exposure were observed in visual evoked response16, however, changes have been demonstrated in somatosensory evoked response17.

Biological Rhythms. A number of investigations have been conducted to examine the effects of ELF fields on natural biological rhythms. The phase and duration of activity and rhythms of oxidative metabolism have been shown to be shifted in male mice by exposure to ELF fields19. Wilson and coworkers19 measured the changing levels of indolamines and enzymes in the pineal glands of E-field exposed rats. A significant reduction in the normal night-time rise of melatonin and it’s associated synthetic enzymes was observed in rats exposed to either 1.5 or 40 kV/m. In more recent studies, nocturnal pineal components in mice and rats were shown to be sensitive to rotated H fields20 and 50-Hz magnetic fields21.

It is difficult to interpret the possible health consequences from the work thus far published on circadian or biological rhythm effects from ELF exposures. However, it is evident that E and H fields may alter the biological timing mechanisms in mammals. It is possible that such changes could mediate significant alterations in other areas where effects have been observed (e.g. behavior, reproduction and development). In addition, plausible mechanisms involving ELF-induced hormonal changes have been proposed as a possible basis for increased risk of adverse health outcomes22.

REPRODUCTION AND DEVELOPMENT

It is generally assumed that the developing organism, including pre- and postnatal mammals, are more sensitive to physical or chemical agents than are adult animals. This greater sensitivity, when it occurs, is thought to originate in subtle effects on the processes and controls which guide the developing cellular interactions. A number of studies have been conducted to examine the effects of ELF exposure on reproduction and development of both mammalian and nonmammalian species. Most of the nonmammalian studies have been performed in birds, either chickens or pigeons. E-field exposure of chicks at several field strengths, both before and after hatching, did not produce any significant effects in viability, morphology, behavior, or growth23. There have been some studies that reported deleterious
effects of E fields on postnatal growth and survival in prenatal mammals, however, these studies are countered by others in which rats, rabbits, or mice were exposed to broad range of field strengths with no evident effects on reproduction, survival, and growth\textsuperscript{23,24}.

Few studies have been performed to examine the effects of ELF H fields on growth and development. In the most comprehensive study published to date, no reproductive or developmental effects of 60-Hz H fields were observed\textsuperscript{25}. These results have been generally supported by a recent study performed in Finland\textsuperscript{26}. A great deal of interest has been generated by reports from Delgado's lab; that significant increases in malformation rates were observed in chick eggs exposed to low levels of pulsed magnetic fields\textsuperscript{27}. Subsequent efforts have partially confirmed these results\textsuperscript{28}.

**BONE GROWTH AND REPAIR**

In animals exposed to 60-Hz E-fields, it appears that bone growth in rats and mice, per se, was not affected by exposure to 100 kV/m, however, bone-fracture repair was retarded\textsuperscript{29}. Pulsed H-field exposure of bone produces quite different responses, with enhanced repair\textsuperscript{30}.

**IMMUNOLOGY**

Exposure of animals to electric fields does not appear to affect the immune system. In a comprehensive investigation of the immune system, no effects of exposure at very low field strengths (150-250 V/m) in mice or rats were observed\textsuperscript{31}. In contrast to the apparent lack of E-field influence in vivo on the immune system, magnetic fields are reported to strongly affect system responses to mitogens and antigens\textsuperscript{32}.

**CARCINOGENESIS AND MUTAGENESIS**

Because of the increasing number of epidemiological reports of positive correlations between ELF fields and cancer, considerable research interest has been generated concerning a possible connection between H-fields and cancer. To date there are few published laboratory animal studies that bear directly on this question, however, an increasing number of investigations are now being conducted.

A number of animal models can be utilized in the laboratory to investigate the issue of EMF and cancer. The selection of a specific model is principally dependent upon the hypothesis chosen to investigate a specific underlying mechanism. For example, if one desires to test EMF for its potential to be a complete carcinogen (an agent, that by its application alone, can give rise to the development of cancer), 1 1/2 to 2 years of exposure of mice or rats to the EMF is necessary. Throughout that period of time exposure of the animals to other possibly confounding agents must be kept to a minimum. In such a study, animals are observed during the major portion of their lifetime and the occurrence of tumors, in number, type, and time of development are the biological endpoints of interest. Complete carcinogen studies require several dose groups and a relatively large number of animals, particularly if the natural occurrence of a tumor type is low. Clearly, studies evaluating complete carcinogenicity are complicated and expensive because of the length of time and the number of animals involved.

Carcinogenesis is generally recognized as a multistep process, therefore, another approach is to assume the EMF acts either as an initiator or a promoter where a two phase
Protocol is required for testing. "Initiation" is defined as a genotoxic event in which the carcinogen causes a direct effect on the DNA. "Promotion" is operationally defined, as an enhancing agent that is applied subsequent to initiation over a protracted time period. Promotion is tied to a number of subcellular events that are usually non-genotoxic and is involved in the conversion of initiated cells to cancerous cells. To evaluate the agent (EMF) as an initiator, one high dose of EMF would be given followed by repeated exposure to a model promoter (e.g. 12-O-tetradecanoylphorbol-13-acetate. TPA). If, on the other hand, EMF were to be investigated as a potential promoter, the animals would be treated with a potent initiator of cancer (e.g. 7,12-dimethyl benz[a]anthracene, DMBA), and subsequently exposed to EMF over a period of several months. These initiation/promotion approaches use fewer animals and involve shorter experimental time and less cost than complete carcinogenicity studies. However, a specific initiation/promotion model is usually restricted to evaluating one or few specific cancers and may provide only limited information on possible biological mechanisms of EMF and development of cancer.

Complete Carcinogen Studies. Few truly long-term animal studies examining EMF as a complete carcinogen have yet been completed, although several are underway (in the US, Italy, Japan, and Canada). However, several studies designed to evaluate EMF as a promoter of cancer have contained control groups that were exposed to EMF without being treated with chemical carcinogen (initiator). These studies include a mammary tumor promotion study in rats, a lymphoma study in mice, and a mouse skin tumor promotion study. A major deficiency of using such "add-on" studies to evaluate complete carcinogenicity is the small group sizes involved. The Beniasjshvili study found an increase in mammary gland tumors in rats exposed to 20 μT for 3 hours per day compared to unexposed animals. The other two studies reported no increase in tumors with long term exposure to magnetic fields (500 or 50 μT and 15 μT, respectively).

Tumor Initiation Studies. No tumor initiation studies have yet been reported in the literature. There is very little motivation for such studies because of the very weak energies involved in extremely low frequency electric and magnetic fields: energies that are too weak to break chemical bonds. Furthermore, invitro studies have provided no evidence that DNA molecules can be damaged by exposure to 50/60 Hz EMF.

Tumor Promotion Studies. Despite the obvious need for EMF promotion studies, based on the suggested associations between EMF and cancer found in the epidemiological results, relatively few animal experiments have been completed. Skin tumor promotion, after initiation with DMBA, was examined in mice exposed to a 2 mT, 60 Hz continuous magnetic field, 6 hr/d, 5 days/wk for up to 21-23 weeks. None of the exposed or sham exposed mice developed papillomas. When magnetic fields were combined with application of TPA, a slightly earlier development of tumors was observed in the magnetic field-exposed animals.

Rannug and coworkers have conducted both skin tumor and liver foci studies in Sweden. In the two-year skin tumor promotion study, mice were initiated with DMBA then exposed to 0.5 mT or 50 μT, 50 Hz magnetic fields for 19-21 hr/d. No evidence of a field-exposure effect was observed in either systemic or skin tumor development or in skin hyperplasia. In the liver foci study, rats were treated with similar EMF exposures over a 12 week period. The exposed animals showed no differences in foci development from the sham exposed rats. In animals treated with a chemical promoter (phenobarbital) as well as the magnetic field, foci formation was slightly inhibited when compared to initiated-only animals.

In a series of experiments conducted in Germany, perhaps the strongest evidence for the carcinogenicity potential of EMF in rats has been reported. Generally, rats...
were exposed for 3 to 4 months to 50 Hz magnetic fields ranging from 0.1 to 30 mT. Initiation was accomplished with repeated oral doses of DMBA and subsequent development of mammary tumors was determined. In some of the experiments, the tumor incidence was increased in EMF exposed animals. In other experiments of this series, the number of tumors per tumor bearing animal was increased but the total tumor incidence was not affected. In still other efforts at replicating the initial results, non-statistically significant trends were seen but the clear enhancement of tumor development with EMF exposure was not observed. These apparent differences in results from the same research group may reflect differences in animal response at the different field intensities used or may simply be a reflection of the differences in group size between experiments. Several efforts are now underway to try and replicate this important studies on EMF and mammary cancer.

Prior to the Mevissen study, a group in Georgia also examined mammary carcinogenesis in EMF-exposed animals that were initiated with N-nitroso-N-methylurea. In the groups of animals exposed to a 20 μT, 50 Hz magnetic field for 3 hr/d, for the lifetime of the animals, there was an increased incidence of NMU-induced mammary tumors over the sham animals or animals exposed to only 1/2 hr of EMF per day.

SUMMARY AND CONCLUSIONS

Numerous studies have been initiated to determine the nature of the physical mechanisms involved in ELF field-induced effects and to what extent an electrical environment containing H or E fields poses a health hazard to living organisms. The biological effects reported in many of the experiments have not confirmed pathological effects, even after prolonged exposures to high-intensity H (10-mT) and high-strength E fields (100 kV/m) fields. However, the question of whether an occurrence of a "biological effect" from field exposure constitutes a health hazard has yet to be answered. Areas in which effects have been demonstrated appear to be associated primarily with the nervous system. In addition, in several instances, unconfirmed or controversial data exist where observed effects may be due to the fields (e.g., changes in brain chemistry and morphology, alterations in reproduction and development). It is not yet known whether confirmed or putative effects are due to a direct interaction of the electric field with tissue or to an indirect interaction, e.g., a physiological response due to detection and/or sensory stimulation by the field. Future research thrusts, at the minimum, need to address the following topics: 1) an investigation of what aspects of field exposure constitutes dose to living systems; 2) a determination of the interaction mechanisms between ELF fields and animals/humans; 3) an exploration of the implications of observed effects on nervous system function; 4) an evaluation of the potential effects of ELF fields on reproduction and early development; and 5) a determination of whether cancer promotion and/or progression is influenced by ELF fields.

Research sponsored today in several countries is designed to provide data to help set maximum levels of ELF field intensities which are biologically acceptable for both workers and the general public. This research also addresses the broader issues of increased electromagnetic fields in the environment from a variety of sources, and the potential health implications for humans.

ACKNOWLEDGEMENTS

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REFERENCES


MILLIMETER-RESOLUTION DOSIMETRY FOR EM FIELDS FROM MOBILE TELEPHONES AND POWER LINES

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INTRODUCTION

Increasingly finer-resolution anatomically based heterogeneous models of the human body are being used for calculations of induced electric fields, current densities, and specific absorption rates for electromagnetic exposures from extremely low frequencies (ELF) to microwave frequencies [1, 2]. Because of the need to extend dosimetric calculations to higher frequencies of several gigahertz, and for small-volume exposure devices such as mobile telephones, hair dryers, hair clippers, etc., resolutions on the order of millimeters are needed. A new mm-resolution model based on the MRI scans of an adult male volunteer has, therefore, been developed [1, 3, 4]. This model, with a resolution of 3 mm in the vertical direction and a pixel size of 1.974 x 1.974 mm has, to date, been used to calculate coupling of EM fields from handheld wireless communication systems operating at centerband frequencies of 835 and 1900 MHz [4], and for spatially varying 60-Hz magnetic fields of a hair dryer and a hair clipper [5].

THE FINITE-DIFFERENCE TIME-DOMAIN METHOD

The numerical method used for all of the calculations given in this paper is the finite-difference time-domain (FDTD) method. This method was first proposed by Yee [6] and later developed by Taflove and colleagues [7-10], Holland [11], and Kunz and Lee [12]. We have extended the method for calculations of the distributions of electromagnetic (EM) fields and SARs in anatomically based models of the human body for whole-body or partial-body exposures due to far-field or near-field irradiation conditions [13-16] and for electromagnetic pulse (EMP) exposures [17]. In this method, the time-dependent Maxwell’s curl equations
\[ \nabla \times \mathbf{E} = -\mu \frac{\partial \mathbf{H}}{\partial t}, \quad \nabla \times \mathbf{H} = \sigma \mathbf{E} + \mathbf{\varepsilon} \]

are implemented for a lattice of subvolumes or “cells” that may be cubical or parallelepipided with different dimensions \( \delta_x, \delta_y, \) and \( \delta_z \) in x-, y-, or z-directions, respectively. The details of the method are given in several of the above referenced publications and will, therefore, not be repeated here.

In the FDTD method it is necessary to represent not only the scatterer/absorber such as the human body or a part thereof, but also any near-field source/s such as an antenna and handset of a wireless telephone by means of the volume-averaged electrical properties \( (\epsilon_r, \sigma) \). The source-body interaction volume is subdivided into the Yee cells. The interaction space consisting of several hundred thousand to a few million cells is truncated by means of absorbing boundaries. Initial fields often assumed to be sinusoidally varying are tracked in time for all cells of the interaction space. The problem is considered completed when a sinusoidal steady-state behavior for \( \mathbf{E} \) and \( \mathbf{H} \) is observed for the interaction for single-frequency irradiation or the incident pulse has died off for transient exposures.

**MILLIMETER-RESOLUTION MODEL OF THE HUMAN BODY**

We have developed a millimeter-resolution model of the human body from the magnetic resonance imaging (MRI) scans of a male volunteer of height 176.4 cm and weight 64 kg. The MRI scans were taken with a resolution of 3 mm along the height of the body and 1.875 mm for the orthogonal axes in the cross-sectional planes. Even though the height of the volunteer was quite appropriate for an average adult male, the weight was somewhat lower than an average of 71 kg, which is generally assumed for an average male. This

![Image](image_url)

**Figure 1.** A typical cross section of the millimeter-resolution model of the human body. This particular cross section is for layer no. 49, which is 12 cm below the top of the head. Shown are the contours of the eyes, the optic nerves, cerebellum, etc., against the background of a grid of cells of dimensions 1.974 \( \times \) 1.974 mm.
problem can, to some extent, be ameliorated by assuming that the cell dimensions for the cross sections are larger than 1.875 mm by the ratio of \((71/64)^{1/2} = 1.053\), i.e., 1.974 mm instead of 1.875 mm. Using a software package from the Mayo Clinic called ANALYZE, the MRI scans were converted into images involving 29 tissue types whose electrical properties \((\varepsilon_r, \sigma)\) can then be prescribed at the frequency of interest. Shown in Fig. 1 is a typical cross section of the new model of the body. This is a section through the eyes and the cerebellum. Also shown in Fig. 1 are the contours for the various features of this cross section such as the eyes, ear, optic nerve, cerebellum, etc.

**SAR DISTRIBUTIONS FOR MOBILE TELEPHONES**

Cellular telephones and mobile wireless communication systems are being introduced into society at a very rapid rate. This has resulted in a public concern about the health hazards of RF electromagnetic fields that are emitted by these devices. We have used the head and neck parts of the above described millimeter-resolution model of the body to study electromagnetic absorption for a number of mobile telephones operating at centerband frequencies of 835 MHz, and some devices that may be designed for use at 835 and 1900 MHz, respectively. The peak 1 cm\(^3\) mass-normalized rates of energy absorption (specific absorption rates or SARs) may be compared with the ANSI/IEEE C95.1-1992 RF Safety Guidelines [18]. These safety guidelines are given in terms of maximum permissible exposures (MPE) of electric and magnetic fields, or of power density for controlled and uncontrolled environments. Though simple to use for far-field, relatively uniform exposures, the MPE limits are not easy to use for highly nonuniform fields such as in the near-field region of a mobile telephone. An alternative procedure given in the following [18] has, therefore, been suggested to satisfy the safety guidelines for uncontrolled environments which are defined as situations where there is exposure of individuals who have no knowledge or control of their exposure.

An exposure condition can be considered to be acceptable if it can be shown that it produces SARs "below 0.08 W/kg, as averaged over the whole body, and spatial peak SAR values not exceeding 1.6 W/kg, as averaged over any 1 cm\(^3\) of tissue (defined as a tissue volume in the shape of a cube)."

Since negligible SARs result for the lower regions of the body, we have used the upper 42-cm height of the body involving head, neck, and the upper torso. Taken from references 19-22, the dielectric properties used for the various tissues at center frequencies of 835 and 1900 MHz are given in Table 1. To simulate a handset that is typically tilted forward by about 33° for a vertically erect head, we have modified the MRI-based model so that it is tilted forward by 33°. With this forward tilt accomplished by displacing each successive layer of the MRI scans separated by 3 mm back by 1 cell, i.e., 1.974 mm, the handset may then be held in the vertical position for SAR calculations. The vertical orientation of the handset and the antenna allows a more accurate modeling of their shapes and dimensions. For commercially used telephones 1-10, given in Tables 2 and 3, we have used the X-ray pictures of the various antennas to model the exact dimensions of the radiating elements. Models for each of the antennas and the handsets were assumed to be covered with insulating materials of prescribed dielectric constants (typically 4.0 and 3.0). Since the coatings of these materials are thinner (generally 1 mm) as compared to the cell size of 1.974 mm, effective dielectric constants \(K_e\) given by the following equation are used for the cells covering the antennas and the handsets.
Table 1. Dielectric properties of the various tissues assumed for the model of the head and neck at midband mobile telephone frequencies of 835 and 1900 MHz

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>Tissue</th>
<th>( \varepsilon_r )</th>
<th>( \sigma )</th>
<th>( \varepsilon_r )</th>
<th>( \sigma )</th>
<th>( \varepsilon_r )</th>
<th>( \sigma )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>muscle</td>
<td>51.0</td>
<td>1.35</td>
<td>50.0</td>
<td>1.08</td>
<td>48.0</td>
<td>1.54</td>
</tr>
<tr>
<td>2</td>
<td>fat</td>
<td>7.2</td>
<td>0.16</td>
<td>11.0</td>
<td>0.17</td>
<td>11.0</td>
<td>0.28</td>
</tr>
<tr>
<td>3</td>
<td>bone</td>
<td>7.2</td>
<td>0.16</td>
<td>21.0</td>
<td>0.33</td>
<td>16.5</td>
<td>0.45</td>
</tr>
<tr>
<td>4</td>
<td>cartilage</td>
<td>7.2</td>
<td>0.16</td>
<td>37.0</td>
<td>0.80</td>
<td>36.0</td>
<td>1.22</td>
</tr>
<tr>
<td>5</td>
<td>skin</td>
<td>35.0</td>
<td>0.60</td>
<td>35.0</td>
<td>0.60</td>
<td>55.0</td>
<td>0.55</td>
</tr>
<tr>
<td>6</td>
<td>brain</td>
<td>43.0</td>
<td>0.86</td>
<td>43.0</td>
<td>0.86</td>
<td>41.1</td>
<td>1.14</td>
</tr>
<tr>
<td>11</td>
<td>blood</td>
<td>64.0</td>
<td>1.25</td>
<td>55.0</td>
<td>1.86</td>
<td>54.0</td>
<td>2.27</td>
</tr>
<tr>
<td>12</td>
<td>eye</td>
<td>70.0</td>
<td>1.90</td>
<td>70.0</td>
<td>1.90</td>
<td>70.0</td>
<td>2.70</td>
</tr>
<tr>
<td>17</td>
<td>cerebrospinal fluid (CSF)</td>
<td>76.0</td>
<td>1.75</td>
<td>78.0</td>
<td>1.97</td>
<td>78.0</td>
<td>2.76</td>
</tr>
<tr>
<td>18</td>
<td>vitreous humor</td>
<td>73.0</td>
<td>1.90</td>
<td>67.0</td>
<td>1.68</td>
<td>74.0</td>
<td>2.35</td>
</tr>
<tr>
<td>19</td>
<td>sclera/cornea</td>
<td>52.0</td>
<td>1.80</td>
<td>51.0</td>
<td>1.13</td>
<td>50.0</td>
<td>2.30</td>
</tr>
<tr>
<td>20</td>
<td>lens</td>
<td>45.0</td>
<td>0.75</td>
<td>33.0</td>
<td>0.79</td>
<td>42.0</td>
<td>1.20</td>
</tr>
</tbody>
</table>

* These numbers correspond to the tissue types used for the model of the whole body.

\[
K_e = \frac{\delta \varepsilon_r}{[\varepsilon_r (\delta - w) + w]}
\]

where \( w \) is the thickness of the coating in millimeters and \( \delta \) is the dimension of the Yee cell which may be \( \delta_x \), \( \delta_y \), or \( \delta_z \), depending on the surface. Because of the proximity of the hand to the telephone, it is essential to also model the hand for numerical calculations.

Table 2. Calculated and measured SARs for the various commercial telephones for the maximum radiated power of 0.6 W or 600 mW at 835 MHz

<table>
<thead>
<tr>
<th>Telephone No.</th>
<th>Calculated whole-body-averaged SAR mW/kg</th>
<th>W/kg</th>
<th>Calculated W/kg</th>
<th>Measured W/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.35</td>
<td>1.29</td>
<td>3.60</td>
<td>1.56-2.80</td>
</tr>
<tr>
<td>2</td>
<td>1.58</td>
<td>0.97</td>
<td>2.20</td>
<td>1.61-2.91</td>
</tr>
<tr>
<td>3</td>
<td>1.60</td>
<td>1.33</td>
<td>2.97</td>
<td>2.80-5.30</td>
</tr>
<tr>
<td>4</td>
<td>1.58</td>
<td>0.59</td>
<td>1.87</td>
<td>1.94-2.84</td>
</tr>
<tr>
<td>5</td>
<td>2.13</td>
<td>1.99</td>
<td>4.03</td>
<td>1.82-3.57</td>
</tr>
<tr>
<td>6</td>
<td>0.89</td>
<td>0.51</td>
<td>1.72</td>
<td>0.80-1.70</td>
</tr>
<tr>
<td>7</td>
<td>0.81</td>
<td>0.49</td>
<td>1.59</td>
<td>0.47-1.28</td>
</tr>
<tr>
<td>8</td>
<td>1.09</td>
<td>0.31</td>
<td>0.96</td>
<td>0.70-0.84</td>
</tr>
<tr>
<td>9</td>
<td>2.30</td>
<td>0.70</td>
<td>1.96</td>
<td>0.86-1.92</td>
</tr>
<tr>
<td>10</td>
<td>0.88</td>
<td>0.51</td>
<td>1.63</td>
<td>1.81-3.07</td>
</tr>
<tr>
<td>4*</td>
<td>1.60</td>
<td>0.35</td>
<td>2.48</td>
<td>1.94-2.84</td>
</tr>
</tbody>
</table>

* Values calculated for telephone no. 4 using the new tissue properties at 835 MHz given in Table 1.
Table 3. Peak SARs for 1 g of tissues of the head, brain, and "hand" for the maximum telephone power of 0.6 W at 835 MHz

| Telephone No. | Peak SAR for any 1 cm³ of tissue¹ | | |
|---------------|---------------------------------|---|---|---|
|               | of the head W/kg                | of the brain W/kg | of the "hand" W/kg |
| 1             | 0.57                            | 0.26                      | 0.66                      |
| 2             | 0.38                            | 0.21                      | 0.41                      |
| 3             | 0.51                            | 0.28                      | 0.59                      |
| 4             | 0.28                            | 0.13                      | 0.49                      |
| 5             | 0.69                            | 0.41                      | 0.71                      |
| 6             | 0.26                            | 0.10                      | 0.15                      |
| 7             | 0.26                            | 0.10                      | 0.09                      |
| 8             | 0.16                            | 0.06                      | 1.90                      |
| 9             | 0.48                            | 0.16                      | 0.33                      |
| 10            | 0.25                            | 0.14                      | 0.24                      |
| 4*            | 0.35                            | 0.15                      | 0.49                      |

¹ Defined as volume in the shape of a cube.
* Same as for Table 2.

For the present calculations we have modeled the hand by a region of 2/3 muscle-equivalent material of thickness 1.974 cm (10 δ₅̅) wrapped around the handset on three sides, with the exception of the side facing the head, up to two-thirds of the height of the handset.

To normalize the calculated SAR distributions for peak operating powers of 600 mW or 125 mW for mobile telephones operating at 835 and 1900 MHz, respectively, the procedure was as follows. A sinusoidal driving voltage of 3 V rms was assumed across the gap for the cell representing the driving point. Starting with this prescribed uniform z-directed E-field of 1000 V/m rms, the various converged components of E and H were calculated for all the cells of the modeled region. From the calculated tangential magnetic fields for the cells representing the antenna, we obtained the z-directed current distributions along the lengths of the various antennas from the expression I = - ∫ H • d₄. The z-directed current calculated for the cell corresponding to the driving point was then used to calculate the complex feedpoint impedance Z = V/I = R + jX and, hence, the power 1/2 |I|² R fed into the antenna. The calculated SARs were subsequently scaled for maximum antenna powers of 0.6 and 0.125 W, respectively.

Table 4. Comparison of the powers absorbed and peak SARs for the λ/4 and 3 λ/8 antennas at 835 MHz. Time-averaged radiated power = 0.6 W. Assumed size of the handset is 2.5 × 6 × 15 cm

<table>
<thead>
<tr>
<th>Antenna Length</th>
<th>Tilt</th>
<th>Peak 1-cm³ SAR W/kg</th>
<th>% power absorbed by</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Head W/kg</td>
<td>Brain W/kg</td>
<td>&quot;hand&quot; W/kg</td>
</tr>
<tr>
<td>λ/4</td>
<td>0°</td>
<td>1.53 (596 mg)*</td>
<td>0.97 (877 mg)*</td>
<td>17.0</td>
</tr>
<tr>
<td>λ/4</td>
<td>33°</td>
<td>1.21 (549 mg)*</td>
<td>0.54 (713 mg)*</td>
<td>15.0</td>
</tr>
<tr>
<td>λ/8</td>
<td>0°</td>
<td>0.67 (526 mg)*</td>
<td>0.35 (795 mg)*</td>
<td>16.5</td>
</tr>
<tr>
<td>λ/8</td>
<td>33°</td>
<td>0.74 (795 mg)*</td>
<td>0.68 (877 mg)*</td>
<td>12.5</td>
</tr>
</tbody>
</table>

* Actual weight of 1 cm³ of volume.
Table 5. Comparison of the powers absorbed and peak SARs for the \(\lambda/4\) and 3 \(\lambda/8\) antennas at 1900 MHz. Time-averaged radiated power = 125 mW. Assumed size of the handset is 2.5 \(\times\) 6 \(\times\) 15 cm

<table>
<thead>
<tr>
<th>Antenna Length</th>
<th>Tilt</th>
<th>Peak 1-cm(^3) SAR W/kg</th>
<th>% power absorbed by Head and Neck</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\lambda/4)</td>
<td>0°</td>
<td>0.86 (503 mg)*</td>
<td>22.5 46.9</td>
</tr>
<tr>
<td>(\lambda/4)</td>
<td>33.3°</td>
<td>0.86 (818 mg)</td>
<td>22.1 45.1</td>
</tr>
<tr>
<td>3 (\lambda/8)</td>
<td>0°</td>
<td>0.71 (503 mg)*</td>
<td>17.7 48.8</td>
</tr>
<tr>
<td>3 (\lambda/8)</td>
<td>33.3°</td>
<td>0.85 (853 mg)*</td>
<td>17.1 46.3</td>
</tr>
</tbody>
</table>

* Actual weight of 1 cm\(^3\) of volume.

The salient features of the results obtained for some of the mobile telephones studied to date are given in Tables 2 to 5. For each of the telephones we have selected the side of the head for which the SAR was higher because of the proximity of the antenna to the head. In Table 2, for the telephones 1, 2, 3, and 5, we have considered the telephone placed against the left ear, while for telephone 4, we have considered the telephone placed against the right ear in order to obtain the highest possible SARs for the model. Because of the symmetrical placement of the antennas in telephones 6-10 and for the \(\lambda/4\) and 3 \(\lambda/8\) antenna devices assumed for the data given in Tables 4 and 5, the choice of the side of the head was immaterial.

Even though older values of \(\varepsilon_r\) and \(\sigma\) given in Table 1 [19-21] were used for the calculations given in Tables 2 and 3, newer values for both 835 and 1900 MHz, given in Table 1, have recently become available [22], and these have been used for the calculated data given in Tables 4 and 5, respectively. An important thing to note for the newly obtained dielectric properties given in Table 1 is that the values of \(\varepsilon_r\) and \(\sigma\), both for skull/bone and cartilage, are much higher than those given previously [19-21]. Using telephone 4 as a test case, we find slight alterations in the calculated values of the SARs when new values of \(\varepsilon_r\), \(\sigma\) are used at 835 MHz. The data calculated for telephone 4 using the new values of the tissue properties are given in the last rows of Tables 2 and 3, respectively. As expected, the peak 1-cell SAR for the higher-conductivity cartilage of the ear is somewhat higher, and the 1-cell SAR for the brain is somewhat lower (see Table 2) because of the additional shielding provided by the higher-conductivity skull.

We have examined the effect of the antenna length (\(\lambda/4\) vs. 3\(\lambda/8\)) on the power absorbed by the head and neck and have determined the peak 1-cm\(^3\) SARs anywhere in the head, including the ear, and the tissues in the brain. The salient features of the calculated results at 835 and 1900 MHz are given in Tables 4 and 5, respectively.

It is interesting to note that the powers absorbed by the head and neck and the peak 1-cm\(^3\) SARs are lower for a 3\(\lambda/8\) antenna vis \(\text{a}\) \(\lambda/4\) antenna at 835 MHz (Table 4), while these quantities are almost the same for corresponding lengths of antennas at 1900 MHz (Table 5). This may be due to the fact that a 3\(\lambda/8\) antenna is substantially longer (13.5 cm) at 835 MHz than that for 1900 MHz (5.9 cm). The peak current region for a 3\(\lambda/8\) antenna is a length of \(\lambda/8\) higher up on the antenna as compared to the \(\lambda/4\) antenna where it is at the base of the antenna and, hence, very close to the ear. For a longer 3\(\lambda/8\) antenna at 835 MHz, the peak current region is, therefore, somewhat removed from the ear and the head, which results in lower absorbed power and SARs as compared to the \(\lambda/4\) antenna, while a similar effect is not obtained for the 3\(\lambda/8\) antenna at 1900 MHz where the high-current region is still
awfully close to the head. Similar arguments can also be given for the calculated lower SARs for 33° tilted antennas vis-à-vis the vertically held antennas, since the somewhat longer antennas at 835 MHz get further away from the head, which results in lower SARs for tilted antennas at this frequency, while a similar effect does not occur at the higher frequency of 1900 MHz because of the smaller lengths of the antennas.

**INDUCED ELECTRIC FIELDS AND CURRENTS FOR POWER FREQUENCY EMFs**

With several epidemiologic studies linking electromagnetic fields (EMFs) with higher rates of incidence of cancer, there is an increasing concern in the public mind regarding the potential health effects of these fields. An important issue that has come up is that coupling of the EMFs to the tissues in the human body is poorly understood for power-line-related frequencies between 10 and 1000 Hz. In the past, a mannequin covered with copper foils has been used to measure the induced currents for the various sections of the body by using breaks in the copper foil [23]. A homogeneous saline-filled, reduced-scale model of the human body (height = 45 cm) has also been used for exposure to vertical electric fields associated with power transmission lines [24]. Assuming a rotationally symmetric model of approximate human dimensions (height = 1.83 m or 6 ft) which is represented by 36 spheres, DiPlacidò et al [25] have calculated the induced currents for the various sections of the body for grounded and ungrounded conditions. While the data generated by these authors are important first steps, and provide approximate understanding of the induced currents, it is felt that heterogeneous models will provide a proper representation of the internal organs and tissue properties leading to knowledge of induced currents and internal electric fields. Such models will also permit inclusion of both the electric (E) and magnetic (H) fields for a variety of exposure conditions rather than uniform E-field exposures that have mostly been considered.

We have previously considered a 1.31-cm (nominal 1/2") resolution anatomically based model of the human body for exposure to purely electric, purely magnetic, and combined electric and magnetic fields at 60 Hz [26]. For this model we have calculated the induced electric fields and current densities for exposure to electric and magnetic fields (E = 10 KV/m, vertically polarized; B = 33.3 μT, oriented from arm to arm) that may be encountered under ultra-high-voltage power lines. Since the heterogeneous model was obtained from anatomical sectional diagrams of several cadavers available in a book on human anatomy [27], it is obviously not as satisfactory as the present millimeter-resolution model obtained from the MRI scans of an individual. Also, resolution of 1.31 cm for the various cells is not as good as the 1.974 × 1.974 × 3 mm cell size used for the present model. We feel that the present model identifying single tissues for each of the cells is capable of modeling the tissue interfaces and identifying regions of peak induced E-field and current densities much better than the previously used coarser model [26]. And yet, it is not possible to use the millimeter-resolution model of the whole body because of the very large number of cells (over 6 million) and the large CPU memories that are not easily available to us today.

Recognizing this limitation of the computer resources, we have combined the 3 × 3 × 2 cells of the mm-resolution model to obtain a new model with resolution of 5.922 × 5.922 × 6 mm along x-, y-, and z-directions, respectively. We have used the previously outlined frequency scaling approach and the FDTD method [26] to obtain the induced currents for grounded conditions of this model for exposure to 60-Hz EMFs with incident electric fields of 10 KV/m (vertically polarized) and for magnetic fields of 33.3 μT polarized from side to side of the model. Taken from references 28-30, the conductivities taken for the various
Table 6. Conductivities of the various tissues assumed for power-frequency EMFs

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Conductivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>muscle (σ_t)</td>
<td>0.068</td>
</tr>
<tr>
<td>muscle (σ_i)</td>
<td>0.068</td>
</tr>
<tr>
<td>muscle (σ_r)</td>
<td>0.86</td>
</tr>
<tr>
<td>fat/bone/cartilage</td>
<td>0.04</td>
</tr>
<tr>
<td>skin</td>
<td>0.11</td>
</tr>
<tr>
<td>nerve</td>
<td>0.12</td>
</tr>
<tr>
<td>intestine/spleen/pancreas</td>
<td>0.12</td>
</tr>
<tr>
<td>heart</td>
<td>0.11</td>
</tr>
<tr>
<td>blood</td>
<td>0.60</td>
</tr>
<tr>
<td>parotid gland</td>
<td>0.11</td>
</tr>
<tr>
<td>liver</td>
<td>0.13</td>
</tr>
<tr>
<td>kidney</td>
<td>0.16</td>
</tr>
<tr>
<td>lung</td>
<td>0.04</td>
</tr>
<tr>
<td>bladder</td>
<td>0.10</td>
</tr>
<tr>
<td>cerebrospinal fluid (CSF)</td>
<td>1.66</td>
</tr>
<tr>
<td>eye humor</td>
<td>1.66</td>
</tr>
<tr>
<td>eye sclera</td>
<td>0.11</td>
</tr>
<tr>
<td>eye lens</td>
<td>0.11</td>
</tr>
<tr>
<td>stomach</td>
<td>0.11</td>
</tr>
<tr>
<td>erectile tissue</td>
<td>0.11</td>
</tr>
<tr>
<td>prostate gland</td>
<td>0.11</td>
</tr>
<tr>
<td>spermatic cord</td>
<td>0.11</td>
</tr>
<tr>
<td>testicle</td>
<td>0.11</td>
</tr>
<tr>
<td>compact bone</td>
<td>0.04</td>
</tr>
<tr>
<td>ligament</td>
<td>0.11</td>
</tr>
<tr>
<td>pineal gland</td>
<td>0.12</td>
</tr>
<tr>
<td>pituitary gland</td>
<td>0.12</td>
</tr>
<tr>
<td>brain</td>
<td>0.12</td>
</tr>
</tbody>
</table>

tissues are given in Table 6. The vertically directed current I, passing through the various sections of the model is shown in Fig. 2. Though the current variation is very similar to that given earlier [26] and, in fact, also by Deno a number of years ago [23], it is now possible to identify the regions of the peak induced E-fields and current densities and the magnitudes of these internal quantities with a degree of precision that was not possible in the past.

In Figs. 3 and 4, we give the calculated maximum magnitudes of the induced electric fields \(E_T = (E + E + E)^{1/2}\) and current densities \(J_T\) for the various cross sections of the body, respectively. It is interesting to note that local induced current densities may be as high as 20 mA/m\(^2\) for the head and trunk and up to nearly 100 mA/m\(^2\) for the lower regions of the body (Fig. 4). These are considerably higher than 4 and even 10 mA/m\(^2\) that have been suggested in the various safety guidelines [31, 32].

**CONCLUSION**

Numerical methods have matured to a level that they are being increasingly used by many laboratories for dosimetric calculations for important and meaningful bioelectromagnetic problems. For certification of mobile telephones to be within the ANSI/IEEE C95.1-1992 RF Safety Guidelines, the approach discussed in this paper may be quite useful. We should also be able to use the numerical approach outlined here to understand coupling of power-frequency high-magnetic-field sources such as
Figure 2. The calculated vertical current passing through the various layers of a 5.922 x 5.922 x 6 mm MRI-based grounded model of the human body exposed to EMFs at 60 Hz. $E = 10 \text{kV/m}$ (vertical) and $B = 33.3 \mu T$ (from side to side of the body).

Figure 3. The calculated variation of maximum induced electric fields for each of the cross sections of the 5.922 x 5.922 x 6 mm resolution grounded model. Incident fields are the same as for Fig. 2.
hair dryers, hair clippers, electric shavers, etc., to the human head. Of particular interest would be the induced EMFs and current densities for the pineal gland which has been alleged to be involved in the biological effects of power-frequency EMFs. With the resolution of the present models being on the order of 11.7 milligrams of tissue for each of the cells of dimension $1.974 \times 1.974 \times 3$ mm, it is possible to define even small glands, such as the pineal, with a great deal of precision. The numerical models may also be used for the design/assessment of important biomedical devices such as implantable cardiac defibrillators, etc.

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INTRODUCTION

Electrical excitation of motor neurons in the brain cortex, or peripheral neurons, and observation of evoked responses have been extensively used in research and medical practice. Until mid 1980’s, excitation had been obtained with current pulses produced either by implanted or external electrodes applied near the neuron. Magnetic field stimulation offers the advantages that it is a non-invasive, non-contact method which produces minimal discomfort to the patient as only low density current flows through skin pain receptors during the procedure (Amassian et al., 1989; Barker et al., 1987; Freeston et al., 1984). Magnetic stimulation of brain, spinal cord, and peripheral nerves has been used to diagnose various medical conditions associated with the abnormal conduction of motor pathways (Barker et al., 1987; Chokroverty, 1990; Evans et al., 1988; Hallett and Cohen, 1989). It has also been used in mapping the motor-cortex (Cohen et al., 1988; Benecke et al., 1988).

In magnetic stimulation, the magnitude, sign and time-course of the spatial derivative of the induced electric field along the axis of the axon (nerve fiber) determine whether stimulation occurs and where along the axon it occurs (Basser and Roth, 1991; Roth and Basser, 1990). Depending on the sign of the field derivative the axon is depolarized (negative derivative) or hyperpolarized. All neurons within the volume where the electric field derivative is negative and above a threshold value for the given neurons are stimulated. In clinical practice, it is important to control the location and size of this volume. Furthermore, it is important to control the location of the volume where hyperpolarization occurs, as it may block the propagation of the action potential.

The induced electric fields have been modeled for some tissue geometries. Two approaches have been used to compute the spatial distribution of induced electric fields from

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coils. Simplified tissue models such as homogeneous semi-infinite plane (Esselle and Stuchly, 1992), cylinder (Esselle and Stuchly, 1994), and sphere (Eaton, 1992) have been used in analytical techniques. Homogeneous and heterogeneous models of varied degree of anatomical fidelity have been used in numerical techniques, (Branston and Tofts, 1991; D'Inzeo et al., 1992; Roth et al., 1990, 1991; Ueno et al., 1992). The numerical techniques are the preferred solution when a final accurate analysis is required. However, for comparative evaluation of various exposure conditions and coil configurations analytical solutions offer considerable advantages. Firstly, the computer resources and time requirements for analytical solutions are significantly smaller than for numerical solutions. This alone makes analytical solutions exceptionally suitable for optimization of coils used in various medical applications. Another advantage is that useful physical insight can be gained by investigating mathematical expressions associated with analytical solutions.

A cylindrical volume conductor model provides a good representation of geometries associated with stimulation of peripheral nerves. This paper describes the method and results of coil optimization for this model. An analytical technique is used. A comparison with numerical computations for the same geometry is also given.

THEORY

Electric Field Due to a Coil Element

This shows a coil carrying a time-varying current $I$, and an infinitely-long homogeneous tissue cylinder with a radius $a$. The coil has an arbitrary shape and $N$ number of turns. Vector $d\vec{l}'$ represents a small element of the coil located at $(r', \phi', z')$. First, we derive an expression for the electric field produced by this coil element assuming that it is located on the $x$-$z$ plane, i.e. $\phi' = 0$. Later, we will generalize it for any $\phi'$. The total electric field is obtained by integrating this expression along the coil.

Under quasi-static conditions (Roth et al., 1991), the primary electric field produced by the coil-elements is given by (Roth et al., 1990):

$$d\vec{E} = \frac{-\mu_0 N (dI/dt) d\vec{l}'}{4\pi R}$$

Figure 1. Geometry of the problem.
where $\mu_0$ is the permeability of free-space and $R$ is the distance between the coil element and the point where the field is calculated, $(r, \phi, z)$, given by:

$$R = \sqrt{r^2 + r'^2 - 2rr'\cos \phi + (z - z')^2}$$  \hspace{1cm} (2)

The secondary quasi-static electric field, which is a result of the surface charge at the tissue-air interface, is derived from a scalar potential function $\psi$ as

$$dE^z = -\nabla \psi$$ \hspace{1cm} (3)

The function $\psi$ is defined only inside the cylinder and it satisfies Laplace’s equation:

$$\nabla^2 \psi = 0$$ \hspace{1cm} (4)

Under quasi-static conditions, the electric field component normal to the tissue-air interface should be zero inside the tissue cylinder (Polk and Song, 1990). That is:

$$\nabla^2 \psi = 0$$ \hspace{1cm} (5)

When $\phi' = 0$ substitution of (1) and (3) in (5) gives the following boundary condition for $\psi$:

$$\left( dE^\nu + dE^\phi \right) \cdot n_{r=a} = 0$$ \hspace{1cm} (6)

where $dl^r$ and $dl^\phi$ are the $r$- and $\phi$- components of $dl^\nu$, respectively.

$$\frac{\partial \phi}{\partial r} \bigg|_{r=a} = \frac{\mu_0 N (dl / dt) (dl^r \cos \phi + dl^\phi \sin \phi)}{4\pi R}$$

To obtain the solution distance $R$ is expanded in terms of modified Bessel functions. Since $\phi$ is a solution of Laplace’s equation, it can be expressed as (Esselle and Stuchly, 1994):

$$\phi = \int_0^\infty \sum_{m=0}^\infty \cos \lambda (z - z') [A_m(\lambda) \cos m\phi + B_m(\lambda) \sin m\phi] I_m(\lambda r) d\lambda,$$ \hspace{1cm} (7)

where $A_m(\lambda)$ and $B_m(\lambda)$ are the unknowns to be determined. The boundary condition (equation 5) is used to determine the unknown coefficients.

The Total Electric Field

The total electric field, $\vec{E}$, produced by the coil can be obtained by integrating $d\vec{E}$ along the coil. For example, the $z$-component of the electric field, $E_z$, is given by
\[ E_z = \oint d\mathbf{E}_z' + d\mathbf{E}_z'' = \oint \left\{ -\frac{\mu_0 N (dI / dt) dl'}{4 \pi R} \right. \\
+ \left. \int^{\lambda} \sum_{m=\pm} \sin \lambda (z - z') \left[ A_m(\lambda \lambda \cos m(\phi - \phi') \right) \right\} I_m(\lambda r) d\lambda \right\} \] (8)

where \( d\mathbf{E}_z \) is the z-component of \( d\mathbf{E}' \).

Detailed derivation and closed form expressions for the electric field are given elsewhere (Esselle and Stuchly. 1994). Because the coefficients that require integration (summation) of Bessel functions are independent of coordinates of the point in which the electric field is computed, they can be determined once for a given geometry and position of the coil, and the results can be stored in memory and re-used in computations of the fields in multiple locations. This feature and the closed form expressions for the induced fields facilitate fast computations. For instance, calculation of axial electric field at 500 points takes less than 5 minutes on a 25 MHz 80486-based PC.

RESULTS

The electric field patterns obtained by this method are qualitatively and quantitively similar to those previously computed by Roth et al., (1990), using the finite difference technique. For example, a 5 cm diameter cylinder was exposed to a 5 cm diameter circular coil carrying a current rising a 100 A/\mu s. When this 10-turn coil was perpendicular to the cylinder and the gap between the two was 1 cm, the electric field at a point 6.25 mm beneath the surface was 39 V/m. When the same analysis was repeated using our method, the result was 41 V/m.

Stimulating coils of circular, square, double-square (DS) and quadruplesquare (QS) shapes were analyzed. The coils analyzed were placed parallel or perpendicular to the cylinder axis or for DS and QS coils with its wings tilted (better conforming to the cylinder surface). For all results presented, the conducting (tissue) cylinder diameter was 8 cm. Circular coil diameter or the length of each square coil section was 5 cm. The nerve, parallel to the cylinder axis, was assumed to be located 1 cm below the cylinder surface. The minimum distance between the coil and the cylinder surface was 1 cm. The time rate of current change in the coil was 100 A/\mu s.

Figures 2 to 6 show contours of the electric field and its spatial derivative along the direction of the nerve for various coil configurations. The surfaces shown are cylindrical and are located 1 cm below the surface of the cylinder, i.e. where the nerve to be stimulated is located. Table 1 gives a summary of the maximum values of the electric field and its derivative at the nerve location for various coils illustrated in Figures 2 to 6.

DISCUSSION

The analysis of the cylindrical model confirmed the previous findings from a planar model regarding properties of various stimulating coils (Esselle and Stuchly, 1992a, 1992b).
Single coils of circular or square shape produce two maxima and two minima of $\delta E_z/\delta z$ (Figs. 2 and 3). Analogously to the previous findings the location of the stimulation can be easily and reasonably well determined by visual inspection for square coils, but not for circular coils. This is because for square coils the maxima of $|\delta E_z/\delta z|$ occur underneath the coil corners close to the coil and somewhat outwards for greater distances (deeper nerves). Square coils also produce a stronger stimulus for the same current pulse (Table 1). Reorienting a circular or square coil with respect to the tissue cylinder, so it is perpendicular, results in only one polarization and one hyperpolarization spot. But the stimulus is much weaker, 0.6 kV/m² compared with 2.1 kV/m² for the same coil parallel to the cylinder.

As illustrated in Figure 5, for a double coil there is one maximum and one minimum of $\delta E_z/\delta z$. Their magnitudes are nearly double that of a single coil with the same number of turns in each section (but twice the total number of turns) (Table 1). This feature makes these coils more advantageous than single coils. A quadrupel coil is even better, as it can be seen from Figure 6 and Table 1. There is only one location of the greatest $\delta E_z/\delta z$, negative or positive.

### Table 1. The maximum $E_z$ and $\delta E_z/\delta z$ at the nerve location for various coils

<table>
<thead>
<tr>
<th>Coil</th>
<th>$E_z$ (V/m)</th>
<th>$\delta E_z/\delta z$ (kV/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>single-circular, parallel, $N = 10$</td>
<td>78.5</td>
<td>1.8</td>
</tr>
<tr>
<td>single-square, parallel, $N = 10$</td>
<td>91.0</td>
<td>2.1</td>
</tr>
<tr>
<td>single-square, perpendicular, $N = 10$</td>
<td>50.4</td>
<td>0.8</td>
</tr>
<tr>
<td>double-square, parallel, $N = 2 \times 10$</td>
<td>167.7</td>
<td>3.8</td>
</tr>
<tr>
<td>quad-square, tilted 110°, $N = 4 \times 5$</td>
<td>79.5</td>
<td>4.7</td>
</tr>
</tbody>
</table>
Figure 3. a. Electric field $E_z$ and its derivative $\partial E_z/\partial z$. b. Electric field $E_z$ for a horizontally oriented square coil, side 5 cm, $N = 10$.

Figure 4. a. Electric field $E_z$ and its derivative $\partial E_z/\partial z$. b. Electric field $E_z$ for a vertically oriented square coil, side 5 cm, $N = 10$.
Figure 5. a. Electric field $E_z$ and its derivative $\delta E_z/\delta z$. b. Electric field $E_z$ for a horizontally oriented double square coil, side 5 cm, $N = 2 \times 10$.

Figure 6. a. Electric field $E_z$ and its derivative $\delta E_z/\delta z$. b. Electric field $E_z$ for a quadruple square coil, side 5 cm, the angle between the sections 110° in x-y Plane (Fig. 1), $N = 4 \times 5$. 
positive, depending on the directions of the currents in the coil sections. Therefore, there is no ambiguity with respect to the nerve depolarization and its location, once the windings of the four sections of the coil are properly connected together. Tilting the sections of the coil to form 110° rather than 180° increases the magnitude of the stimulus.$\delta E_i / \delta z$

Since the anatomy of a human arm is heterogeneous rather than homogeneous, differences in magnitudes of the electric field and its derivative can be expected from those shown in Table 1. This issue has recently been addressed (D'Inzeo et al. 1994). An admittance method (numerical) was used to analyze an anatomically correct three-dimensional model of a human arm. The resolution was 2.5 mm, and conductivities of the following tissues were represented: skin, fat, muscle, bone, tendon and nerve. The results are given in Table 2. Lower values of both the electric field and its spatial derivative are clearly evident when compared with the corresponding values in Table 1. The reductions are due to the electric charge at the interfaces between various tissues. However, the superior performance of quadruple coils remains unaffected.

**ACKNOWLEDGEMENT**

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THE APPLICATION OF ELECTROMAGNETIC ENERGY TO THE TREATMENT OF NEUROLOGICAL AND PSYCHIATRIC DISEASES

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INTRODUCTION

Recent progress in the application of electromagnetic energy has revolutionized many areas of medicine. Nuclear magnetic resonance imaging (MRI) continues as a leading noninvasive modality for diagnostic examination of tissues to detect or localize disease. Other emerging functional imaging modalities include magnetoencephalography and magnetoencephalography. Magnetic stimulation for the diagnosis of neurological disorders represents yet another noncontact application of electromagnetic energy that is gaining in popularity (Lin, 1993). Magnetocardiography (MCG) is a promising noninvasive modality for obtaining functional information on the electrical activity of the heart. The small biomagnetic signals (1 pT or less) are recorded using multi-channel superconducting quantum interference devices (SQUID) with subjects in the supine position and the SQUID sensors placed directly over the thoracic surface (Stroink, 1989). It has been explored as a mapping tool to localize noninvasively cardiac electrical sources responsible for atrial flutter and ventricular fibrillation (Ribeiro et al., 1992, Williamson et al., 1989). In a like manner, high resolution magnetoencephalography (MEG) measures the minute magnetic fields generated by the ionic currents in the brain (Barth, 1993). MEG correlates well with abnormal

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electrical activity measured by electroencephalography (EEG), and sometimes shows abnormalities not seen on EEG. It has been successfully used to localize cerebral sources of electrical activity (Pantev et al., 1991; Sandyk and Anninos, 1992; Squires et al., 1990; Stefan et al., 1989). MEG is more accurate in spatial localization, as the magnetic field passes through the scalp and skull unimpeded, while the electrical signal of EEG is attenuated and dispersed.

In magnetic stimulation of the nervous system, a time-varying magnetic field, produced by passing a current through a wire coil, gives rise to an induced electric field in proximity to excitable tissues of the central and peripheral nervous system. The currents are delivered as 50-200 microsecond pulses with peak values of several thousand amperes. The technique is used to study the neuromuscular system in humans (Amassian et al., 1989; 1990; Chokroverty, 1990; Evans, 1991; Ueno et al. 1991). The principal advantages of magnetic stimulation are that it is noninvasive and less painful than applying electrical currents through surface electrodes, and it has the ability to reach nerves lying well below the skin surface. The main disadvantages are that magnetic stimulation is not selective enough to restrict the region of excitation and it has relatively poor controllability and reproducibility.

This paper reviews the pathophysiology of several common neurologic and psychiatric diseases, and the effect of electromagnetic field (EMF) on these conditions. Studies of this kind are sparse and are mostly not yet replicated in a different laboratory. Other recent diagnostic and therapeutic applications of electromagnetic energy have been described elsewhere (Hand and Cardossi, 1993; Lin, 1993a; b).

The brain is an electric organ. Its major cellular components, the neurons, can be viewed as combinations of capacitors in parallel. The lipid dielectric between the neuronal membranes maintains a potential of -60mV in the cell's resting state. The change in potential is mediated by transmembrane flow of ions. Electromagnetic fields of different frequencies could affect this system by induced-current or direct field effect on molecular interactions. The therapeutic effect of EMF has been studied on a variety of neurologic diseases. In these studies, extremely low frequency (ELF) magnetic or electric fields have been used. Thorough clinical trials assessing the true efficacy of EMF in specific neurological or psychiatric illness are lacking. However, experimental models and some studies on humans offer intriguing results.

**EPILEPSY**

Epilepsy is a neurologic disorder characterized by paroxysmal attacks of brain dysfunction due to excessive neuronal discharge. This abnormal activity is often associated with trauma to brain tissue, or to genetic predisposition. The physical and cognitive manifestations of these discharges vary depending on their locations in the brain, and to what extent the abnormal activity spreads to adjacent tissue. This abnormality depends on the extracellular environment, the supporting cell populations, and interaction with other neurons (Schwartzkroin, 1993). The electronic structure of the neuron is composed of both "passive" membrane structural components and an active component of transmembrane potential mediated by intra- and extracellular ion concentrations and their fluxes. The ions involved in electrical activity are potassium, sodium, chloride, and calcium. Anticonvulsant medications affect the ion channels and modulate the repetitive discharges which occur in hyperexcitable neurons during epilepsy.

A recent case report involved treatment of a 20 year old female with idiopathic seizure disorder, with onset in childhood (Sandyk and Anninos, 1992). She was having generalized convulsions and averaged three times a day while on anticonvulsant medications with therapeutic serum levels. She also had intellectual decline and violent
behavior directed against herself and others. An interictal EEG was normal. External magnetic fields were applied for 2 minutes on 3 consecutive days. The patient was continued on her anticonvulsant medication (Valproic acid). She was seizure free for 2 weeks. She was then given a device to administer “magnetic smoothing” treatments at home each evening. At a 3 month follow-up, her seizure frequency declined to 1 per week, and her aggressive behavior had abated. Time spent in sleep also increased 1-2 hours a day.

**MULTIPLE SCLEROSIS**

Multiple sclerosis (MS) is classified as a demyelinating disease, with onset in young adulthood. Diagnosis can be made on a clinical basis. Initial physical complaints are often paresthesias and visual changes: decreased visual acuity or diplopia. Other neurologic systems may be affected, and the patient may have weakness of one or more extremities, with loss of balance and difficulty or inability to walk. Urinary incontinence, fatigue and depression are often features. The disease is characterized by a waxing and waning course of either of two patterns. One is acute exacerbations of symptoms with resolution, and the other is a chronic progressive course. Diagnosis can be confirmed by Magnetic Resonance Imaging (MRI) of the brain which on T2 signal will show demyelinating lesions, often involving the corpus callosum. Visual evoked potential studies show slowing of cortical electrical response to visual stimuli.

The exact mechanism of the disease is unknown. It is generally thought to be an autoimmune response, perhaps related to past viral exposure. Some patients respond favorably to treatment with steroids during an exacerbation (methylprednisolone or ACTH). It is not clear that the demyelinating lesions on MRI account for the clinical course of the disease, and there is speculation that the clinical manifestations are due to impaired serotonin activity (Sandyk and Iacono, 1993). A cohort study of 25 patients with multiple sclerosis found pineal gland calcification in 96%, abnormal melatonin levels in 52%, and abnormal alpha-melanocyte stimulating hormone in 70% (Sandyk and Awerbuck, 1992).

Several cases of treatment of multiple sclerosis with magnetic fields have been reported in detail (Sandyk 1992; Sandyk and Iacono, 1993; Sandyk, 1994). The main features of these cases will be mentioned here. The patients were age 50-64 with a history of MS who were referred for experimental treatment based on their poor physical condition and failure to respond to other treatments. They were treated with an external array of magnetic field generating coils at 7.5 pT, 5 Hz, for durations of 20 to 30 minutes. After a single treatment, and over a period of several days, the patients reported significant (50-60%) improvement in all symptoms. This included improved vision, balance, sleep, clearness of thinking and speech. One measure of improvement was pre- and post-treatment drawing tests for assessment of motor control and psychological constructional ability. Patients were asked to draw an Archimedes spiral and a house. In pre-treatment drawings, the lines were irregular and showed poverty of content. In post-treatment drawings the lines were more regular and the pictures more detailed. More objective evidence of a change in neurologic function was obtained in one patient by measurement of visual evoked potentials (VEP). Prior to treatment, the latency response time of the occipital cortex to stimulation of the eyes with a flashed checkerboard light pattern was slowed in both eyes to an abnormal range. The VEP was remeasured and showed a normalization of latency 24 hours after treatment. Up to a 3 month follow-up was done showing the improved condition to be stable.
PARKINSON’S DISEASE

Parkinson’s Disease is a movement disorder with peak onset in the sixth decade of life. It is characterized clinically by a resting tremor, muscle rigidity, akinesia, stooped posture and masked faces. The disease is progressive and can be debilitating. There is no clear etiology for this disease. Pathology shows loss of pigmented cells in the substantia nigra and other pigmented nuclei (Adams and Victor, 1993). This is associated with a loss of dopamine producing cells. The mainstay of symptomatic treatment is dopamine replacement therapy with the dopamine precursor L-dopa.

The symptoms of Parkinson’s Disease are associated with an increase in activity of striatal neurons which project to the globus pallidus. The system is normally balanced by the inhibitory nigral dopamine input interacting with the excitatory cortical glutamate input. In this disease the excitatory input dominates, and may contribute to induced cell death along the striatal pathway (Mitchell et al., 1994). The substantia nigra has elevated iron levels compared to other areas of the brain which is bound by neuromelanin, with electron paramagnetic resonance showing unbound iron to be in paramagnetic valence states (Zecca and Swartz, 1992). Iron contributes to oxidative stress in the brain tissue by being a catalyst for lipid peroxidation reactions, with resulting alteration in calcium hemostasis (Youdin and Ben-Shachar, 1991).

There are reports of low-level magnetic fields improving the symptoms of Parkinson’s Disease. (Sandyk, 1992). We will summarize one typical case. A 71 year old man had a 3 year history of progressive symptoms of Parkinson’s Disease. At the time of treatment he had moderate generalized bradykinesia, mild resting tremor, frontal lobe release signs, oily face and forehead, hypophonic speech, decreased blink rate of 5-8/min, micrographia, cogwheel rigidity, and stooped posture. He had been treated with the medication bromocriptine (16 mg/d) with mild improvement of symptoms. He was treated with a sham exposure session with no change in symptoms. He was then treated with a 2-7 Hz magnetic field at 7.5 pT for seven minutes. Ten minutes after initial treatment, the patient’s blood pressure dropped from 140/80 to 130/70 mm Hg. Heart rate dropped from 76/min to 68/min. Thirty minutes after treatment facial muscles showed improved expression and blink rate had increased to 18-21/min. His voice was louder and he was less bradykinetic. Posture had improved. There was complete resolution of his hand tremor. The initial benefit lasted 4 days. The patient assessed his improvement at 60%, which remained stable for three months with weekly magnetic treatments.

Further assessment of cognitive improvements were done using picture drawing and a word-fluency test (Sandyk, 1994). Subjects were asked to produce a list of words beginning with the letters “S” and “C”. Parkinson patients tended to show a decline in the number of words produced; about half of that expected when compared with controls. After a series of two magnetic field treatments, word fluency on the same tasks improved to normal range. When drawing objects such as a daisy flower or a bicycle, the pre-treatment subjects had drawings with irregular lines and poverty of content. Post-treatment subject showed smoother lines and more detailed drawings; an indication of improved tremor and visuoconstructual functioning. Micrographia has also been shown to improve with magnetic field treatments (Sandyk and Ilocono, 1994).

STROKE

A stroke is a sudden focal neurologic deficit. This is usually caused by the interruption of oxygenated blood flow to an area of the brain by an embolus or thrombosis.
of an artery. The neurons in that region undergo ischemic necrosis. Treatment of stroke was traditionally providing supportive medical care and treating the risks for vascular disease or cardiac disease. It was suggested that the neurons surrounding the core area of ischemic damage were being injured by glutamate which was being released by the excess calcium released by the dying cells (Choi, 1990). These surrounding cells, dubbed the "ischemic penumbra", were thought to be potentially salvageable. Indeed, several pharmacologic agents have been known to decrease the volume of brain injury, and clinical trials are ongoing (Ginsberg, 1993). Many of these agents block the release of excess glutamate by preventing calcium influx to neurons. The presence of iron in the brain also contributes to propagation of injury by acting as a cofactor in the lipid peroxidation reaction. This produces malignant free radicals which the brain is poorly equipped to deal with, due to the low level of the endogenous free radical scavenger superoxide dismutase (Davalos, 1994). This mechanism is also an area of research in pharmacologic intervention.

Electromagnetic fields have been shown to alter calcium flux in the brain (Bawin and Adey, 1976; Blackman, 1988). This led to attempts to reduce the amount of brain injury from stroke in animal models using electromagnetic fields. Using a pulsed electromagnetic field (parameters: 27.1 MHz, 585 W peak power, 65 us pulses, 400 pulses per second) on rats undergoing permanent middle cerebral artery occlusion, it was found that animals treated for 2 hours had reduced edema, but no significant reduction in the volume of injury (Rappaport and Young, 1990). In a more recent study, using a model of transient ischemia in the rabbit, certain zones of ischemia in the treated group showed marked improvement, but overall injury was not significantly different (Grant, 1994). The field used was 2.8 mT at 75 Hz with pulse width of 1.3 ms. Of additional interest was that somatosensory evoked potentials were done to assess ischemic change, and the magnetic field treated animals tended to show less attenuation of conduction compared to controls.

ELECTROMAGNETIC FIELDS, MELATONIN PRODUCTION AND PSYCHOLOGICAL DISORDERS

Secretion of the hormone melatonin by the pineal gland in vertebrates is affected by exposure to EMF (Reiters, 1992; 1993). Visible light had been shown to inhibit melatonin synthesis. Acute exposure to light induces a T 1/2 of 10 minutes. During periods of darkness the levels increase 5-10 fold. This response is mediated via the visual system. Light on the retina of the eye is transduced to an electrochemical signal communication with the pineal gland via suprachiasmatic nuclei of the hypothalamus, preganglionic neurons in the intermediolateral column of the upper thoracic spinal cord, and post ganglionic neurons in the superior cervical ganglion. The release of norepinephrine in the pineal gland augments the production of cAMP and melatonin, and may be mediated by protein kinase C (Ca²⁺ activated).

Different magnetic field manipulations have been shown to reduce melatonin levels by 50% (Reiters, 1992; 1993) Experiments in rodents have shown intermittent static magnetic fields interfered with the nocturnal metabolism of melatonin. An inversion of the geomagnetic field reduces pineal cAMP and melatonin production. Instantaneously inverted fields with induced current depress pineal melatonin production. Since 50-60 Hz fields also affect melatonin production, conceivably, magnetic fields can be used to alter melatonin metabolism for the treatment of various psychological disorders, such as changes in circadian cycle.
ELECTRICAL STIMULATION IN MENTAL ILLNESS

Although controversial, as many of 50,000 Americans receive electroconvulsive therapy (ECT) annually so that they obtain relief from several depression and related illness. Because of such frequency of use, its persistence in clinical practice, and surrounding controversy we will review ECT in some depth followed by sections on use of ECT in mental illness.

In 1938, Cerletti and Bini from Italy proposed the use of electrical stimulus to induce grand mal seizures, which from 1934 until then were induced by drugs such as camphor, to "cure" schizophrenia. Both patient and physician acceptance was so high that the newly "discovered" ECT rapidly spread through war-torn Europe and North America within a few years. Research in the past 25 years has led to the conclusion that ECT is probably the most effective for depression although it may also be effective in many types of other mental illnesses such as mania, schizophrenia, delirium, and neuroleptic malignant syndrome (Kahn et al., 1993).

Besides induction of grand mal seizures, electrical stimuli were used to induce electro-narcosis and electric sleep to treat mental illness. However, neither of these attempts were shown to be effective when studied systematically (Similar to insulin coma, psychosurgery or hydrotherapy). Type of electrical stimulus and its delivery for ECT have undergone considerable development. Research in the 1960's and 1970's convincingly showed that ECT's unwanted effects such as loss of memory, disorientation, and delirium were significantly related to the amount of electrical energy used to induce seizures (Fink, 1989). Because of concern over ECT's adverse effects, newer and possibly more benign electrical stimulus were considered. The past 20 year's research has focussed ways to deliver the lowest but effective amount of electrical energy to induce seizures. The present commercially available ECT machines offer the following types of delivery system. Instead of sine waves the newer machines deliver alternating current (40-180 Hz) as brief pulses (20 to 100 mA) ranging in duration from 0.5 to 2 ms. The electrical charge can be delivered at intervals of 0.5 to 3.0 seconds. Administration of 8 to 12 ECT's in a period of 3 to 4 weeks leads to patient improvement.

As a rule, such transcranial stimulation needs to overcome a resistance of 100 to 300 ohms, and in humans grand mal seizures are induced with a electrical current of about 150 to 250 mA. However, recent data suggest that men generally need a larger charge, and there is a linear relationship between increasing age and electrical charge to induce seizure. A significant proportion of older persons need 2 to 5 fold higher energy.

One of the unresolved issue is the mechanism by which ECT works. Accepted scientific rationale relies on the assumption that seizures per se may be the relevant factor. This is based on the finding that seizures induced by such chemical means as camphor, pentylenetrazol, indoklon, or ECT are only effective when they lead to grand mal seizures. However, recent data suggest that induction of a grand mal seizure with very low levels of electrical stimulus (50 to 100 mA) by placing electrodes on one side of the head may not lead to any patient improvement (Sackei m et al., 1991).

Although many psychological theories were proposed for the reason by which ECT works, they have lost support since many excellent studies have shown that a electrical stimulus followed by a grand mal seizure are needed for clinical improvement. Thus, most physicians consider that biological effects are the key to ECT's effects. Attempts at a detailed study of human brain at autopsy in patients who have had ECT shown no structural changes (Devanand et al., 1994). Moreover, large spike-like increases in most brain neurotransmitters such as norepinephrine, serotonin, acetylcholine, and dopamine occur immediately after ECT (Khan et al., 1993). Repeated ECT administration, as in routine medical practice, leads
to a decrease in concentration of receptors for most of these neurotransmitters and is one of the leading contenders as to how ECT works since most antidepressants also lead to such effects. An alternative possibility is the stimulation and re-regulation of hypothalamic-pituitary endocrine functions since ECT leads to major changes in this system. A third possibility includes reorganization and resynchronization of circadian rhythms as a possible mechanism by which ECT works.

An alternative approach to induce seizure for the treatment of depression is the use of rapid-rate transcranial magnetic stimulation (George and Wasserman, 1994). This approach could also allow selective stimulation of brain sites which are involved in depression and thus reduce side-effects, e.g. memory deficits, due to electrical disruption of function in unrelated sites.

CONCLUSION

While the application of ELF electromagnetic fields to neurologic and psychiatric disease may appear promising, it is in its infancy. A great deal of research must be conducted to demonstrate its safety and efficacy. The mechanism of interaction of magnetic fields with the nervous system is still unclear. Apart from seizure induction, the flux density used in the cited studies was very low (pT), the effects seemed to occur very fast and were long-lasting. Magnetites have been identified in cells in the human brain (Kirschvink et al., 1990), however, it has been calculated that magnetic fields higher than 5mT are required to generate sufficient effect on these particles (Adair, 1993). There may be a common pathway as to how EMF improves the conditions in the mentioned diseases. The role of calcium is fundamental in neuronal functioning and may play a key role in elucidating mechanisms of effect. It's involvement was mentioned in all the diseases, and could probably be associated with the neurotransmitter glutamate. Iron also plays a key role in the pathophysiology of neurologic disease (Sachdew, 1993), but to what extent magnetic field affects iron metabolism in the main remains to be elucidated. Endogenous opioids may also play a role. Magnetic fields have been shown to activate endogenous opioids in the brain (Lai, 1993). In addition, naltrexone has been shown to attenuate the antiparkinsonian effects of magnetic field (Sandyk, 1994). Magnetic field also shows an effect on immune responses, which may be significant in multiple sclerosis (Tanovic, 1991).

An important criticism of the human case reports is the lack of control subjects. Even though, the treatments included sham exposures, late improvement in tasks could be in part from practice. In most cases, the small numbers yield weak statistical power. Animal models of stroke are variable in reproducibility, and further systematic studies of different treatment parameters would be beneficial. While some mechanisms of action have been proposed, their substantiation is needed.

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1. INTRODUCTION

Some species of bacteria possess the ability, termed a taxis, to move from an unsuitable environment to a more suitable one. Chemotaxis is the ability to detect a specific chemical substance and then swim either away from or towards it. A similar ability with respect to light is termed phototaxis. Some of bacteria swim in the direction of a magnetic field, and they were therefore called magnetotactic bacteria. The intracellular magnetic particles synthesized by a magnetotactic bacterium form a chain-like structure which functions like a single bar magnet, causing the bacterium to orient in an external magnetic field. As a result of this orientation, the bacterium is directed to swim towards microhabitats of lower oxygen concentration deeper in the sediment of lakes, swamps and other aquatic habitats due to the inclination of the earth's magnetic field. North-seeking bacteria swim downwards in the northern hemisphere and upward in the southern hemisphere (south-seeking bacteria do to opposite). However, culture of magnetotactic bacteria is now possible, and cultured magnetotactic bacteria swim towards both poles. Magnetotactic bacteria have also been discovered which can grow under conditions of high oxygen concentration, although they do not synthesize magnetic particles under these conditions. Since the discovery of these bacteria, the category of magnetotactic bacteria appears less clearly defined, and perhaps a more useful category would be magnetic bacteria, that is, all bacteria which synthesize magnetic materials. A further area of uncertainty is the reason why magnetic bacteria synthesize magnetic particles. One suggestion is that under anaerobic conditions it is necessary for these bacteria to metabolize iron, and magnetic particles are simply by-products of the metabolism. However, research has not yet provided any clear information about bacterial magnetic particle synthesis or related aspects of metabolism.

Magnetic materials have also been found in migratory fish, such as salmon and tuna, and in other higher organisms. Magnetic materials have been extracted from the heads of these types of fish, and it has been proposed that these materials function as compasses to enable the fish to determine direction in order to migrate vast distances across the open ocean. It has been suggested that the magnetic material may form a composite structure which
functions as a magnetosensory organ. However, it is unclear whether these magnetosensory organs actually exist. There is still little known about the possession and synthesis of magnetic materials in fish, but they are very similar to the magnetic particles of magnetic bacteria, and it has therefore been suggested that there is an evolutionary relationship between synthesis of magnetic materials in bacteria and in animals. The evolutionary relationship between magnetic bacteria and higher animals has not been confirmed, but the mutual ability to synthesize magnetic materials is an important point they have in common. We hope that by investigating the genes that code for enzymes and proteins involved in the synthesis of magnetic materials, the connection between magnetic materials in various organisms can be elucidated.

Some bacteria isolated from fresh water synthesize intracellular magnetic particles which consist of magnetite, *Magnetospirillum magnetotacticum* MS-1 (1), *Magnetospirillum* sp. MGT-1 (15), *Magnetospirillum* sp. AMB-1 (Fig.1) (14) and *Magnetospirillum gryphiswaldense* (20). Phylogenetic analysis of 16S rRNA sequences of *Magnetospirillum* sp. have shown their close evolutionary relationships to some photosynthetic bacteria (2,9).

We are presently using AMB-1 as a model system for the study of magnetite biomineralization at the molecular genetic level(13). In this review, we describe a genetic approach to investigating AMB-1, and analysis of the MagA protein of AMB-1, which is thought to be involved in the magnetic particles synthesis.

2. GENE TRANSFER AND TRANSPONSON MUTAGENESIS IN AMB-1

Because a system for gene transfer into magnetic bacteria has not been studied, a basic molecular genetic approach was required. Among magnetic bacteria, *Magnetospirillum magnetotacticum* MS-1 was the first isolated, but it can only grow under microaerobic conditions. *Magnetospirillum* sp. strain AMB-1, however, can grow under microaerobic or aerobic conditions, and forms colonies on agar. To investigate the mechanism of magnetite biomineralization in magnetic bacteria, we chose AMB-1 as our study material.

To search for genes involved in magnetite synthesis, we used transposon mutagenesis of AMB-1. Transposon Tn5 is inserted into the genomic DNA at only one point, and transconjugants have kanamycin resistance. When one of the genes involved in magnetite synthesis is cleaved by Tn5, the transconjugant will lose the ability to synthesize magnetite.

![Figure 1. Transmission electron micrograph of Magnetospirillum sp. AMB-1. Scale bar indicates 1 μm.](image-url)
Figure 2. Southern hybridization analysis of pRK415 isolated from transconjugant. Lanes: M, λ-PstI; 1,2, pRK415 purified from E. coli by ultracentrifugation; 3,4, pRK415 isolated from AMB-1 transconjugant; 5,6, pRK415 from AMB-1 transformed and reisolated from E. coli; 7, genomic DNA from wild type AMB-1. DNA samples in lanes 1,3,5,7 were digested with EcoRI and samples in lanes 2,4,6 were digested with SmaI. Probe: purified pRK415.

In this section, gene transfer into AMB-1, by conjugation and nonmagnetic transposon mutagenesis of AMB-1, are discussed.

2-1. Conjugal Gene Transfer between E. coli and AMB-1

We chose a broad-host-range mobilization system to attempt gene transfer in AMB-1. We used E. coli S17-1 as the host in the gene transfer system. E. coli S17-1 contains the broad-host-range plasmid RP4 (tra') in its genomic DNA, and can transfer plasmids containing mob* to donors such as AMB-1. The broad-host-range plasmid, pKT230, is derived from RSF1010 and belongs to the IncQ group of incompatible plasmids. pRK415 is also a broad-host-range plasmid, is derived from RK2, and belong to the IncP group of incompatible plasmids. Both plasmids were transferred into AMB-1 using S17-1 as a donor. Each
transconjugant was confirmed to have antibiotic resistance, and was shown by southern hybridization to maintain the plasmid (Fig. 2) (13).

Moreover, we optimized transfer of pKT230 by performing conjugation at different conditions. Optimum conditions for this vector were at a donor-recipient ratio of 10:1 and for 6 h at 25°C (Fig. 3). For pRK415, they were at the ratio of 100:1 for 6 h and the maximum efficiency was $5.0 \times 10^3$ (transconjugants / donors). Therefore, all conjugations of AMB-1 were done under the conditions.

This efficiency is fairly high when compared with *Aquaspirillum* species (5), so this transfer method is considered useful for future transposon mutagenesis.

2-2. Random Transposon Mutagenesis of AMB-1 and Cloning of Chromosomal Regions Involved in Synthesis of Magnetic Particles

Selection of mutants which lose magnetite synthesis ability is the easiest way to find genes involved in the synthesis of magnetic particles in magnetic bacteria. However, mutants due to UV or chemicals have various mutations in their genomic DNA, so it is difficult to find which mutation is involved in magnetite synthesis. On the other hand, transposons are inserted at only one point in the genomic DNA. Transposon Tn5 also gives kanamycin resistance, and the fragment where the transposon is inserted is detected by southern hybridization. Therefore, we next made a transposon mutant of AMB-1 by conjugation with pSUP1021 (22). pSUP1021 contains Tn5 and mob* to transfer in AMB-1.

We selected Tn5 transconjugants by kanamycin resistance. Among 118 colonies of transconjugants, 5 were found to be completely nonmagnetic while 2 showed reduced magnetite production compared with the wild type. The nonmagnetic transconjugants were denoted NM1, NM2, NM3, NM5, and NM7. The nonmagnetic phenotypes of these mutants were confirmed by transmission electron microscopy (Fig. 4). Moreover, genomic DNA from each mutant was probed with a Tn5-specific *XhoI* restriction fragment purified from pSUP1021 (Fig. 5). In each lane, the probe hybridized with only one band. This means that insertion by Tn5 was at only one point in the genomic DNA of each mutant.

Three Tn5-containing chromosomal *EcoRI* fragments were cloned into *E. coli* DH5α. Three clones, plasmids pCN1, pCN3, and pCN5, were obtained from the nonmagnetic transconjugants NM1, NM3, and NM5, respectively.
3. GENETIC ANALYSIS OF magA GENE

As indicated in the previous section, 5 nonmagnetic mutants of Magnetospirillum sp. strain AMB-1 were obtained by transposon mutagenesis, and three Tn5-containing genomic DNA fragments were cloned. Among these clones, pCN5 had an 8.8-kb EcoRI fragment which contained inserted transposon Tn5. About 3-kb of the genomic DNA fragment from NM5 was sequenced and two open reading frames were analyzed. Genetic analysis of these two genes is discussed in this section.

3-1. Sequence of Genomic DNA from AMB-1 Nonmagnetic Mutant

2975 bp of the genomic DNA fragment from the Tn5 flanking region of NM5 was sequenced. The physical map of this region is shown in Fig. 6. The transposon insertion site is also shown. Two open reading frames with putative ribosomal binding sites which resemble a SD sequence (21) were found. In Fig. 6, the black line represents one of the open reading frames, ORF1, named magA, which is interrupted by the Tn5 insertion and followed by ORF2, which overlaps magA by 25 bp. Fig. 7 shows the nucleotide sequence of the genomic DNA fragment containing magA and ORF2. Within magA gene, there is a Tn5 target sequence, TTCTGACC, at nucleotide (nt) 1524 which was duplicated by Tn5 integration in the mutagenized genome of NM5. The magA gene 1305 bp in size and a putative promoter

Figure 5. Southern hybridization analysis of EcoRI-digested genomic DNA from nonmagnetic mutants with Tn5 as a probe. Lanes: M, 1-HindIII, 1, pSUP1021; 2, wild type; 3, NM1; 4, NM2; 5, NM3; 6, NM5; 7, NM7.

Figure 6. Localization of the Tn5 insertion site within the open reading frame ORF1 in the mutagenized genomic fragment from nonmagnetic mutant NM5.
Figure 7. Nucleotide sequence of the mutagenized genomic fragment of NM5. Arrows indicate inverted repeats and hairpin loop.
region is located 75 bp upstream from the start codon of magA at nt 883. Two sets of putative -35 and -10 domains are shown as boxed regions and inverted repeats occur near the -35 and -10 domains which are indicated by arrows in Fig. 7. However, no promoter-like region was found upstream from the start codon of ORF2 at nt 2162. Furthermore there is a region of dyad symmetry at nt 2227 downstream from the stop codon of magA which resembles a Rho-independent terminator (19). No terminator structure was found downstream from the stop codon of ORF2. The hypothetical protein encoded by the magA gene, MagA, consists of 434 amino acids with a predicted molecular weight of 46.8 kDa. ORF2 consists of a 606 bp section which would encode a 21.6 kDa protein.

3-2. Homology Analysis of magA Gene and ORF2

MagA was found to have high homology with the KefC protein from E. coli (16), with 25.4 % similar amino acid residues. Fig. 8 shows an alignment of MagA and KefC. KefC functions as a potassium efflux protein for control of turgor. Moreover the NaH-antiporter, NapA of Enterococcus hirae (26) is homologous to MagA, with 24.1 % similarity. The similarity between KefC and NapA has been described (18). These proteins form a group of cation efflux antiporters. This result suggests that the magA gene may encode a cation efflux transport protein.

Moreover, KefC consists of two domains, a hydrophobic membrane-binding domain, and a strongly hydrophilic carboxy-terminus. The region homologous with MagA corresponds to the hydrophobic domain, which is thought to be a potassium-translocating channel domain (16). Furthermore the hydrophathy plot of MagA was compared with KefC. a similar pattern of hydrophobicity of amino acid sequence was observed (Fig.8). The sequence of hydrophobic amino acids could form eight to ten transmembrane α-helices. The similarity and structure suggest that MagA may also be located in the membrane of AMB-1.

The putative protein encoded by ORF2 was also analyzed. A strong homology (47.2 % similarity) with E. coli RNase HII (8, 25) was exhibited (Fig. 9). RNase HII degrades the ribonucleotide moiety on RNA-DNA hybrid molecules. This strong homology suggests that the ORF2 coding protein has a similar function to RNase HII.

3-3. Measurement of magA Promoter Activity

The function of the promoter which was found upstream from magA was investigated. A 540 bp KpnI-PstI fragment containing the promoter region was cloned upstream from the CAT reporter gene of the promoter probe vector, pXCAT, in the orientation which the putative promoter transcribes the CAT gene. This plasmid was designated pMKP. pXCAT and pMKP

| Table 1. Average CAT activity of exponentially growing transconjugant cells |
|-----------------|-----------------|
|                | pXCAT | pMKP  |
| AMB-1           | N. D.  | 45.7  |
| E. coli         | 2.0    | 2.0    |

unit ; nunit/cell, 1 unit of CAT activity acetylates 10 nmol chloramphenicol per minute. N. D. ; not detected.
Figure 8. Amino acid sequence homology of MagA with KefC of E. coli. Identical amino acids are indicated as bar. Equivalent amino acids are shown as one or two dots. Two dots indicate higher similarity than one dot. Homology exists between the MagA protein and the KefC hydrophobic region (16).

were transferred into wild type AMB-1 cells by conjugation (13, 23) and change in CAT activity with cell growth was measured (24). Table 1 shows average CAT activities of AMB-1 transconjugants containing either pXCAT or pMKP in log-phase. The pXCAT transconjugant did not show CAT activity. In contrast, high CAT activity, 44.8 nunit/cell was measured in
Figure 9. Amino acid sequence homology of the MagB protein with RNase HII of E. coli. Identical amino acids are indicated with a bar. Equivalent amino acids are shown as one or two dots. Two dots indicate higher similarity than one dot (8).

4. EXPRESSION OF PROTEIN ON THE SURFACE OF AMB-1 MAGNETIC PARTICLES USING magA GENE

Attempts to express proteins on the surface of bacteria using various genes which encode proteins located in the outer membrane have been made. For example, using ompA or iga gene, proteins have been expressed on the cell membranes of gram-negative bacteria (6, 7, 11). Moreover, ‘phage display’, which is the method of forming fusion proteins on the surfaces of bacteriophages by fusing with coat protein, has been applied to gene screening (3, 4, 12).

The putative protein of MagA seemed to be localized on the cell membrane from the homology between magA and kefC gene. We try to localize MagA protein by fusion gene method and immnoelectron microscopy localization method. Since magnetic particles in AMB-1 is also covered with membrane, MagA fusion protein will also be localized on the surface of AMB-1 magnetic particles.

4-1. Localization of magA Protein

In order to investigate localization of the MagA protein, the gene fusion method of magA and luciferase was employed. The firefly luciferase gene, luc (Toyo Ink co. ltd., Tokyo, Japan), was used as the fused reporter gene. The strategy of fusion gene construction is shown
in Fig. 10, magA is 1305 bp long. Firstly, luc was cloned into the plasmid vector, pRK415 (Tc, lacZ, mob') (10), which is a broad-host-range vector for gram-negative bacteria, and was maintained in Magnetospirillum sp. AMB-1. 886 bp of the EcoRI-NcoI fragment containing the magA promoter region and excluding the magA open reading frame was connected with the luc gene. This plasmid was designed pKPL. Then, 1135 bp of the EcoRI-SphI fragment was ligated with the luc gene after blunting treatment. The SphI restriction enzyme site is in the hydrophilic amino acid coding region in magA. By this ligation the magA-luc fusion gene was successfully constructed, with no translation frame shift. The constructed plasmid was termed pKML. Both plasmids were transferred into wild type AMB-1 cells by conjugation.

The luciferase activities of cytoplasm, cell membrane and bacterial magnetic particles (BMPs), of transconjugants were measured. The luciferase assay of each fraction was carried out using PicaGene (Toyo Ink co., ltd., Tokyo, Japan). The luminescence of the luciferin-luciferase reaction was detected using Luminescence Reader. pKPL and pKML transconjugants were grown to stationary phase. Cells were harvested and resuspended in 10 mM Tris buffer (pH 7.0). Harvested cells were broken by sonication, and BMPs were collected using a magnet. Cells which were not lysed were separated from the cell debris by centrifugation at 5000 x g for 20 min. Then the supernatant was ultracentrifuged at 96,600 x g for 1.5 h to separate the membrane fraction as a precipitate from the supernatant containing the cytoplasmic fraction.

Results of the luciferase assays on pKPL and pKML transconjugants are shown in Table 2. Luciferase activity was determined from the integrated luminescence yield for 3 minutes per µg of protein. In the pKPL transconjugant the highest luciferase activity was
Table 2. Luminescence yields of cell fractions of transconjugants

<table>
<thead>
<tr>
<th>Cell fraction</th>
<th>pKPL</th>
<th>pKML</th>
</tr>
</thead>
<tbody>
<tr>
<td>cytoplasm</td>
<td>35.2</td>
<td>0.9</td>
</tr>
<tr>
<td>cell membrane</td>
<td>2.6</td>
<td>13.0</td>
</tr>
<tr>
<td>magnetic particle</td>
<td>0.2</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Unit: kcounts/mg protein, Luciferase activity was determined from the integrated luminescence yield for 3 minutes.

detected in the cytoplasmic fraction. Luciferase is a soluble protein, so it should localize in the cytoplasmic space when it expresses independently. In contrast, most of the luciferase activity in the pKML transconjugant localized in the membrane fraction. This result indicates that the MagA protein localizes in the cell membrane, and the luc-connected site in MagA protrudes from the membrane. Moreover, the BMP fraction showed weak luciferase activity. This result suggests that the MagA-Luc fusion protein is expressed on the BMP surfaces.

Iron must be translocated across the BMP membrane during the crystallization of magnetite. We consider that MagA is probably an iron channel protein which is integrated

Figure 11. Transmission electron micrographs of BMPs extracted from transconjugants which were treated immunogold. Photograph A shows BMPs of the pKML transconjugant. B shows BMPs of the pKPL transconjugant. Gold colloid particle has 15 nm diameter in size.
in the cell and BMP membranes of AMB-1, and translocates iron from the environment into the cytoplasmic space across the cell membrane, and from the cytoplasm into the lipid vesicles of BMPs across the BMP membrane. In order to elucidate the synthesis mechanism of the lipid vesicles of BMPs, it is important to know whether the MagA protein localizes on the outer or inner membrane. We assume that the inner membrane invaginates to form the lipid vesicles of BMPs.

4-2. Detection of Luciferase Protein on the Bacterial Magnetic Particles

To detect the localization of the MagA-Luciferase fusion protein, immunoelectron microscopy localization method was used. Anti-Luciferase polyclonal antibodies which bind to gold particles (15 nm) were used to localize the fusion protein at the ultrastructural level. BMPs were extracted from pKPL or pKML transconjugants of AMB-1 by French press and magnetic separation, and resuspended in PBS buffer (pH 7.4). Then the suspension was incubated for 1 hour after adding anti-Luciferase antibody and was washed twice. Samples were observed by electron microscope. The results are shown in Fig. 11. Antibody-gold particles were bound to magnetic particles extracted from pKML transconjugants. This means that the MagA-Luciferase fusion protein is localized on the lipid membranes of BMPs. Moreover, it will be possible to express various proteins on the surfaces of magnetic particles using fusion proteins with MagA.

1. Production of BMPs possesses new function
2. Development of new protein engineering system

- Production of IgG-bind BMPs and construction of new immunoassay system
- Development of micromachine bound to protein which enable to induce by magnet

Figure 12. Technological application of protein product on BMPs.
4-3. Protein Expression on the Surfaces of Magnetic Particles

Examples of protein expression of the surface of magnetic particles is shown in Fig. 12. In a previous novel fluoroimmunooassay method using BMPs, fluorescein isothiocyanate (FITC)-conjugated monoclonal antibody was immobilized onto BMPs using a heterobifunctional reagent. N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP) (17). However, conjugation of antibodies to magnetic particles is difficult and the quantity of antibody per magnetic particle was unstable. On the other hand, magnetic particles from MagA-Antibody-fusion-gene transconjugants will be useful, and will eliminate the necessity conjugate antibodies and particles chemically.

Moreover, expression of fusion proteins with MagA protein can be applied to the system of protein production. Magnetic particles with fusion proteins can be collected easily and quickly by magnet after breaking the cells. We will be able to obtain purified proteins by this system by inserting a gene between magA and the target gene in order to cleave the target protein from the fusion product.

5. SUMMARY

Establishment of gene transfer has enabled study of analysis of genes in magnetic bacteria and protein production on BMPs using magnetic bacteria as the host cell. Gene transfer into magnetic bacteria, and transposon mutagenesis, are very useful for future analysis of the mechanism of magnetic particle production. The magA gene was the first discovered gene involved in magnetite biomineralization in magnetic bacteria, and the product of magA seems to be a kind of cation channel protein localized on the cell and magnetic particle membranes. Gene analysis of Magnetospirillum sp. AMB-1 will determine the mechanism.

Moreover, protein expression on the surfaces of magnetic particles using the fusion gene method was studied. Magnetic particles which have fusion proteins on the surfaces can be collected very easily, and production of purified protein will be applied in future.

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EFFECTS OF STATIC MAGNETIC FIELDS ON ERYTHROCYTE RHEOLOGY

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ABSTRACT

The acute influence of an external static magnetic field on the erythrocyte rheology is briefly reviewed. 1) The magnetohydrodynamic action may be effective to alter the fast blood flow in aorta at a field over 5 T, but no experimental study has been made. 2) The diamagnetic interaction, between the normal blood component and uniform magnetic field, has been found: i.e., the biconcave disk-shaped erythrocytes and platelets orient their flat plane parallel to the field direction above several T. 3) The paramagnetic interaction, effective only on a slow flow of deoxygenated erythrocytes, affects the distribution of erythrocytes within a vessel by the paramagnetic attractive force, which is proportional to the product of [flux density] x [spatial gradient], the magnetic susceptibility of erythrocytes, and the reciprocal of the flow velocity. Our model study showed that the paramagnetic interaction may be effective under an anemic situation and under an inhomogeneous magnetic field over above 100 T²/m for the product of [flux density] x [spatial gradient].

INTRODUCTION

The static magnetic field has been known to interact with the cellular components of blood: for example, the magnetic separation of phagocytes loaded with ferromagnetic material [1], the orientation of sickled erythrocytes in a uniform magnetic field of 0.35 T [2, 3], and so forth. An external magnetic field may affect the blood flow even in the normal state through three distinct physical mechanisms: 1) The magnetohydrodynamic action: i.e., when charged particles pass at a high speed through a strong magnetic field, a force against the flow develops (Lorentz force). Lorentz force is predicted to increase the pressure drop...
by 10% in the ascending aorta under an uniform magnetic field of ca. 5 T [4]; but no experimental proof has been given yet. 2) The diamagnetic interaction, which affects the orientation of erythrocytes in an uniform magnetic field with normal discocytes at several T [5]. 3) The paramagnetic interaction, which acts only on the paramagnetic erythrocytes at an inhomogeneous magnetic field with a strong spatial gradient. The first trial to trap the Malaria-infected erythrocytes from flowing blood was not satisfactory [6], but later the idea is successfully applied to the magnetic separation of paramagnetic erythrocytes [7].

Among these mechanisms, we have concentrated our attention to the paramagnetic attraction of flowing erythrocytes under an inhomogeneous magnetic field [8,9,10,11], in order to establish the physical characteristics of the phenomena, to find out the experimental condition for the basis of safety criteria and for possible animal experiments, and to apply the techniques for the hemorheological study. We have already demonstrated the magnetic attraction of a flow line of the paramagnetic erythrocytes, using three experimental procedures: i.e., 1) displacement of a narrow stream line of erythrocyte suspension in a wide laminar flow [8,9], 2) a small change in the distribution of erythrocytes flowing through a cylindrical vessel [10], and 3) an acceleration of the erythrocyte sedimentation rate [11], due to an inhomogeneous magnetic field.

This paper briefly reviews these interactions between erythrocytes and magnetic field, with emphasis on our own studies of paramagnetic attractive interaction.

A. MAGNETIC PROPERTIES OF ERYTHROCYTES

Blood contains various electrolite ions and negatively charged proteins. The erythrocyte is a biconcave disk-shaped cell containing hemoglobin as a major constituent, which occupies a volume fraction of 45% (called hematocrit, Ht) thus determines the rheological properties of blood. The surface of erythrocyte membrane is covered with negatively charged polysaccharides.

The erythrocytes have a unique feature, i.e., the magnetic susceptibility of hemoglobin can be varied by changing the valency and the spin state. The deoxygenated erythrocytes are paramagnetic due to ferrous deoxy-hemoglobin (S=2), while the oxygenated erythrocytes are diamagnetic due to oxyhemoglobin (S=0); and the erythrocytes containing high- and low-spin (ferric) methemoglobin are both paramagnetic (S=5/2 or 1/2, respectively) but with different magnetic susceptibility (Fig. 1).

![Figure 1](image_url)

**Figure 1.** The spin states of hemoglobin (Hb) derivatives. Depending on the ligand to the 5th position (L) the spin state of central iron ion varies: i.e., (a) deoxy-Hb is in ferrous high spin state [S=2], (b) oxy-Hb is diamagnetic ferrous [S=0], (c) metHb-H2O is in ferric high spin state [S=5/2], and (d) metHb-CN is in ferric low spin state [S=1/2].
Four types of erythrocytes, containing various spin states of hemoglobin, were prepared from freshly drawn human blood after removal of plasma and buffy coat [8-11].

(a) The washed erythrocytes were re-suspended in an isotonic buffered saline (90 mM NaCl, 5 mM KCl, 50 mM Na-phosphate, 5.6 mM glucose; pH 7.4), and employed as the erythrocytes with oxygenated hemoglobin. (b) The suspension was deoxygenated by adding sodium hydrosulfite, to obtain the erythrocytes containing deoxy-hemoglobin; (c) after an addition of sodium nitrite to oxidize the hemoglobin in erythrocytes, the erythrocytes were washed and resuspended at pH 5.7 to establish methemoglobin in high spin ferric state, or (d) the erythrocytes containing methemoglobin were treated with potassium cyanide and washed with buffer at pH 7.4 to obtain the erythrocytes with low spin methemoglobin.

**B. MAGNETOHYDRODYNAMIC ACTION (LORENTZ FORCE)**

A static magnetic field interacts with flowing blood in a vessel, because blood contains various ionic species.

*B-1. Action on Blood Flow.* When a solution, containing the ionic species (electric charge: q), e.g., blood, flows rapidly at a velocity (u) in a tube (diameter: d) placed perpendicularly to a static magnetic field (magnetic flux density: B), the Lorentz force (\( F_B \)) appears towards the direction perpendicular to both the flow direction and the magnetic field as follows:

\[
F_B = q(u \times B).
\]

Blood is a conducting fluid (electrical conductivity, \( \sigma \)), thus an electric current (\( j \)) is generated in a homogeneous magnetic field: \( j = \sigma (u \times B) \). The current produces a force (\( f_L \)) to reduce the blood flow,

\[
f_L = j \times B = \sigma (u \times B) \times B
\]

Combining the Navier-Stokes equation and the Lorentz force, a calculation predicted that 10% retardation of blood velocity may occur with ascending aorta under the static field of 5 T [1], but no change with small arteries [12]. However, no experimental proof has yet been made.

*B-2. Flow Potential.* In addition, the induced electric potential (\( V \)) is provided by blood flow with a diameter (d) in a static magnetic field: \( V = |u| |B| d \). The amplitude of T-wave in electrocardiogram can be modified by the superposition of the induced potential (synchronizing to the pulse flow) due to aortic blood flow in a static magnetic field [13,14,15].

**C. DIAMAGNETIC INTERACTION**

Most of the biological materials are diamagnetic, and for a rod-like macromolecule placed in a strong uniform magnetic field the diamagnetic anisotropy may lead an orientational change along its rotation axis. In the case of erythrocyte, a disc-like material (volume, \( V \)) with the anisotropic diamagnetic susceptibility (perpendicular to and parallel with the disc surface, \( \chi_p \) and \( \chi_{d} \)) under an uniform magnetic field (\( H \)), the field-induced energy (\( U \)) is given by the following equation:

\[
U = - (V H^2/2) (\chi_p + \chi_d \cos^2 \theta),
\]

where \( \Delta \chi = \chi_d - \chi_p \) and \( \theta \) is the angle between \( H \) and the disc plane of erythrocyte as shown in Fig. 2.
However, the magnetic interaction energy is theoretically predicted to be small compared with the thermal energy $kT$, thus the degree of orientation of only 1% was achieved for calf thymus DNA at 14 T [16]. For the blood components, Torbet et al [17], later Yamagishi et al [18], demonstrated the orientation of fibrin polymerization in a strong magnetic field.

Recently, Yamagishi et al [19] have shown an orientation of normal, biconcave erythrocytes under a high uniform field (the effect saturates above 4 T): i.e., human erythrocytes are oriented with their disk plane parallel to the magnetic field direction. Furthermore, the spin state of hemoglobin inside the erythrocytes does not influence the degree of erythrocyte orientation in a strong magnetic field. For example, the deoxygenated erythrocytes contain the paramagnetic hemoglobin (high spin ferrous Fe, $S=2$) while the oxygenated cells possess diamagnetic hemoglobin (low spin ferrous, $S=0$), the degree of orientation is unaffected by such changes in spin state of hemoglobin-Fe. Therefore, Higashi et al [20] explained the magnetic orientation of normal discocytes taking account for the contribution of anisotropic susceptibility of lipid bilayer membrane and transmembrane proteins. Membrane phospholipids are oriented with their hydrocarbon chain perpendicular to the magnetic field and the anisotropic diamagnetic susceptibility ($\Delta\chi$) is about $1 \times 10^{-20}$ emu/molecule [21,22]. On the other hand, the $\alpha$-helix portion of the transmembrane proteins (e.g., Band 3, glycopolins, etc) is oriented their long axis (the longitudinal direction of the helix-chain) in parallel with the magnetic field and $\Delta\chi$ is about $7 \times 10^{-19}$ emu/peptide group [23]. Comparing these two opposite forces, the estimated contribution of total membrane phospholipid ($13 \times 10^{-22}$ emu/cell) is greater than that of transmembrane proteins ($7 \times 10^{-22}$ emu/cell), thus intact erythrocytes oriented their disk plane parallel to the magnetic field. Further, the observed, anisotropic diamagnetic susceptibility of the erythrocytes ($\Delta\chi = 8 \times 10^{-22}$ emu/cell) agrees with the above rough estimation [20,24].

Similar phenomenon is also observed with human platelets [25], which are oriented their disc surface parallel to the magnetic field direction. In the case of platelet, however, the observed $\Delta\chi$ cannot be explained by counting only the estimate of membrane lipids but the contribution of cytoskeletal microtubules should be accounted for.

In addition, a death was reported, possibly due to the dislodgment of intracranial aneurysm clip placed in 1.5 T magnetic field [26].

**D. PARAMAGNETIC INTERACTION**

Early in 1946, Heidelberger et al [6] made the first trial to separate or concentrate the Malaria infected erythrocytes (paramagnetic due to deoxygenation and/or oxidation of hemoglobin) from the un-infected (presumably diamagnetic) erythrocytes with an inhomogeneous magnetic field. They could concentrate the paramagnetic erythrocytes in some extent, and finally they wrote that the Malaria-infected erythrocytes were separable from
unparasitized erythrocytes in 5 to 25 blood samples in a strong, unsymmetrical magnetic field, but the method does not appear suited to the preparation of large quantities.

In 1975, Melville et al [7] succeeded to separate the paramagnetic erythrocytes from the flowing blood in a column filled with stainless-steel wire, which produces a strong inhomogeneous field in an externally applied uniform magnetic field. Various methods of the magnetic separation have been widely applied in biotechnology [27]; e.g., Rous and Beard (1933) used magnetic separation technique to select the living Kupffer cells which phagocytized ferromagnetic iron oxide [28]. Similar techniques are employed for separating the activated leukocytes which phagocytized iron granules [1].

We have been studying the effect of paramagnetic attractive force on the flowing erythrocytes under an inhomogeneous magnetic field. Our aims for the following three types of hemorheological experiments were (i) to establish a theoretical basis for the safety criteria of static magnetic field on the blood circulation and (ii) to find the appropriate conditions for the possible animal experiments under magnetic fields. The essential parts of our results are summerized: in the order 1) displacement of a narrow stream line of erythrocyte suspension in a wide laminar flow [8,9], 2) a small change in the distribution of erythrocytes flowing through a cylindrical vessel [10], and 3) an acceleration of the erythrocyte sedimentation rate [11].

D-1. the Displacement of Erythrocyte Stream Line in Laminar Flow [8,9]. (i) Using the flow channel shown in Fig. 3, the position of the narrow stream line was measured on microscopic photographs before and during the application of the magnetic field and the displacement of the stream line due to the magnetic attraction was obtained by comparing the two values. Fig. 4 shows the displacement of the stream line of erythrocyte suspension, whose diameter is ca. 80 μm in a wide laminar flow (Fig. 3a). The displacement is detected only for paramagnetic erythrocytes, but not for the oxygenated erythrocytes containing diamagnetic oxy-hemoglobin. The displacement became larger with the increase of: (a) the product of magnetic field strength and its spatial gradient, (b) the paramagnetic susceptibility of hemoglobin in erythrocytes, and (c) the reciprocal of the flow velocity. Further, it increased in parallel with (d) hematocrit of the suspension.

(ii) The forces acting on a flowing paramagnetic particle are shown in Fig. 5. The force \( \mathbf{F}_{\text{mag}} \) is due to the attractive magnetic interaction.

\[
\mathbf{F}_{\text{mag}} = \chi \mathbf{V} (\mathbf{H} \cdot \mathbf{V}) B + q(\mathbf{V} \times B + \mathbf{E})
\]

the first term is obtained by differentiating the potential energy \( \phi = \mathbf{V}^2 \mathbf{H} \mathbf{B}/2 \) of a paramagnetic particle in the magnetic field, using \( \mathbf{V} \times B \) (and \( \mathbf{H} \)) =0. Here \( \mathbf{V} \) (nabla) is a differential operator. This term describes the magnetic interaction of erythrocytes (suscepti-

![Figure 3. Flow channels and an iron block attached to an electromagnet. (a) For the observation of the displacement of an erythrocyte stream line in the laminar flow [8,9]. The dimension of the channel was 4 x 0.4 x 150 mm, and the width of the stream line (shown with a dotted line) was ca. 80 μm. (b) For the detection of excess flow of erythrocytes to the side branch [10]. The inner diameter of the main cylinder was 2.0 mm and that of the side branch 1.0 mm. The inhomogeneous magnetic field was made using an electromagnet and an iron block with one side tapered, to which the channel or the tube is attached tightly. The magnetic flux density around the model channel was measured point by point with a gauss meter, then the spatial gradient and the “averaged” value of the product, \( B \times dB/dz \), were calculated [8,9,10].](image-url)
Figure 4. Displacement of the erythrocyte stream line. Top panels show the position of erythrocyte stream line in the absence of a magnetic field; bottom panels are those in the presence of a magnetic field, with erythrocytes (hematocrit of 5 %) containing [from left] (a) oxy-hemoglobin, (b) low spin methemoglobin, (c) deoxy-hemoglobin, and (d) high spin methemoglobin. The microphotographs were taken at the position y=150 mm. The unfocused spots were small dust attached outside of the flow channel. Standard experimental conditions: flow velocity 0.7 mm/s, average value of B x dB/dz 29 T/m [For details, see ref 9].

Figure 5. Displacement of the paramagnetic erythrocytes due to the interaction with an inhomogeneous magnetic field in a flow experiment (see text).
Although the displacement of the erythrocyte stream line occurred in accord with the above theory, the observed displacement is much larger than the calculated value for a single erythrocyte. Moreover, the displacement becomes large with an increase in the hematocrit of erythrocyte suspension. These results indicate that a group of erythrocytes (in a "volume") is attracted as a whole by the magnetic force, as discussed previously [8,9]. Actually a good agreement was obaining between the observed and theoretical values of displacement, when we calculate the displacement for a volume of spheric droplet, of which the diameter R is the same as the diameter of narrow stream line and the magnetic susceptibility \( \chi \) as that for the flowing erythrocyte suspension.

**D-2. Asymmetric Distribution of Flowing Erythrocytes in a Vessel [10].** (i) With the setup shown in Fig 3b, which is similar flow channel tested by Heidelberger et al in 1946 [6] but with much higher magnetic field, a displacement of erythrocytes due to a strong inhomogeneous magnetic field was observed [10]. In order to separate the paramagnetic cells from blood, an extremely high gradient magnetic field was necessary [7]. As we have shown, with paramagnetic erythrocytes containing the high spin methemoglobin, the skimmed erythrocytes into the side branch of the flow channel of Fig 3b increased by the magnetic attraction [10]. The degree of attraction increased linearly (a) with the magnetic susceptibility of erythrocytes, (b) with the product of magnetic field strength and its spatial gradient, but it saturated above ca. 20 T/m, and (c) with the reciprocal of the flow velocity. Further, (d) a peculiar hematocrit dependence was found: i.e., at the hematocrit of ca. 5% the maximal attraction took place, while at 45% no attraction was detected. In the case of mixed suspensions containing erythrocytes with high spin methemoglobin (paramagnetic) and oxygenated erythrocytes (diamagnetic), the attraction reached maximum at the "partial hematocrit" for the paramagnetic erythrocytes of ca. 5% and remained nearly constant with a further increase of the "partial hematocrit" [10].

(ii) In order to quantify the degree of the magnetic attraction of deoxygenated erythrocytes, we have to measure anaerobically the amount of erythrocytes skimmed into the side branch. Therefore, a spectrophotometer with long optical fiber was attached at the rectangular side ranch just below the magnetic field, taking the difference spectra before and after exposure to the magnetic field. The changes for the skimmed erythrocytes (\( \Delta V \)) are defined as: \( \Delta V = (\text{amount with field})/(\text{amount without field}) - 1 \).

The following results were obtained [29]. \( \Delta V \) increased proportionally to (a) the product of magnetic field strength and its spatial gradient, but saturated at weak magnetic field (20 T/m); (b) the magnetic susceptibility of hemoglobin derivatives in erythrocytes; and (c) the reciprocal of the flow velocity. (d) The effect is maximal at the optimal hematocrit of 5%. Further, (e) \( \Delta V \) varied with the degree of deoxygenation, proportionally to the estimated magnetic susceptibility.

(iii) These phenomena can be interpreted as due to the asymmetric distribution of flowing erythrocytes made by its paramagnetic interaction with the magnetic field in the cylindrical vessel. The saturation phenomena, at high magnetic field and/or at high hematocrit, may be arisen from the collision among erythrocytes and to the vessel wall, which produces the local eddy currents and turbulent flow, and the magnetic field-induced asymmetry in the distribution tends to disappear. Therefore, as the collision rate increases with increasing the magnetic field and/or the hematocrit, the magnetic effect (asymmetric distribution) is apparently suppressed.

**D-3. Acceleration of Erythrocyte Sedimentation Rate [11].** The sedimentation rate of paramagnetic erythrocytes in the Westergren tube increased in a spatially inhomogeneous magnetic field, but not in a homogeneous field. Moreover, no magnetic effect was detected for diamagnetic erythrocytes. This phenomenon is similar to the so-called Boycott effect.
[30], i.e., the accelerated sedimentation in an inclined cylinder [31]. However, the detailed mechanism of Boycott effect is not fully understood.

D-4. Summary for Paramagnetic Attraction. The effect of an external inhomogeneous magnetic field on the flow of erythrocytes containing paramagnetic hemoglobin was systematically studied with three experimental setups. The results are summarized as follows:

1) Paramagnetic erythrocytes are attracted by an inhomogeneous magnetic field.
2) The degree of attraction is proportional to (a) the product of magnetic field strength and its spatial gradient, (b) the magnetic susceptibility of hemoglobin, and (c) the reciprocal of the flow velocity. The attraction is detectable with the flow channels under an inhomogeneous magnetic field above 10 T/m.
3) The degree of attraction depends on the hematocrit, with the maximum effect at ca. 5%.
4) Such asymmetric distribution of venous, deoxygenated blood flow in an inhomogeneous magnetic field of above 100 T/m may induce some biological effect.

CONCLUDING REMARKS

The effect of a static magnetic field on erythrocyte rheology was summarized. In order to detect circulatory effects by the magneto-hydrodynamic action, a considerably strong field will be needed. Minor effects due to the diamagnetic interaction may appear at the field strength of several T. On the other hand, the paramagnetic attraction affects only the venous blood flow, when the product of magnetic field strength and its spatial gradient exceeds above 100 T/m, especially in anemic situation under slow flow.

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REFERENCES


PUBLIC HEALTH ON ELECTROMAGNETIC FIELDS AND MAGNETIC SHIELD OF LINEARMOTORCAR (EDS) MAGLEV

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1. SAFETY

1.1. Denotation of “Safety” as a Legal Term

As a legal term, the Labor Safety and Hygiene Law (LSHL; enforced October, 1972) uses it with a denotation of laborious disaster prevention. Activities for disaster prevention of workplace are divided into safety management and hygienic management by a narrow sense. Here, we think that it is prevention of an emergent danger with a task of “safety” of a narrow sense concerning LSHL, and we are allowed to think it is prevention of a health impairment that occurs chronically with an issue of “hygiene”. We state about “safety” related to magnetic field or electromagnetic field. Safety issues about a case of MRI taking a superconducting magnet (SCM) as an example, are as follows: 1. a strong magnetic field attracts an iron-made oxygen bomb and a stretcher (simple bed with wheels that carries a patient prostrate) or causes a hammer and operative instruments to fly, injuring personnel nearby; 2. danger of an asphyxia for a personnel nearby, when a SCM is quenched under insufficient ventilation; and 3. when dB / dt change in magnetic field of super-high-speed MRI equipment is large, induced currents stimulate nerves, muscles and directly act on the heart.

In an industrial scene, we can easily understand “safety” issue as an accident which is visible directly. “Safety” issue is generally well known, since we were concerned about it earlier than about “hygiene” issue. As we are interested in weak influences of the factors that shift and persist chronically, the present revised Act for Labor Safety and Hygiene (ALSH) puts stress on a hygienic matter. We see not only “safety” matter that the ALSH treats, but also “hygiene” issue. We discuss on safety issue with a wide denotation that includes professional and general personnel.
1.2. Division of “Safety” Conception with Hygienic Management

When we speak of safety as a basis to hygienic management we mean that we do not meet an accident, and that there is no worry even though we are exposed to various factors over a long term. To be specific, we aim at each factor by which we can manage to hold the exposure amount below the level that causes no health impairment, when an 8-hour per day exposure is supposed to last 30 years. A theme that comes next is what level of exposure amount is safe and over what level is harmful. As the basis to determine the exposure-dose level, the concept called dose-response relation [1] is known for hygienic management. This concept shown in Figure 1 indicates that as exposure dose increases, the human response progresses from the first step of homeostasis that can be compensated, to sickness able to recover from, to sickness unable to recover from, and finally to death. How to divide the levels of this stimulus factor is a difficult job involving not a few physical factors which are unlike chemical materials. With a chemical substance, for example, a carbon monoxide, combines with hemoglobin and makes the whole body short of oxygen, division between harm and no harm is very clear. However, physical stimuli, except strong ones like radioactive rays, show no clear-cut border between safety and danger.

Recently an interest is aroused in influence of the factors such as non-energy or non-ionizing agents. Because an action of such factors is extremely weak, dose-response relation is hardly found in many cases. For such weak physical factors, we can count other factors besides electromagnetic fields that we treat here. These are radio-frequency radiation (especially 300 MHz from 30 kHz), ELF radiation (from 3 to 300 Hz or up to 3 kHz), cold stress, and change of pressure, and distinguishing between safe and unsafe is considered is also a difficult job within either of them. As far magnetic exposure dose (frankly speaking on this concept there is no definition as yet), we try to think of 4 steps in evaluation of the responses: i.e. 1. safe without specific anxiety, 2. indistinct, 3. harmful, and 4. dangerous. However, from a point of view of hygienic management, a magnetic issue is considered an object that involves a safe and indistinct step. Then, there remains the division of these first and second steps in a very difficult situation of these days. About how to distinguish between second and third step is similarly difficult.

![Figure 1. Dose-response relation, indicating changes upward in response as magnitude of dose is increased. A level of non response exists below a threshold dose level of above zero. Threshold dose of electromagnetic fields with no health risk is also unknown yet.](image-url)
2. PROGRESS OF MAGNETIC APPLICATION

2.1. Classification of Magnetic Fields

Application of magnetic fields has advanced with scientific-technological progress. Energy employed for the machines utilizing magnetic fields gets very large. With utilization of magnetic fields extended further, we have to think about safety in various applied scenes.

Today, concern for magnetic fields as a matter of safety may be involved in an application of static-magnetic field; 1. a super-strong magnetic field of the equipment using a superconducting magnet, in an application of a time-varying magnetic field, 2. the magnetic field applying an induced current by large dB/dt, 3. a time-varying magnetic field of ELF (extremely low frequency), and 4. high-frequency electromagnetic fields. In these four large classifications, however, as we think about safety of magnetic fields to the human body, we see that mechanism of each action is different. Next, we consider for what the magnetic field in each equipment is used.

2.2. Equipment Using Static Magnetic Field

It seems to be thought generally that a static-magnetic field does not affect deleteriously a living body. That might be a mere concept, however, in the age when there exists none of such gigantic equipment using a static-magnetic field with several T (tesla).

As an opportunity that average people encounter today, it is a rare case that MRI (magnetic resonance imaging) equipment for the whole body of 4 T is trially made. A lot of MRI equipment with 0.5 T- and 1.5 T-superconducting magnet are sold as mass production machines. We were not able to imagine that we might have an opportunity of our head and body trunk being exposed to a magnetic field of 0.5 T, before MRI has come to be actually used for medical diagnoses. As other large superconducting magnet equipment, there are EDS (linearmotorcar)-maglev, MHD (magnetohydrodynamics), SMES (superconducting magnetic energy storage), and a fusion reactor of a tokamak- and a mirror-type. However, only some specialists have an opportunity of access to strong magnetic field of the central portion. For others the equipment using a large magnetic flux density are NMR (nuclear magnetic resonance). MRS (magnetic resonance spectroscopy) and hybrid type super-strong magnetic field equipment. However, with these equipment, there seems to be no chance of an experimenter being exposed to a super-strong magnetic field. The reason is due to this structure with a super-strong magnetic field being installed in a secluded narrow space.

2.3. Magnetic Fields of Large dB/dt

Generally such a magnetic field is called a pulse magnetic field and it is intended for a special purpose. As one expected to have an effect on a living body, the first equipment produced for a medical treatment of bone-fracture was Bi-Osteogen system. This experimental equipment was built 20 years ago[2] to apply electric currents in the human body induced by a time-varying magnetic field. Early in 80’s devices began to be made which could generate so large induced currents as to stimulate nerve and muscle[3]. Barker et al. [4] after that stimulated a human cerebral cortex and invented use of this equipment for pathological diagnosis of the nerves. It is a merit of the machine that it can stimulate nerve-cells of cerebral cortex from the outside without craniotomy, free from any unpleasant action of an amperage such as in direct stimulation by electrodes. As an application of this merit, a direct stimulation of volitional muscles and/or a respiratory muscle (diaphragm) is also tried. Further, an
apparatus for magnetic stimulation is produced in large quantities as a clinical test machine (e.g. SMN-1100 of Nihon Kohden Corp.)

2.4. ELF Time-Varying Magnetic Fields

ELF (extremely low frequency)-band indicates a frequency range of 3 kHz from 3 Hz in electric waves. As frequency of a power transmission line is 50 Hz or 60 Hz, a time-varying magnetic field affecting the residence near the transmission lines belongs to this frequency band. Additionally, there are EMF of low-frequency band from the heaters of an industry use, harmonic ingredient that occurs from transport facilities, and other noise-like electromagnetic fields with this band.

2.5. High Frequency Electromagnetic Fields

When high-frequency electromagnetic field is used only near the generative source, the electromagnetic field has a disposition that differs from that of electromagnetic waves used for communications covering a long distance. Electromagnetic field within a distance of \( \sqrt{2} \) from a wave source is called a near field. There, however, seems to be no unity about the definition and character of this field.

Usually, as high-frequency electromagnetic field sources, the cite an induction heater (180 Hz \( \sim \) 1 MHz) applying Joule’s heat of induced currents, a dielectric heater (3 MHz \( \sim \) 100 MHz), and a microwave heater (2.45 GHz, 5.85 GHz) utilizing dielectric loss. Representative equipment of the former is an electric furnace used for iron manufacture and metal processing, those of the latter are a plastic sealer, diathermy, and microwave oven. As electromagnetic waves of far field with high energy area, there are microwave communication and radar. There are some cases where a radar is treated as belonging to ELF band because it is remodulated with ELF.

3. EMF ENVIRONMENTS AND THEIR SAFETY

3.1. Static Magnetic Field

3.1.1. Static Magnetic Field of Superhigh Intensity: Though a superstrong static magnetic field is used widely with application of a superconducting magnet, the studies of a super-strong magnetic field for its influence on a living body are surprisingly few. The reason might be speculated that the interest in recent years is concentrated on influences of ELF electromagnetic fields.

An obvious effect of a static-magnetic field on composing ingredients of biota is to cause the diamagnetic macromolecules to be oriented in the magnetic direction. For this mechanism, the following phenomenon is attributed: that when a diamagnetic substance has different magnetizing rates by crystal axes, this material orients itself along the weak magnetized axis to an outside magnetic field direction. In a gigantic molecule structure with a similar configuration of repeating diamagnetic substance such as biomembranes and DNA, an orienting power times the integer of a molecule number has a chance to turn itself toward the direction of a magnetic field. Murayama [5] is known as the person who reported on this phenomenon in early stage. A theory of nuclear acids orientation is discussed by Maret et al. [6]. Recently super-strong magnetic fields are used in the experiments, some reports saying that a blood clot gets weak by an orientation of fibrin at coagulation [7].
The probable effects of this mechanism on a living body are a change of metabolism accompanied with mechanical distortion of biomembranes, or direct effect on gene functions and expression. It is difficult to prove a substantial effect of static-magnetic field changing the serum-chemical substances found in mice exposed for long term to a super-strong magnetic field [8], though some relation could be suspected. An effect on genes is also one of large themes in recent years. However, the effect seems not to be so strong as to be suspected from the mechanism in a static-magnetic field. A growth of strong bacteria on fertility is not affected even in a strong magnetic field of 11.7 T, unless the condition of cultures is so specific [9]. We found that the effect of a magnetic field is extremely weak by our sharp mutation detection system of pyrimidine dimers using mutant fruit fly that lacked repair functions of DNA damage. A mutation rate caused by 24-hour serial exposures to 0.6 T, was almost the same value of 5-s irradiation (12.5 Jm⁻²s⁻¹) at a distance of 50 cm from a usual 15 W germicidal lamp [10].

A real case that human whole body, especially head and body trunk, is exposed to a strong magnetic field is a medical diagnosis using MRI. Though usual flux density of mass production machines is 0.50 - 1.5 T, it is said that three trial production equipment of MRI for a whole body diagnosis with 4 T are available in the world. Schenck concerned in development of 4-T-MRI for the whole body of General Electric Corp. tested exposures of 11 volunteers for a year according to a protocol of Pennsylvania University [11]. The total exposure time of these volunteers was 150 hours that is less than 1 hour to 40 hours per one volunteer. Though healthy men and women were selected for the test, nothing objective was found by a check before and after the exposure in a medical examination by a medical doctor. However, some personnel conspicuously complained of unusual conscious perception in a reply to the questionnaire. There were reactions of vertigo, nausea, and metallic taste that differed with 2 groups of 4 T and 1.5 T depending of the strength of a magnetic field significantly, while there was no difference about headache, vomiting, tinnitus, balance difficulties, numbness, or hiccuping. Magnetophosphenes were also noticed in both groups. However, the perceptions felt at 4 T were clinically all light grade, and they did not occur at all unless the subject abruptly moved the head inside a magnet or they were weak if ever occurred.

"Safety" issue of magnetic field in medical examination and equipment treatment seems to remain usually at the extent comparable with any other high-energy equipment. After all, comparing electromagnetic fields for X-ray CT, electromagnetic field is recognized to be safer. Even though an exposure to a super-strong magnetic field of 1.5 T of MRI is brief and repeated examinations may be a few, we can say that safety in medicine is not a matter of extent.

3.1.2. Estimation of Minimum Amount of Static Magnetic Exposure That Produces a Detectable Effect on Mammals, and Calculation of Safety Exposure Levels.

(1) Introduction. In recent years, we occasionally come close to a strong magnetic field. For example, magnetic resonance imaging (MRI) technique is practiced in many hospitals for diagnosis. Some of the newest models of MRI device are equipped with superconductive magnet, and they generate 1.5-T flux density. Advance in medical engineering is so rapid that we have little time to discuss potential effect of strong magnetic field on human body before MRI-diagnosis is widely spread. In contrast, the US Department of Energy proposed very low flux densities as safety standard for workroom environment. The strict standard is, however, sometimes criticized for hampering normal operation in factories and laboratories equipped with strong magnets. The author shall illustrate biological effects of magnetic fields, estimate minimum amount of magnetic exposure that produces a detectable effect on mammals, and propose a tentative safety standard.
Inconsistent results on biological effects of magnetic fields have been reported from experiments involving various combinations of experimental variables, such as the species of animals, exposure environment, frequency and flux density of the magnetic field, duration of exposure and biological end point. Among these variables, the most critical factor appears to be the amount of exposure as defined by flux density and exposure duration, with more weight on the latter. In general, brief exposure of biological materials to a static magnetic field, even under highly intense flux densities, has produced no biological effects. Null examples are the nerve-conduction velocity after a 20-30 min exposure to 1.2 T [12] and the growth rate of mammalian cells exposed for 2 h to 1.75 T [13]. In contrast, positive results have been found in studies in which biota were exposed to a static magnetic field for a prolonged period: e.g., Drosophila for 3 days to 0.44 T [14], mice for 35 days or more to 0.42 T [15], mice for 30 days to 1.6 T [16], rabbits for 5 weeks to 0.06 T [17], and mice for 4 weeks to 0.42 T [18]. Even at high densities used by many investigators, the static magnetic field is probably too weak to cause substantial changes in animals exposed only for a short period. Therefore, lengthy exposures and prolonged periods of observation after exposure are indispensable if one is to obtain reliable results by which the existence of an effect can be ascertained or denied. Magneto-electromotive force, magnetic anisotropy [19], and modification of chemical reactions [20] are regarded as the primary factors involved in the mechanism underlying the biological effects of static magnetic fields. Even a flux density of 0.2-0.5 T is effective at the molecular level. However, there is a wide gap between the understanding at the molecular level and that at the whole-body level. Selye [21] demonstrated that the homeostatic responses of organisms to external stimuli follow a characteristic pattern, which he named "general adaptation syndrome" (GAS). The progress of GAS takes rather long time, so a persistent observation for days or weeks is necessary to ascertain this pattern. Barnothy & Barnothy [22], Barnothy & Sümegi [15], Laforge [23], and Nakagawa & Matsuda [24] have demonstrated that reaction of animals to a static-magnetic field shows the GAS-type pattern.

The activity of an animal is depressed immediately after a stressful stimulus is given (shock phase), then it recovers beyond the original level of activity (counter-shock phase). Both phases constitute "the stage of alarm reaction": a characteristic feature of the GAS. When the intensity of stimulus is moderate or exposure period is short, specific reactions disappear after the counter-shock phase without showing a "going-to-death pattern" described by Selye. Therefore, identification of "stage of alarm reaction" following a magnetic exposure was taken as a criterion for successful detection of a biological effect of magnetic field.

(2) Method and Case Traces. Barnothy & Barnothy [22] identified a typical pattern of the GAS at the "stage of alarm reaction" by a body mass change in mice that had been exposed to a field at 9.400 Oe (ca. 0.94 T) for 4 consecutive days. They specified it as a "shock effect" caused by magnetic field.

Figure 2 illustrates the result of our experiment. It shows changes in the body mass of mice that were subjected to a field of 0.6 T for 16 h a day for 4 days [14]. The double dotted line indicates data obtained from control mice and the solid line from exposed ones. The changes of body mass caused by the exposure were rather small, and the weight returned to pre-exposure level on the second day. This is probably because exposure amount (which is operationally defined here as the product of magnetic-flux density and exposure duration) was less than that employed by Barnothy and Barnothy [22]. The magnitude of mammals' response to the magnetic field, in general, seems to be positively related to the exposure amount, and there may be a threshold of exposure amount below which animals do not show an observable reaction to the magnetic field.
Figure 2. Rate of changes in body mass of mice in the exposure week, under three conditions of control (○), 0.6 T magnetic field (●), and 4°C temperature (▲), with half bars of the standard error.

Figure 3 shows frequency of shock received in Sidman’s conditioning schedule. Rats in the experimental group were exposed to 0.6 T for 16 h a day for 4 days [24]. The frequency of shock received was counted during the final 30 min of a one-hour session and averaged for each experimental condition (exposed/control, 10 rats each). Asterisks mean a significant difference between the two conditions (*: p<0.05, **: p<0.01). Shocks received by the exposed rats were more frequent than those by the control rats during and after the exposure period.

Author filed the reports in which magnetic-field density and minimum exposure time have been determined with certain effects produced at such intensities or densities.

(3) Results and Conclusions. There are only nine reports that found the GAS-like responses of mammals by magnetic exposure: five from our laboratory: A [26], B [27], D [28], F [24] and G [24], and four from others: C [23], E [29], H [22] and I [16] as shown in

Figure 3. Frequency of shocks received in the final 30 min of sessions in the SA schedule [13]. Exposed rats (▲) received more electroshocks than did control (○) during the exposure period and thereafter. (*: p<0.05, **: p<0.01).
Figure 4. The minimum total exposure durations employed in these studies are plotted as a function of flux density. A power model without a constant term was used to search for a regression equation. It was found that the solution was \( y = 34x^{-0.48} \). Although this solution is a preliminary one due to the small number of data, half of the exposure amount indicated by the above-mentioned function (\( y = 17x^{-0.48} \)) can be considered the best estimation now available of the minimum exposure that produces detectable biological effects. However, in order to think about safety standards of magnetic fields, we are compelled to push these values lower. In that case, generally we decide the values as one tenth of the real effect. We reached the standard values taking about one tenth of a dotted line in Figure 4 (\( y = 1.7x^{-0.5} \)). According to this inference, these values are 7.6 h with 0.05 T, 5.4 hours with 0.1 T, 2.4 hours with 0.5 T, and 1.7 h with 1 T and 1.2 h with 2 T.

It is difficult to estimate the effects of high-density magnetic fields, since there is no report on mammals exposed to a magnetic field over 2 T in which prolonged observation was carried out to obtain the GAS patterns. It seems that a considerable amount of exposure is required to make the changes on molecular level appear outside of the body exceeding the limits of homeostasis. As demonstrated in Beischer’s report [21], he did not detect any effect on mice during one hour exposure to a magnetic field at extremely high flux density of 14 T. A certain length of exposure time would be needed for mammals, even a magnetic field of ultra-high flux density, because magnetism, unlike ionizing radiation, does not destroy biological structures instantaneously.

### Figure 4.

Minimum exposure duration was plotted as a function of flux density employed in the studies in which a GAS-like pattern was observed in mammals in response to the magnetic field. Responses observed in Studies A to I are as follows: A: \( \ln(\text{HD-cholesterol})/\ln(\text{total cholesterol}) \) in rabbits, B: food consumption by mice, C: delayed reinforcement of low rate by rats, D: urination in mice, E: body mass in mice, F, G: operant responding by rats, H: body mass in mice, I: adrenal corticosterone in mice. The power model of regression on density of magnetic field and exposure time (\( y = ax^b \)), half of which was presumed to be the minimum amount of exposure that produces a detectable effect in combination with exposure time against flux density of magnetic field. Values of about one-twentieth (\( a = 1.7, b = -0.5 \)) can thus be used as safety standards for whole body exposure.
This chapter was rewritten based on the paper "Mammals' response and adaptation to static magnetic fields as a nonspecific stressor" [31].

3. 1. 3. Some Recommended Safety Standards for Static-Magnetic Field. By 1986, some so-called safety standards on magnetic field proposed were all the objects regarding a static-magnetic field. It seems likely that these standards have been influenced by a series of results from the study with extremely prolonged exposure period by Barnothy et al. at the beginning of work on this area [15, 22]. They considered that the research results at the beginning stage had evidence that a magnetic field caused plenty of organic changes.

On the other hand, reports on effects of static MF from the United States in recent years and experimental reports concerning MRI diagnosis are almost negative. These reports were based on the experiments that were faithfully performed according to a method of radiobiology, relying on the concept of safety in clinical radiology. Consequently, clinical radiobiology seems to adopt that of kind of a clinical medicine. That is to say, clinical medicine takes precedence in a medical application of magnetic fields. It can be said that this concept became a big propulsive power, boosting a clinical application of MRI (NMR-CT) that appeared as a powerful tool in X-ray diagnosis. Table 1 shows supposed main examples of safety standards published so far. In 1970's, we thought that conventional research results were added for consideration. And after early stage in 80's, we could hardly find a report on this issue discussing from standpoints of environmental hygiene and ecological ideas. The limited marginal curve of Nakagawa (figure 4) by magnetic field strength and exposure time was drawn using only the endpoints that noticed a pattern of GAS (general adaptation syndrome) after a long-term observation made before and after the exposure period. This limited exposure time is quite long as stated before. Values E (Nakagawa) in Table 1 shows one tenth of that time for a reference. Three years after that recommendation ACGIH published a value 60 mT, time-weighted average of a day, as a threshold limited value of static-magnetic field as given in the table (values F). Safety standards of a static-magnetic field look rather rised back.

3. 2. Magnetic Field with High dB/dt

Goodman et al. [32] reported series of results that indicated that comparatively weak (< 0.2 mT) low-frequency (15 Hz or 72 Hz) time-varying magnetic fields increased a transcription of RNA, which explains an effect of the Bi-Osteogen System described before. We think that weak time-varying magnetic fields come to be considered to promote cellular proliferation from the series of their studies. We refer to evaluation on this matter later, which is related to the effects of ELF electromagnetic fields. However, there remain difficult points in plausible estimation. We do not know clearly about what level of dB·dt of a time-varying magnetic field affects deleteriously a living body.

An obvious effect of MF with high dB/dt is to stimulate nerves and muscles as MF is intended for this purpose. Ueno et al. recorded smarting pain and decrement of blood flow when they brought the palm close to the coil of an induction heater with 3.8 kHz[16 to 48 mT][33]. \( \tau \) (rising time) was 130 \( \mu \)s, and dB/dt was \( 1.5 \times 10^5 \) T s\(^{-1} \) with a strength of 20 mT. We have tested whether it is effective or not to use as a respirator to stimulate a diaphragm with a pulse magnetic field applied from the exterior of lateral abdomen [34]. The condition for the abdominal muscles and deep diaphragm to be contracted enough, in our equipment, was that dB/dt = \( 5.6 \times 10^2 \) T s\(^{-1} \) with a rising time of 250 \( \mu \)s.

Figure 5 shows the concept of a safety zone of dB/dt from the 6th plan of IEC's draft [35] for making a safety treating plan regarding MRI for a medical diagnosis. Our equipment [34] also holds in C area of this figure, as an output or total energy is large enough. To stimulate a phrenic nerve at a cervical portion with MF of this strength is rather harmful.
Table 1. Some safety standards and guidelines on static-magnetic fields recommended today

A. Safety standards by Beischer (1962)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 3 d/2</td>
<td>5,000 G</td>
</tr>
<tr>
<td>Duration of 15 min</td>
<td>20,000 G</td>
</tr>
</tbody>
</table>

B. Safety standards by Vyalov (1967)

<table>
<thead>
<tr>
<th>Field</th>
<th>Field Gradient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole body</td>
<td>300 Oe</td>
</tr>
<tr>
<td>Hands</td>
<td>700 Oe</td>
</tr>
</tbody>
</table>

C. Safety standards by Stanford Linear Accelerator Center (1971)

<table>
<thead>
<tr>
<th>Period</th>
<th>Whole body or head</th>
<th>Arms and hands</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extended periods (h)</td>
<td>200 G</td>
<td>2,000 G</td>
</tr>
<tr>
<td>Short periods (min)</td>
<td>2,000 G</td>
<td>20,000 G</td>
</tr>
</tbody>
</table>

D. DOE® interim guidelines (1979)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Time-weighted average for a work day</th>
<th>Ceiling</th>
</tr>
</thead>
<tbody>
<tr>
<td>For 8 h work day</td>
<td>For exposure &lt;1 h</td>
<td>For exposure &lt;10 min</td>
</tr>
<tr>
<td>Whole body</td>
<td>0.01 T</td>
<td>0.1 T</td>
</tr>
<tr>
<td></td>
<td>0.5 T</td>
<td></td>
</tr>
<tr>
<td>Extremities</td>
<td>0.1 T</td>
<td>1 T</td>
</tr>
<tr>
<td></td>
<td>2 T</td>
<td></td>
</tr>
</tbody>
</table>

E. Safety standards by Nakagawa (1986)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Time</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.05 T</td>
<td>&lt;0.1 T</td>
<td>&lt;0.5 T</td>
</tr>
<tr>
<td>&lt;0.5 T</td>
<td>&lt;1 T</td>
<td>&lt;2 T</td>
</tr>
</tbody>
</table>

| Whole body     | 7.6 h         | 5.4 h     |
|                | 2.4 h         | 1.7 h     |
|                | 1.2 h         |           |

F. Threshold limit values by ACGIH (1989)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Time-weighted average for a work day</th>
<th>Ceiling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole body</td>
<td>60 mT</td>
<td>2 T</td>
</tr>
<tr>
<td>Extremities</td>
<td>600 mT</td>
<td>2 T</td>
</tr>
</tbody>
</table>

\[ T(\text{tesla})=10^4 \text{G(gauss)}=10^7 \text{Oe(oersted)} \]

because a sternocleidomastoid muscle contracts spastically and the vagus of the cervical portion is also stimulated. dB/dt and also duration time \( \tau \) are related with a stimulation of the living body. Nerve and muscle cannot respond to electromagnetic fields, when the \( \tau \) is short and so the frequency is high, even though dB/dt might be large. This is the reason why Figure 5 illustrates a longer portion than 1 \( \mu \)s. From a point of view of safety for heart muscles, an experimental stimulation was carried using a dog [36]. After temporarily stopping the canine heart with a strong stimulation of the vagus, it was possible to move it again by stimulating with a pulse magnetic field. However, a persistent stimulation as long as 10 ms is necessary to cause a fibrillation with an electric stimulation of a normal heart.
This energy is so great that dB/dt needs to be as high as $6 \times 10^3$ T s$^{-1}$. Even a future-type MRI (echo planar imaging) demanding very high energy cannot come up to that power. It is supposed difficult to cause cardiac arrest with magnetic stimulation by a real equipment.

As a problem of an unexpected obstacle arising from a large dB/dt of magnetic field, we cite the case of a cardiac pacemaker. There are many investigations reporting that temporary abnormal conditions of pacing often cannot be noticed by a pacemaker user. It is reported that it is feared that an elimination equipment of EAS (electronic article surveillance; cheap watching patch to be attached to the recent products to protect from robbery) system causes malfunctioning of a mode switch of pacemakers [37].

3.3. ELF-Electromagnetic Fields

Though a frequency band is of the highest interest, the opinion is probably divided over it. As for electromagnetic fields of the levels of transmission lines, not a few reports state that leukemia incidence increased in children who live under powerlines, or that cancer and leukemia occur frequently at electrical workplace, and only epidemiological surveys indicate positive view [38]. On the other hand, if we want to ascertain on this matter by experiments, we will get almost invariably negative results concerning cancer initiation or promotion under the effect of weak electromagnetic field with flux density of less than $2 \sim 5$ mT [39]. Therefore, many experimental scientists negate it.

Figure 6 cites some graphs from safety guidelines proposed nowadays about electromagnetic fields ranging from ULF (ultra low frequency) to microwave. In the first half of 80's safety values on higher phase of frequency were published and shifted to the lower one around 1990. We might say that these values are safe enough considering the experimental evidence. IRPA (International Radiation Protection Agency, see Fig. 6) is reviewing the values at present in order to add and draw new lines for lower and higher frequency bands, though it has published relatively lower guidelines. Furthermore, such values that Florida and New York recommended come around the figures measured under the real transmission lines, or Sweden is ready for to recommend strictly low values comparable with Swedish regulation on stray fields from VDT. However, Japanese official opinion reported in recent years states that reports by epidemiological method are too defective to satisfy these data for judgment of cancer-relating effects of ELF-electromagnetic fields [40].
Figure 6. Main recommendation on safety standards or guidelines of electromagnetic fields with frequency of ULF (ultra low frequency) to microwave range. Some researchers in advanced industrial countries think these values are rather high compared with those of real fields where positive results are reported on high risk of leukemia or some kinds of tumors by epidemiological methods.
3.4. High Frequency Band

A dielectric heating effect of a high-frequency EMF has been known from early stage, and in a corresponding plan for safety, ANSI (American Standard Research Institute) made a recommendation on safety standard in 1966. The facilities with high-power output exceeding this standard are not a few, and plastic sealers used in industry may be one of the objects calling for careful watch at present [41]. A short wave diathermy and microwave diathermy for clinical use are said to have surprisingly large straying electromagnetic field around the cable [42]. Furthermore, in highly energized MRI, RF output for signal giving and receiving is so large as to exceed the standard. In the case exceeding the safety standard in a medical examination and treating equipment, with the same concept as for a static-magnetic field of MRI, “the medical treatment business” is managed with an idea to prefer one of higher merit. On safety in heat generation an IEC draft mentioned before [35] is reviewed, and equipment will be employed under proper watch of a medical doctor.

4. INTRODUCTION FROM OUR DATA

4.1. Case of Bacterial Mutation Assay (Ames Test)

This test is one of the most commonly used assays in genetic toxicology to detect mutagenicity and carcinogenicity of chemicals newly produced, drugs and environmental samples. We applied this method in estimating the ability to detect genotoxicity for some kinds of electromagnetic fields. Until now, some studies reported about weak time-varying magnetic field using bacterial mutation assay at 20nT of 100Hz (Juutilainen and Liimatainen [43]) and 20mT of 0.3Hz (Moore [44]). The results suggest that those magnetic fields revealed no mutagenic activity. Our report shows the result about mutagenicity test used 50Hz-timevarying and 1.3T-static magnetic fields.

The tests were carried out with the plate incorporation method using preincubation modification described by Maron and Ames [45]. We used two types of magnetic field exposure systems. One is for a static magnetic field with ESR (electron spin resonance) equipment i.e. JES-RE2X (JEOL). This magnet can generate a quite homogeneous static field up to 1.3 T. The other system (made by IDX) is for a time-varying magnetic field, and has a capability to generate the flux density of up to 35 mT and has a frequency of zero to 100 Hz. For the first stage experiment, we used a commercial 50 Hz frequency in east area of Japan. Scale and both sections of generating coil for time-varying field are shown in Figure 7. Figure 8 indicates a specially made shaker used in the JES-RE2X, which can be shaken in the field and the other side of the rod is also connected for the sham exposure incubation. For the preincubation method the shaker maintains the temperature of bacterial suspension tubes both in exposure and sham space at 37±0.1. These system is very unique and ensure the high quality condition of these bacterial mutation assay.

Strains used for the test were Salmonella typhimurium TA98 and TA100. The cultures of test strains were incubated overnight at 37°C until the cell concentration ranged 1-3×10^9 viable cells per ml. Cultured cell suspension (0.1 ml) is added to 0.1 M-phosphate buffer (0.5 ml) in sterilized tubes. Half of the tubes were incubated shakenly at 37°C in a 1.3 T-static or 50-Hz- various density of time-varying magnetic field for 20 min and the others in a sham space for the control. After that 2 ml of molten top agar was added to each of the tube and the contents were immediately poured onto Vogel-Bonner E minimal agar plates. In the static magnetic field test, experiments and also carried out for 100 min exposure to magnetic field by the same procedure. The plates were incubated upside down at 37°C for 48 h, and the numbers of His⁺ revertant colonies were counted. Table 2 shows the result of the test in 1.3
T-static magnetic field. Table 3 indicates the result of the test at various flux densities of time-varying magnetic field. In both data, no significant difference between exposed and control groups was observed. These results suggest that 1.3 T-static and up to 35mT-50Hz time-varying magnetic fields have no mutagenic activity for these bacteria. However, Shimizu et al. [46] reported a high density static magnetic field (up to 11 T) which affected mutagenicity of some chemical mutagens and its effect depended on density of magnetic flux in the Ames test with modified (not shaken) preincubation method. Now we made a new 5 T-superconducting magnet. This magnet generates homogenous static magnetic field in 20cm-diameter and 20cm-long space, and is placed in the constant temperature room. We
Table 2. Mutagenic activity of 1.3 T static magnetic field

<table>
<thead>
<tr>
<th>Tester strain</th>
<th>Exposure period (min)</th>
<th>Exposure group</th>
<th>Sham group</th>
<th>Internal control</th>
<th>Positive control a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TA98</td>
<td>20</td>
<td>29.8 ± 10.3</td>
<td>32.3 ± 9.9</td>
<td>34.7 ± 10</td>
<td>865 ± 98.5</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>37.1 ± 11.5</td>
<td>36.8 ± 11.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA100</td>
<td>20</td>
<td>178.3 ± 17.4</td>
<td>186.9 ± 23.3</td>
<td>164.5 ± 13.2</td>
<td>879.0 ± 88.6</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>153.2 ± 28</td>
<td>152.8 ± 28.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a) Positive control used AF-2 (0.1 μg/plate for TA98 and 0.01 μg/plate for TA100) and control used DMSO.

This experiment did not use S9 mixture.

These data shows mean of more than six independent experiments using more than 3 plates per test point and S.D.
Table 3. Mutagenic activity of 50Hz time-varying magnetic field. Number of revertant of control and exposed group (colonys per plate)

<table>
<thead>
<tr>
<th>Tester Strain</th>
<th>TA98</th>
<th>TA100</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Exposure</td>
</tr>
<tr>
<td>ELF field(mT)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>24.3 ± 6.0</td>
<td>25.4 ± 7.0</td>
</tr>
<tr>
<td>10</td>
<td>34.4 ± 14.4</td>
<td>32.6 ± 10.1</td>
</tr>
<tr>
<td>15</td>
<td>25.3 ± 3.8</td>
<td>26.6 ± 5.2</td>
</tr>
<tr>
<td>20</td>
<td>25.1 ± 5.2</td>
<td>26.5 ± 3.7</td>
</tr>
<tr>
<td>25</td>
<td>29.1 ± 11.5</td>
<td>31.4 ± 7.1</td>
</tr>
<tr>
<td>30</td>
<td>23.1 ± 6.9</td>
<td>22.5 ± 7.0</td>
</tr>
<tr>
<td>35</td>
<td>25.6 ± 4.4</td>
<td>25.3 ± 3.0</td>
</tr>
<tr>
<td>sham</td>
<td>23 ± 11.5</td>
<td>25.3 ± 7.1</td>
</tr>
</tbody>
</table>

positive control a) 18.3 ± 4.4 981.7 ± 117.5 166.2 ± 15.4 716.5 ± 97.6

a) Positive control used AF-2(0.1 μg/plate for TA98 and 0.01 μg/plate for TA100) and control used DMSO.

This experiment did not use S9 mixture.

These data shows mean of more than six independant experiments using more than 3 plates per test point and S.D.
can perform both of plate incorporation and preincubation method in it. We have started a test for detection of mutagenicity under combination of high static magnetic field with chemical mutagens.

4.2. Detection Of Mutagenic Activity Using Fruit Fly (Somatic Cell Test)

4.2.1. Method and Result. To estimate genetic effects of static magnetic field we applied the mutant fruit fly *Drosophila melanogaster* that lacked repair function of damage to their cellular DNA (deoxyribonucleic acid). The mutant was sc z\(^{1}\) w\(^{-TT}\) mei-9\(^{a}\) mei-41\(^{D5}\) that was defective in both excision repair and post-replication repair. Young larvae of mutant and normal genotypes were exposed to 0.6 T magnetic field for 24 h, and then allowed to continue development in normal culture condition until they molted and finally emerged from pupa cases. After their eclosion, the number of surviving adults was counted. Table 4 shows the adults of mutant genotype (male) which decreased by about 8 %, while normal siblings (female) remained unchanged. When FM6 with normal repair function was used instead of the mutant, its survival rate was not affected by magnetic field. It is inferred that exposure to static magnetic field caused damage to larval cellular DNA, and that somatic cells without normal DNA repair function failed to continue cell division, which resulted in developmental lethality of mutant larvae. DNA damages occurring in normal larvae must have been repaired, so that their survival rate was not altered. Similar effect was observed when mutant larvae were subjected to ultraviolet (UV) light of conventional 15 W germicidal lamp at 50 cm distance for 5 s (12.5 Jm\(^{-2}\)). We estimated that mutagenic activity of 0.6 T static magnetic field was the same as that of 0.14 mJm\(^{-2}\) UV lights.

4.2.2. Discussion

(1) Detection and Estimation of DNA Damaging Effect. It was found that repair-defective, mutagen-sensitive larvae were sensitive to a 0.6T static magnetic field. When first and second instar larvae were exposed for 24h, about 8% of mutagen-sensitive males failed to grow up to adult, while female siblings with normal repair function eclosed normally. As a result, the male/female ratio showed a statistically significant decrease compared with the non-exposed control (Table 2). It is inferred that static magnetic field caused, directly or indirectly, damages to cellular DNA of the exposed larvae, which were repaired in female somatic cells, but could not be repaired in male cells. As DNA molecules with damages could not be duplicated normally, male somatic cells with damaged DNA may not divide, which finally resulting in developmental lethality of mutagen-sensitive male larvae.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>sc z(^{1}) w(^{-TT}) mei-9(^{a}) mei-41(^{D5})</th>
<th>FM6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condition</td>
<td>0.6 T for 24 h unexposed</td>
<td>0.6 T for 24 h unexposed</td>
</tr>
<tr>
<td>Mutant (male)</td>
<td>7,938</td>
<td>8,514</td>
</tr>
<tr>
<td>Normal (female)</td>
<td>7,135</td>
<td>7,038</td>
</tr>
<tr>
<td>M/F</td>
<td>1.12</td>
<td>1.22</td>
</tr>
<tr>
<td>Odds ratio (95% CI)</td>
<td>0.88-0.96</td>
<td>-</td>
</tr>
</tbody>
</table>
The apparent genotoxicity of static magnetic field was compared with that of UV light. When second instar larvae were irradiated with various doses of UV light, the viability of the females with normal repair function was more than 90% of untreated control at 180s irradiation (450 Jm⁻²). Mutagen-sensitive males were, on the other hand, sensitive to even 10s irradiation. The number of male survivors decreased exponentially as the irradiation period was lengthened. Exposure of the mutagen-sensitive larvae to 0.6T magnetic field for 24h and to 12.5J m⁻² UV caused the same decrease of male survivors. Assuming a linear relationship between the UV effect and exposure dose, genotoxicity of a 0.6T static magnetic field was estimated to be the same as that of 0.14mJ m⁻² s⁻¹ UV light.

It can be argued that exposure to magnetic field gives general physiological effects on larvae rather than effects of DNA damage. If female larvae were more resistant to such stress than males, the exposure to magnetic field should decrease male/female ratio. This is not probable, if not impossible, because FM6 males with normal repair function were not killed by the exposure to magnetic field. The relative viability of FM6 males to y f: = females is similar to, but a little bit smaller than that of sc z¹ w⁺¹ ma mei-9⁹ mei-41¹⁵ males, the former are expected to have similar or higher sensitivity to general physiological effects than the latter. Therefore, the resistance of FM6 males to a 0.6T magnetic field strongly suggests that repair-defective males were actually killed by induced damages in cellular DNA, rather than due to general physiological effect of the exposure to which males were more sensitive than females.

(2) Possible Mechanisms of DNA Damaging. However, it is not probable that a 0.6T static magnetic field directly attacked DNA molecules forming pyrimidine dimers or other DNA damages. Dissociation energy of 4 to 5 eV is necessary to disrupt chemical bonds and form a pyrimidine dimer but the interaction of an electron spin with a 0.6T magnetic field creates electromagnetic energy of 10⁻¹⁵ of it. Other kinds of DNA damage need 0.1 to 10 eV and they are impossible to occur too. It is possible, on the other hand, that exposure to a 5T magnetic field suppresses the photoreactivation process. In somatic cells of sc z¹ w⁺¹ ma mei-9⁹ mei-41¹⁵ males, excision repair and post-replication repair are defective while photoreactivation, another DNA repair function, is still active. It is well known that some enzyme reactions are affected by static magnetic field exposure[47], Drosophila photolyase can be another example of magnetic field sensitive enzyme.

Another possible hypothesis is that the magnetic field interacts with mutagenic free radicals which appeared occasionally in larval somatic cells. It is well known that static magnetic field affects singlet-triplet transition in the unpaired electron. This effect is especially prominent in a reaction in micelle. Tanimoto et al. [48] showed that the life time of 2-naphthylphenylmethy1 radicals in polyoxyethylene(44)dodecanol (Brij 35) micelles was elongated by several times in a 0.6T static magnetic field. As micelle can be considered a simple model of cell, it is possible that the life time of radicals in living cells is also affected by static magnetic field. If its life is elongated, the same amount of radicals may have more mutagenic activity.

Still another possibility is that the membrane permeability of cells is affected by magnetic field, which results in influx of mutagenic agents which exist in the haemolymph at the background level. Permeability change by magnetic field exposure has been reported in cultured cells [49] and in liposome vesicles [50]. Although experiment was done at a temperature near the phase transition region of lipid bilayer, this hypothesis is not absurd at physiological temperature. This hypothesis predicts that the dose-response relationship between flux density and genotoxicity is not linear. Deformation of lipid bilayer or channel protein causes influx of mutagens while the flux density is not too high. When the flux density exceeds a certain level, an over-deformation may occur which prevents influx of chemicals. This is compatible with the “window” theory of the magnetic field effect. This should be
confirmed by an experiment at a higher flux density using a superconducting magnet. For the time being, we can only say that static magnetic field has, in a practical sense, a weak genotoxicity.

(3) Sensitivity and Limitation of Dna Repair Test. The average dose of genotoxic UV component in the sunlight is 10 to 20 kJ m$^{-2}$ in Japan. As an exposure to a 0.6T magnetic field for 24h and to 12.5J m$^{-2}$ UV caused the same decrease of male survivors, the genotoxicity of a 0.6T static magnetic field is considered to be 1/2 to 1/4 of that of sunlight. It is surprising that such weak genotoxicity could be detected by an individual animal test. In normal cells, almost all DNA damages induced by a weak mutagen are repaired by DNA repair functions. Occasional error in the post-replication repair process causes mutations which could be detected by mutagenic activity tests such as the sex-linked recessive lethal test. In our experimental system, on the other hand, we detected DNA damage which was much more frequent than occasional error in the repair process. The mutant used in our experiment was defective in both excision repair and post-replication repair. In *Escherichia coli*, a single damage in the whole genome was sufficient to kill a mutant cell that lacked both of excision repair and recombination repair (bacterial version of the post-replication repair, see ref [51]). Developmental lethality of individuals occurs at a high frequency if DNA damages are induced in even a few percent of larval or embryonic cells. This “amplification” makes the sensitivity of our test system much higher than in vitro tests. Even if repair-deficient Xeroderma pigmentosum cells were used, it is hardly possible to detect a mutagenic activity that causes DNA damages in a few percent of the cultured cells.

However, it is not clear whether this genotoxicity is equal to mutagenic activity. Mutations are created by occasional errors in the DNA repair process. Our test animals carried mei-41$^{D5}$ mutation and therefore were defective in the error-prone post-replication repair, so that mutation could not occur. The DNA damages caused by UV light are mainly pyrimidine dimers which are known to be repaired, in the cells with normal repair functions, by excision repair and/or post-replication repair process. Errors are expected to occur at a frequency proportional to the number of damages and therefore the genotoxicity determined by the DNA repair test using a repair defective strain can be considered equal to, or at least proportional to the mutagenic activity of UV light. Mutagenic activity of chemicals whose DNA damaging mechanisms are known is also determined by this test. The genotoxicity of magnetic field determined by our test system will be also considered a mutagenic activity, when its DNA damaging mechanism will have become clear.

5. STRUCTURE OF SUPERCONDUCTING MAGLEV TRAIN AND MAGNETIC FIELD SHIELDING

5.1. Introduction

Powell and Danby bore the original conception of a magnetically suspended train in 1966 [52]. In Japan study on conception design of EDS (electro-dynamic suspension)-maglev began from 1962, and it led to construction of the preliminary test track in Miyazaki in 1975. Vehicle MLU001 with some research staff on board recorded a speed of 400 km/h in 1987. In June 1990 the Ministry of Transport Japan declared the construction of an experimental track for maglev in Yamanashi adjoining Tokyo authorized, which will go into practical operation in future. Test train will consist of 3 and 5 vehicles and have ability of developing a maximum speed of 550 km/h.
Here, we introduce a first trainset to be used for the running test in Yamanashi aiming at business operation of the next phase. We state position and size of the SCM (superconducting magnet), related position of a propulsion coil, and shielding method to control a leakage magnetic flux inside of the car and near the door where passengers get on and off.

5.2. Principle of Magnetic Propulsion and Levitation

Linearmotor may be equated to a stator of the conventional motor being spread out on the level, and the vehicle with magnet serving as a rotor. To propel linearmotor smoothly, the frequency of 3-phase alternating current has to match the polarity of the magnets on vehicle. Linearmotor N-pole must always be located at the same position of the vehicle where N-pole attracts the S-pole of the vehicle, and at the same time linearmotor S-pole after the N-pole repels the S-pole of the vehicle. This relation holds for the linearmotor S-pole, too. Because superconductive magnets are arranged with specific pitch and the polarities of the magnets alternate, some ripples (time-varying magnetic fields) remain in the vehicle. In order to make these ripples as small as possible, creativity was displayed in various ways. In Yamanashi test track, “a formation with 240-degrees double layer placement of alienated poles” will be adopted for coil arrangement and wiring of propulsive linearmotor. This arrangement of coil-positions is shown in Fig. 9. As shown at right side, it is a complicated structure with levitating and guidance coils arranged on the front side of the surface where propulsive coils are set in double layer. A mechanical meaning of this ripple decrement plan is to reduce vibration of SCM, to improve stability and prevent a quenching. The feature of “guidance” is that combined coils and wiring are made to cooperate to bring the vehicle from extremely right or left side track to the center of guideway by virtue of electromagnetic force.

The principle of levitation is rather simple. With propulsion of the vehicle, magnetic flux density of SCM changes relative to cross section of the levitating and guiding coils arranged along the side-beam of the guideway, so that induced currents in the coil produce new magnetic flux and cause the vehicle to float. Fig. 10 shows the relation between SCM on the vehicle and the propulsion coil and levitation-guidance coils along the guideway. An arrangement of side wall levitation in the left is adopted in Yamanashi test track. While the speed is low, the vehicle runs on wheels. As the speed reaches 150 km/h approximately, however, the vehicle can levitate just by a repulsion of magnet fluxes with induced currents in the surface coils.

5.3. a Trainset Using Yamanashi Test Track. The first trainset will be composed of three vehicles as shown in Fig. 11, their production to be completed in 1995. There are 3 cars that is a Kofu-side leading car, a standard middle coach, and a Tokyo-side leading car, and every vehicle is combined with connecting bogies. Big feature with respect to outside

Figure 9. Arrangement of positions and wiring of propulsive linearmotor used in Yamanashi test track, named “formation with 240-degrees double layer placement of alienated poles”.
appearance is a forefront shape. The Kofu-side leading car "double-cusp" and the Tokyo-side "aerowedge". Performance of these two forefront shapes will be tested by real running. One aerodynamic brake is provided above every bogie. Fig. 12 is an imaginative figure drawn with computer graphics seeing from the Tokyo-side leading car. With no driver aboard, there is no window in front of the leading car.

In the machine chamber of Tokyo-side forefront, batteries for main power supply, gas turbine generator, DC/AC converter, and a helium buffer tank are mounted. Middle coach has a passenger room at the center and an end body structure above the bogie. The connecting division is provided for a person to pass through the center, and the exterior of passage makes the machine chamber. In the machine chamber at Tokyo-side bogie, we load an aerodynamic brake equipment, helium buffer tank, and inverters for machine power supply. In the Kofu-side machine chamber, we load a helium buffer tank, ventilator, and air conditioning outdoor machines. Large fairing for both sides of the connecting portion is done to rectify the air flow aerodynamically.

About the magnetic shield inside of the car, less than 1 mT in passenger rooms, and less than 20 mT on the passage of the connecting division, are considered design features. Fig. 13 shows the arrangement of shielding materials with pure iron of industrial use at connecting portion. We confirmed the real shield effect by a mock-up of a full size by comparing with calculated values and investigating whether they were controlled at marked levels of a magnetic shield. An equipment shown in Fig. 14 is the conception figure of a boarding bridge to unanable passengers to approach stray magnetic fields from outside of the vehicle. As the maglev has SCMs at both exterior sides of connecting portion, it allows passengers to avoid from the outside of the bogies. Two doors of the equipment are designed
Figure 12. A constructed 3-car trainset for Yamanashi test track seeing from the Tokyo-side leading car. aerowedge-shaped.

to open only to guide passengers to the door of vehicle when they get on or off the train. The floor of the equipment consists of a partial bridge, which serves as to shield people against the magnetic field from under the floor. Moreover, end of doors of the equipment are designed in the shape of a cross section closely adhering to the vehicle with an increased shielding effect.

5.4. Theme of the Future

Given today's safety concept of electromagnetic fields, we do not believe that many people are of the same opinion necessarily. A consensus seems to be reached on the difference in an exposure amount of EMF among general public, occupational workers, and on medical

Figure 13. An arrangement of shielding materials with pure iron for industrial use at connecting portion.
treatment use. It is not fully agreed, however, that any level of magnetic field should be regulated for the vehicles utilized by people for short time. Levels of a time-varying magnetic field of electrified railroads and electric vehicles are quite large comparing with those of power transmission lines. As for the evaluation of an exposure amount of electromagnetic fields, it is not argued yet that an exposure amount might be multiplied by a simple magnetic flux density (whether it is a total amount of magnetic flux or not that a whole-body receives) and by time (or the square of it or a square root of it). Therefore, about the safety of electric trains nobody can say anything definite. Either it is not solved about ELF frequency whether more than a certain Hz should be considered controversial (e.g. whether we should think that the effect of less than 3 Hz is as weak as a static-magnetic field in IEEE frequency designation).

We can say about safety of EDS maglev that magnetic shield marks meet the values in Table 1 of a static-magnetic field. About the ripples mentioned before, however, magnitude and frequency of them may become an issue. Frequency, strength, and exposure time must be reviewed for the safety of a time-varying magnetic field, and further arguments are going to begin from now. In parallel to the argument, we are progressing in development of maglev such as the reform of a running mechanism, countermeasures for magnetic resistivity, stabilization of the SCM, and development of power supply method. In the same way as in development of electric vehicles, we expect that scientific technology and safety aspects keep abreast of maglev development.

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RECENT BIOLOGICAL STUDIES RELEVANT TO CARCINOGENESIS

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INTRODUCTION

Technological advance and changing social behaviour have been responsible for driving the massive increase in the production and consumption of electricity during the 20th century. One inevitable consequence of this process has been increased environmental exposure to electromagnetic fields both at work and in the home. While there have been many undoubted benefits from the widespread use of electricity, there has been growing concern, especially during the last thirty years, that prolonged exposure to electromagnetic fields at even low levels could have detrimental consequences on human health, and in particular could be associated with some cancers. This possibility has aroused persistent interest and concern, and continues to be investigated by a substantial number of laboratories throughout the world. Much of this work has been comprehensively reviewed elsewhere (Sienkiewicz, et al., 1991; Saunders, et al., 1991; NRPB, 1992; Cridland, 1993; WHO, 1993), and it is clear that many of the older observations were essentially phenomenological in nature. Within the last few years, however, a more coherent research strategy based on powerful cellular and molecular approaches has started to emerge (Sienkiewicz, et al., 1993).

ELECTRIC AND MAGNETIC FIELDS UP TO 100 kHz

Initiation

The available evidence suggests that exposure to extremely low frequency (ELF) fields is not genotoxic (Murphy et al., 1993; McCann et al., 1993) and is therefore unlikely to be directly associated with initiation. Although a few studies have reported a higher incidence of chromosome aberrations following exposure to pulsed (Garcia-Sagredo et al., 1990; Khalil and Qassem, 1991) or intermittent (Nordenson et al., 1994) magnetic fields, most recent studies have failed to find any evidence for chromosome aberrations or DNA damage after exposure to 50 Hz electric fields at up to 20 kV m⁻¹ or magnetic fields at up to
5 mT (Garcia-Sagredo et al. 1990; Garcia-Sagredo and Monteagudo, 1991; Livingston et al. 1991; Saalman et al. 1991; Scarfi et al. 1991, 1994; Fiorani et al. 1992; Fairbairn and O'Neill, 1994; Nordenson et al. 1994). In agreement with previous studies using microbial systems, Tabrah et al (1994) found that exposure to 0.2 mT, 60 Hz magnetic fields did not affect the mutation frequency in the Ames test; azide-induced mutations, however, were increased by exposure to the magnetic field. The frequency of dominant lethal mutations was not increased in male mice exposed for 14 days to 50 Hz electric fields at 20 kV m⁻¹ (Kowalczyk and Saunders, 1990).

**Tumour Promotion**

A number of studies have examined the possible effects of electromagnetic fields on proliferative responses at the cellular level, as an indicator of tumour promotion. This evidence is considered here at the various stages in the signalling pathways which control cellular behaviour.

**Early Signals.** A number of cell signalling pathways produce transient increases in the intracellular concentration of free Ca²⁺, initially by stimulating release from intracellular stores, and subsequently by influx across the cell membrane from the extracellular fluid. A number of studies have sought to investigate the possibility that electromagnetic fields act to stimulate calcium ion movements, and thereby influence signalling pathways. Using rat thymocytes incubated in the presence of ⁴⁰Ca²⁺, Walleczek and Liburdy (1990) reported that a 22 mT, 60 Hz magnetic field increased the influx of Ca²⁺ which resulted from stimulation with the mitogen Concanavalin A (Con A). In an extension of this work, annular ring dishes were used to expose the cells to different induced electric fields at the same magnetic flux density; the induced current depends on the radius and is therefore larger in the outer rings of such dishes. These experiments indicated that the influx of calcium depended on the induced electric field and demonstrated that the direct application to the culture of a similar electric field produced the same effect (Liburdy, 1992). Furthermore, the fluorescent dye, Fura-2 was used as a probe to make real-time measurements of intracellular free calcium concentration. The data indicated that exposure to a 60 Hz electric field in the culture of 170 mV m⁻¹ increased the influx of calcium ions from outside the cell, but did not affect release from intracellular stores.

Exposure of Jurkat human lymphoblastic T-cells to a 50 Hz, 100 μT magnetic field has been reported to elicit an increase in intracellular free Ca²⁺ concentration which was similar in magnitude to that induced by stimulation with an anti-CD3 antibody (Lindström, et al, 1993). Preliminary reports from other groups using fluorescent indicators to monitor intracellular ion concentrations in Jurkat cells have indicated that the response to a 60 Hz, 2 mT magnetic field (inducing an electric field of 1.8 mV m⁻¹ in the medium), may be dependent on the biological status of the cells (Walleczek et al, 1994). This observation is in agreement with previous findings from the same authors on the Con A-induced calcium response of rat thymic lymphocytes exposed to 3 Hz pulsed magnetic fields (Walleczek and Budinger, 1992); significant effects on Ca²⁺ influx were observed at peak flux densities of 6.5 and 28 mT, and appeared to be field strength dependent. Exposure of HL-60 human leukaemia cells to complex fields generated in a magnetic resonance imaging unit has been reported to induce a small increase in intracellular free Ca²⁺ concentration, as monitored using the fluorescent indicator indo-1. (Carson et al, 1990).

It has been reported that ⁴⁰Ca²⁺ uptake was increased following exposure of a variety of lymphocytic cells to combined static and time-varying magnetic fields 'tuned' to resonant conditions (Lyle et al, 1991; Yost and Liburdy, 1992), although neither of these studies examined the dependence on resonance, for example, by 'detuning' the fields. Furthermore,
two other studies found that exposure of either human (Prasad et al., 1991) or mouse (Coulton and Barker, 1993) lymphocytes to resonant fields did not affect \( ^{42} \text{Ca}^{2+} \) uptake or intracellular free \( \text{Ca}^{2+} \) concentration respectively.

**Effects on the Nucleus.** Early reports of increased RNA synthesis from several chromosomes in cultured *Sciara coprophila* salivary glands exposed to a variety of ELF magnetic fields (see Goodman and Henderson, 1991) has recently been extended (Goodman et al., 1992) to include an analysis of transcription from the right arm of chromosome 3 in a similar preparation from *Drosophila melanogaster*. Exposure to a 72 Hz pulsed magnetic field at a flux density of 3.5 mT has been reported to induce a short-term increase in the synthesis of both total and messenger RNA (mRNA) in CCRF-CEM lymphoblastoid cells (Phillips and McChesney, 1991). Exposure of HL-60 cells to a 1 mT, 60 Hz sinusoidal magnetic field was reported to produce a temporally similar, though quantitatively smaller, increase in total RNA synthesis which was dependent on the magnitude of the induced electric field (Greene et al., 1991).

The significance of the reported short-term increases in gross transcription is difficult to assess. Clear and unequivocal evidence for effects on the transcription of specific genes, particularly those known to be important for regulating cellular behaviour would be of far greater importance. Unfortunately the evidence for this is much less convincing. In particular, the absence of internal loading controls renders the data extremely difficult to interpret. Furthermore, the published studies have mostly been based on dot-blot assays rather than more analytical assays such as Northern blots or RNAase protection assays, and examples of raw data have rarely been shown. Even where the evidence for magnetic field effects on gene expression appears to be reliable, the magnitude of the response tends to be small and should be viewed in the context of the response to other agents. For example, in cultured fibroblasts, c-myc expression can be induced about 20-fold by serum (Campisi et al., 1984), 40-fold by platelet-derived growth factor, 15-fold by fibroblast growth factor and 10-fold by the chemical tumour promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) (Kelley et al., 1983). Similarly, treatment of B-lymphocytes with the mitogen lipopolysaccharide results in a 20-fold induction of c-myc expression, whilst treatment of T-lymphocytes with Con A elicits a 10-fold induction (Kelley et al., 1983). For the proto-oncogenes c-fos (Shah et al., 1993) and c-jun (Stein et al., 1992), inductions of up to 200-fold have been reported following treatment with serum and ultraviolet radiation respectively. Thus the biological significance of much smaller inductions by EMF must be questionable.

It has been reported that exposure of HL-60 human lymphocytic cells to either pulsed (1.5 - 72 Hz, 0.38 - 19 mT peak) or sinusoidal (5 - 150 Hz, 0.57 - 570 \( \mu \)T rms) magnetic fields increased accumulation of transcripts from the \( \beta \)-actin, histone H2B, c-myc, c-src, and \( \beta \)-tubulin genes (Goodman et al., 1989, 1992, 1994; Wei et al., 1990; Goodman and Henderson, 1991; Gold et al., 1994); one gene, \( \alpha \)-globin, did not respond to magnetic fields, but expression of this gene is normally cell-type specific anyway. Exposure to a 5.7 \( \mu \)T rms, 60 Hz sinusoidal magnetic field has also been reported to induce expression of the heat shock protein gene, *hsp70* in HL-60 cells (Goodman et al., 1994), and large T antigen in SV40-transformed human fibroblasts (Gold et al., 1994). In general these data suggest the existence of frequency, time and field strength windows for magnetic field effects on transcription. However, excessive reliance on the use of dot-blot assays, and the absence of internal loading controls renders interpretation of these data difficult, if not impossible. Moreover, the results for histone are puzzling since the expression of histones is normally tightly regulated within the cell cycle, whilst the reported increase in accumulation of mRNA from the proto-oncogene c-myc may have little significance since the c-myc gene in these cells is abnormally regulated following a gene amplification event.
There is some support for magnetic field effects on the expression of \( c-myc \), and other regulatory genes, from work in other laboratories. For example, exposure of CEM-CM3 T lymphoblastoid cells to a 100 \( \mu \)T, 60 Hz magnetic field for up to 2 hours resulted in transient changes in the expression of the \( c-myc \), \( c-fos \), \( c-jun \), and protein kinase C (PKC) genes (Phillips et al. 1992). In this study the principle approach was to assess the rate of transcription directly using nuclear run-off assays rather than indirectly by estimating the accumulation of transcripts, a parameter which may be influenced by other processes. Slot-blot analysis of cytoplasmic RNA from the same cells indicated that accumulation gave a reasonable estimate of transcription. In general exposure was reported to increase transcription from the \( c-fos \), \( c-myc \), and PKC genes, whilst the effect on expression of \( c-jun \) was variable and dependent on cell density. Time course data indicated that these responses were all observed within an hour, whilst longer exposure was reported to inhibit expression of PKC. It should be noted, however, that in the absence of appropriate internal loading controls it is difficult to reliably assess such small responses (generally around 2-fold).

Effects on gene expression, if they exist, are unlikely to result from direct effects of the applied field on transcription complexes, but would instead result from activation of an appropriate signalling pathway. Data on early signalling events (see above) indicate that there could be effects on calcium ion influx, and it is therefore logical to investigate possible links between this and the activation of genes such as \( c-myc \). Liburdy, et al (1993a) have reported such an association in cultured rat thymocytes stimulated with a suboptimal concentration of Con A and exposed to a 22 mT, 60 Hz magnetic field for 60 minutes. Calcium ion influx and accumulation of \( c-myc \) RNA were measured in the same cells, and whilst both were increased by a combination of magnetic field exposure and suboptimal Con A, neither agent on its own produced an effect. This study appears to have been better conducted than most; \( c-myc \) transcripts were quantitated by Northern analysis, and glycer-aldehyde-3-phosphate dehydrogenase was used as an internal loading control. However, although this study provides evidence for an association between calcium ion influx and \( c-myc \) expression in response to magnetic fields, it does not demonstrate that one leads to the other. It is to be hoped that future extensions to this work will address this question, possibly through the use of appropriate inhibitors and ionophores.

The activation of a gene in response to earlier signalling events occurs as a result of changes in the interaction of protein transcription factors with regulatory elements in the promoter region of the gene. There may be several such elements in the promoters of genes which are responsive to a number of signalling pathways (activated by different stimuli), and identification of the element conferring responsiveness to a particular stimulus may be helpful in elucidating the pathway through which the signal is transduced. It has been reported that a magnetic field responsive element resides between positions -353 and -1257 upstream of the \( c-myc \) P1 initiation site (Lin, et al, 1994). The element was identified by deletion analysis of the human \( c-myc \) promoter fused to a chloramphenicol acetyl transferase (CAT) reporter gene, and mediated transcriptional activation in HeLa cells exposed to 60 Hz magnetic fields at 8 \( \mu \)T, but not 80 \( \mu \)T. However, 900 base pairs is a relatively large fragment of DNA, and there therefore remains considerable scope for defining the location of the element more precisely. Moreover, it should be noted that the CAT activity appeared to be extremely low for all the constructs, suggesting that they were all essentially unresponsive under the conditions tested.

In contrast to those discussed above, other studies have failed to find consistent effects on proto-oncogene expression. For example, Parker and Winters (1992) examined transcription in a variety of human and mouse cell lines exposed to 0.1 mT, 60 Hz magnetic fields and found no evidence for increased accumulation of mRNA from the proto-oncogenes \( c-fos \), \( c-myc \), \( c-raf \) and \( c-ras \); there was also no effect on transcription from \( hsp70 \) or, in the case of cells infected with either mouse mammary tumour virus or murine sarcoma virus,
viral genes. It should be noted, however, that in addition to suffering many of the methodo-
logical flaws criticised above, the minimum exposure time would have been too long to
detect transient effects.

In another study, it was found that exposure of HL-60 cells to a 1 mT, 60 Hz field for
up to 90 minutes did not affect the accumulation of either c-myc or β-actin transcripts; the
steady state level of the 28 S ribosomal RNA was also unaffected (Greene et al., 1993). Using
a unique assay involving pulse labelling of cellular RNA in combination with a nuclease
protection step, it was also shown that the transcription rates for c-myc and β-actin were
unaffected by exposure. There did, however, appear to be an effect on synthesis of the 45 S
precursor ribosomal RNA, although this appeared to be associated with a concomitant
reduction in the half-life of the 45 S fraction and its mature products the 18 S and 28 S RNAs.

A number of studies currently in progress which appear to have been carefully
designed and performed, have not yet detected any effect of exposure to magnetic fields.
Preliminary reports indicate that in one study accumulation of c-myc RNA was not affected
by exposure of HL-60 cells to 60 Hz magnetic fields at 1 and 10 μT (Lacy-Hulbert et al.,
1994), whilst in another careful and extensive study, neither c-myc nor β-actin appeared to
be affected by exposure of either HL-60 or Daudi cells to 60 Hz fields at flux densities from
8 μT to 1 mT (Saffer and Thurston, 1994). Similarly, it has been reported that exposure to
50 Hz magnetic fields at either 6 μT or 2 mT did not affect the interleukin-3 induced
expression of c-fos, c-jun or jun-B in FDCP-mix murine haemopoietic stem cells (Reipert et
al., 1994).

There is some evidence from studies of calcium influx that magnetic field effects on
cells are the result of the induced electric field (see above). It is therefore pertinent to consider
whether electric fields applied directly to the cultures can affect gene expression. It has been
reported that exposure of HL-60 cells to electric fields of 0.3 - 3 V m⁻¹ induced expression
of the c-myc, histone H2B, and hsp70 genes; cells were exposed to frequencies between 15
and 120 Hz (Blank et al., 1992; Goodman et al., 1994). Under the same conditions, transcription
from the β2-microglobulin was unaffected. It should, however, be noted that the
magnitudes of the reported responses were modest and are therefore of questionable
significance.

**Effects on Cell Proliferation.** Recent studies of DNA synthesis, a measure of cell
growth, have failed to establish effects in CCRF-CEM cells exposed to a 3.5 mT magnetic
field pulsed at 72 Hz (Phillips and McChesney, 1991), or HF-19 normal human fibroblasts
exposed to a 2 mT, 50 Hz field (Cridland et al., 1993). The proliferation of K562 human
myeloid leukaemia cells was similarly unaffected by exposure to 50 Hz electric fields of 20
kV m⁻¹ (in air) or magnetic fields of 200 μT (Fiorani et al., 1992). In contrast, it has been
reported that a 14 Hz electric field of 10 μV m⁻¹ applied directly to the culture increased both
DNA synthesis and insulin-like growth factor-II (IGF-II) production in TE-85 human
osteosarcoma cells (Fitzsimmons et al., 1992). Exposure to a 2 mT, 50 Hz magnetic field
induced a small but significant increase in several measures of proliferation, although this
was dependent on the field generating system (Schimmelpfeng and Dertinger, 1993).

Several studies have been performed under resonant conditions, with the flux density
of the static field and the frequency of the time varying field "tuned" for Ca²⁺ ion resonance.
Under these conditions, rabbit-ligament fibroblast proliferation was dependent on the
amplitude of the time-varying field, an effect which could be abolished by detuning the static
field (Ross, 1990). In another study, the proliferation of 3T3 mouse fibroblasts was increased
in a resonant field, whereas human Raji lymphoma cells were unaffected. The proliferation
of the human cells could, however, be inhibited by exposure to field conditions which were
tuned to K⁺ (Rochev et al., 1990). Exposure of FDCP-Mix murine haemopoietic stem cells
to various field conditions, including fields tuned to Ca\(^{2+}\) did not affect proliferation or cell viability (Reipert et al., 1992).

A recent study of ornithine decarboxylase (ODC) activity in L929 mouse fibroblasts indicated that basal activity was elevated by 55-65 Hz magnetic fields at flux densities of 1-100 μT (Litovitz et al., 1991). The process appeared to be dependent on the maintenance of a coherent signal for at least 10 s.

In Vivo Studies. Tumour promotion has also been examined directly using animal carcinogenesis models. Three studies have employed the well-established mouse skin promotion model. McLean et al. (1991) initiated skin tumours in groups of 32 juvenile female SENCAR mice by painting with 7,12-dimethylbenz(a)anthracene (DMBA). Exposure to 60 Hz magnetic fields at 2 mT for 6 hours a day over 21 weeks did not affect tumour development.

Other studies have investigated whether magnetic field exposure can exert promoting effects on the development of skin tumours in female NMRI (Rannug et al., 1993a) and SENCAR (Rannug et al., 1994) mice. Tumours were initiated in 7-8 week old mice by painting shaved dorsal skin with DMBA and groups of 30 (NMRI) or 40 (SENCAR) mice were assigned to each treatment. Exposure to 50 Hz magnetic fields at flux densities of 50 or 500 μT commenced one week after initiation and continued for 2 years. Fields were either continuous or intermittent (15 s on/off, SENCAR mice only) and mice were exposed for 19 hours a day (21 hours at weekends). Comparison of exposed animals with control mice, which had received only DMBA, did not reveal any significant differences in either the number of tumour bearing animals or the total number of tumours per group thus demonstrating that magnetic fields do not act as tumour promoters in this system; treatment with the chemical tumour promoter TPA as a positive control greatly enhanced the yield of tumours. These findings were further confirmed by analysis of epithelial hyperplasia, a marker for tumour promotion in mouse skin. In contrast to TPA, which produced a marked hyperplastic response both on its own and in combination with DMBA, magnetic fields did not have any effect.

Exposure to a 20 kHz, 15 μT (peak to peak) pulsed magnetic field did not affect the development of lymphoma in female CBA mice which had previously been exposed to 5.24 Gy of x-rays; magnetic field exposure commenced when the mice were 40 days old and continued for the remainder of their lives (Svendenståhl and Holmberg, 1993). It should be noted, however, that the high incidence of x-ray-induced lymphomas would preclude the detection of magnetic field effects in this study. Exposure to the magnetic field alone did not affect the spontaneous lymphoma incidence although it did significantly decrease survival; the survival data for exposed mice were unusually variable thus casting doubt on the validity of the latter finding.

Three studies have investigated the possibility that magnetic fields might promote the development of mammary tumours initiated with DMBA in female Sprague-Dawley rats. In these studies exposure to magnetic fields started at the time of the first treatment with DMBA and continued for 91 days, at which time the animals were sacrificed for histopathological examination. In one of these studies, a group of 99 rats exposed to a 100 μT, 50 Hz field exhibited a significantly higher tumour incidence than controls after 8 weeks of treatment, a trend which continued for the remainder of the exposure (Lösch et al., 1993). The number of tumours per tumour bearing rat was the same for both groups, although the final tumour size was greater in exposed animals. In the other studies, however, groups of 18 - 36 rats which had been exposed to various magnetic fields did not exhibit significant differences in tumour incidence when compared with controls; some measures were altered in individual experimental runs, but these changes were not consistent across the study (Mevissen et al., 1993; Lösch et al., 1994). The field conditions included a 15 mT static
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field, a homogeneous 50 Hz field of 30 mT, and a gradient 50 Hz field of 0.3 - 1 \( \mu \)T. It is possible that small field-dependent effects would not have been detected due to the restricted sample size used in the latter studies (Löschler and Mevissen, 1994).

Beniashvili et al (1991) reported that exposure of rats to a 50 Hz, 20 \( \mu \)T magnetic field for 3 h per day enhanced the induction of mammary tumours initiated with nitrosomethyl urea (NMU). Exposure apparently enhanced the rate of tumour development in addition to increasing both the number of rats with tumours and the number of tumours per rat increased. A more complete description of the experimental protocol would, however, be useful for a proper evaluation of this study.

Co-Promotion

The possibility that magnetic fields may act as tumour co-promoters by enhancing the inhibitory effect of tumour promoters on gap junctional communication, thereby releasing premalignant cells from the inhibitory effect of adjacent normal cells has been investigated. Cain et al (1993a) employed an experimental model in which untransformed mouse fibroblasts inhibit focus formation by transformed cells, an effect which can be relieved by the addition of the chemical tumour promoter 12-0-tetradecanoylphorbol-13-acetate (TPA) to the culture medium. Exposure to a 100 \( \mu \)T, 60 Hz field for four 1 hour periods every day for 29 days significantly enhanced focus formation by sub-optimal concentrations of TPA, although the effect was not apparent at concentrations of TPA which induced little or no focus formation. It should be noted, however, that there was considerable interexperimental variation. Moreover, preliminary results from the same authors (Cain et al. 1993b) indicated that in an extension to this study 60 Hz magnetic fields of 1 - 200 \( \mu \)T inhibited rather than enhanced focus formation in a field strength-dependent manner.

Magnetic field co-promotion has also been investigated in a whole animal study (Stuchly et al, 1992). Tumours were initiated in 6 week old female SENCAR mice by painting DMBA onto shaved dorsal skin and promoted by application of a sub-optimal dose of TPA at weekly intervals thereafter. The mice were split into two groups of 48, one of which was exposed for 6 hours per day and 5 days per week to a 2 mT, 60 Hz magnetic field, whilst the other was sham exposed in the same room. The results for the first 24 weeks of the 54 week study indicate that exposure produced a significant increase in the rate of tumour development but not in the yield of tumours at the end of the study. However, preliminary reports from the same authors have indicated that the co-promotion effect is not reproducible and may have been confounded by variable exposure to light (Thansande et al. 1993). Earlier studies had failed to establish a direct tumour promoting effect of magnetic fields under the same conditions (see above).

Magnetic field exposure does not appear to have any effect on the formation of chemically-induced preneoplastic lesions in rat liver (Rannug et al. 1993b, 1993c). Groups of 9 - 10 male Sprague-Dawley rats were partially hepatectomised and treated 24 hours later with the chemical initiator diethylnitrosamine (DENA).

Rats were exposed to 50 Hz magnetic fields for 19 hours per day (21 hours at weekends), starting one week after initiation and continuing for 12 weeks. After sacrifice of the animals, preneoplastic lesions, characterised by expression of \( \gamma \)-glutamyl transpeptidase (GGT) and the placental form of glutathione S-transferase (GST), were detected by histochemical staining of liver sections. There were no consistent differences in the number of preneoplastic lesions identified in rats exposed to flux densities between 0.5 and 500 \( \mu \)T when compared with unexposed rats; treatment with the chemical tumour promoter phenobarbital increased the yield of preneoplastic lesions almost 20-fold (Rannug, et al. 1993b). Similarly, exposure to magnetic fields at flux densities of 0.5 and 500 \( \mu \)T did not have any consistent effect on tumour promotion by phenobarbital in this system (Rannug et al. 1993c).
Peptide Hormones

Another avenue where exposure to electromagnetic fields could influence tumour promotion is via an effect mediated by circulating peptide hormones. The best developed hypothesis relates to effects mediated by melatonin. This hormone is produced by the pineal gland in a distinct circadian rhythm controlled by the photoperiod: synthesis and secretion of this hormone is high at night and minimal during the day.

*Melatonin.* Stevens (1987) first suggested that chronic exposure to electric fields may affect melatonin secretion and thereby increase the risk for breast cancer. This possibility has aroused wide interest and attention and produced a large number of reviews, monographs and books on this topic (for example, Gupta *et al.*, 1988; Wilson *et al.*, 1990a; Stevens *et al.*, 1992, Moore-Ede *et al.*, 1992).

Melatonin is known to affect seasonal (reproductive) rhythms, although more recently it has been implicated in a range of other physiological functions, and several possible mechanisms have been proposed which suggest that melatonin may be able to influence oncogenesis, and particularly breast cancer. The most convincing of these suggests that decreased melatonin levels, which cause elevations in circulating estrogen and progesterone and so increase cellular proliferation within the stem cell population of the breast, would increase the risk of cancer of these cells (Cohen *et al.*, 1978). It must also be remembered that significant increases in estrogen concentration would also increase the proliferation of any estrogen-responsive tumour cells. An alternative mechanism suggests that melatonin directly suppresses the growth of (estrogen receptor-positive) tumours (Blask and Hill, 1986; Hill and Blask, 1988). In addition, there is some evidence to suggest that melatonin acts as a scavenger of free radicals (Tan *et al.*, 1993a) and so protects DNA molecules from oxidative damage (Tan *et al.*, 1993b). In this case, reductions in melatonin could increase the likelihood of the initiation of cancers, and possibly of their promotion. To be effective as a radical scavenger, however, melatonin is required at a supraphysiological concentration of about 20 μM. This is many orders of magnitude greater than normal serum melatonin concentrations of 100 - 400 pM. For comparison, glutathione, an established radical scavenger which is claimed to be less effective than melatonin, is present in human skin at concentrations of about 1 mM. For these reasons, it is possible that only melatonin in pharmacological quantities would provide protection against free radicals. Finally, it is possible that melatonin may have a modulatory effect on immune function (see Stevens *et al.*, 1992) and decreases in circulating melatonin could compromise immune surveillance and leave the animal more vulnerable to carcinogenic attack.

All the proposed mechanisms require that exposure to electromagnetic fields cause reductions in pineal or serum levels of melatonin. However the experimental evidence to support such a statement is somewhat equivocal: some studies have reported an effect, but others have failed to find consistent field-dependent changes in pineal function. This may mean that either the effect is not very robust or it may only occur under highly specific conditions which have yet to be fully described.

Studies using 60 Hz electric fields (reviewed in Wilson and Anderson, 1990) provided the first indication of a field-dependent effect on pineal function. Suppression of the normal nocturnal rise in the production of melatonin by the pineal gland was observed in adult rats exposed for three weeks to electric fields in the range of 2-40 kV m⁻¹: normal melatonin rhythms returned within three days of cessation of the field. However, the results were somewhat variable, and magnitude of the effects were independent of field intensity. Similar, if more modest effects have been reported in rats exposed to electric fields from conception until 23 days after birth. In contrast, a more recent study (Sasser *et al.*, 1991) reported that exposure to electric fields failed to modify nocturnal levels of melatonin in adult male and
female rats. It is of interest to note that the exposure parameters were identical to those used in the previous studies which found positive effects. Similarly, no change in pineal function was found in ewe lambs following chronic exposure to a combined 60 Hz electric and magnetic field (at 6 kV m⁻¹ and 4 μT) associated with a 500 kV transmission line (Lee et al., 1993). It is possible that the original positive observations were the result of some artifact, possibly associated with the methods used to assay melatonin levels (Reiter, 1993) and may not be related to exposure to the electric field per se. Few attempts to replicate the original findings using electric fields have been made in recent years.

Exposure to magnetic fields has also been reported to disrupt pineal function. A number of studies have found that inversion of the geomagnetic field or exposure to pulsed static magnetic fields modifies nocturnal melatonin metabolism in a variety of rodents (see Reiter, 1993). In contrast, only a handful of studies have investigated the effects of power frequency magnetic fields, and the results are somewhat variable. Chronic exposure to a rotating 50 Hz magnetic field has been reported to depress both nocturnal and diurnal pineal and serum melatonin levels in albino rats exposed at between 1 and 250 μT (spatial vector rms) (Kato et al., 1993) and in pigmented rats exposed at 0.02 and 1 μT (Kato et al., 1994a). The unusual exposure conditions appear to limit the relevance of these studies to humans, and exposure to a (more usual) horizontal or vertical field at 0.02 and 1 μT was found to be without effect in albino rats (Kato et al., 1994b). As part of an experiment to determine if magnetic fields affect mammary carcinogenesis induced by application of DMBA, albino rats were exposed to a 50 Hz field at 1 μT for 8 - 9 weeks (Löscher et al., 1994). This treatment caused a significant but rather conservative reduction in nighttime serum melatonin levels. The effects of DMBA itself on pineal physiology were not described.

Occasionally large, but inconsistent effects have been reported in Djungarian hamsters (Yellon, 1994). An initial experiment found that the nighttime peak of pineal and serum melatonin levels was both reduced and delayed following exposure to a 0.1 mT field for 15 minutes beginning 2 hours before dark, although replicate experiments failed to reproduce these effects, and in one experiment, exposure had no effect on melatonin levels. The differences in responsiveness were attributed to differences in age of subjects and other seasonal factors. No other study has indicated that such a brief exposure before dark could be disruptive to melatonin rhythms, and replication of this study by an independent laboratory appears warranted.

Very few studies appear to have used non-human primates: one preliminary study (Rogers et al., 1991) reported inconsistent changes in serum melatonin levels in baboons exposed to a combined electric and magnetic field (at 30 kV m⁻¹ and 0.1 mT). With few laboratories possessing the necessary facilities to work with primates, it is important that this study appears in the peer-reviewed literature.

It has not been established that exposure to electromagnetic fields is able to affect the circadian production of melatonin from the pineal in humans, although several large scale investigations are underway to help resolve this issue. The available data are very limited. Wilson et al. (1990b) reported a reduction in urinary levels of a stable metabolite of melatonin in some volunteers following nighttime exposure to a very weak (0.4 - 0.7 μT) 60 Hz magnetic field from an electric blanket. The results were highly variable, and were only found in 7 out of 28 subjects, so the robustness of the effect and the implications for human health are not at all clear. It is recognised that individuals exhibit wide differences in sensitivity of melatonin levels to light at night, and it may be that individuals will also show similar differences in sensitivity to electromagnetic fields, although further investigations are required before any firm conclusions can be drawn.

Of more direct relevance to carcinogenesis, Liburdy et al. (1993b) investigated whether a 60 Hz magnetic field could influence the inhibitory effects of melatonin on the growth of cultured breast cancer cells. Growth curves for estrogen receptor-
positive MCF-7 breast cancer cells were determined over 6 - 7 days in the presence or absence of melatonin and magnetic fields. It was found that exposure to the magnetic field did not affect the growth of cancer cells in the absence of melatonin. The presence of melatonin at physiological concentrations (1 nM) produced a small but significant inhibition of cell growth which was abolished by exposure to a 1.2 μT field. However, it should be noted that the melatonin-induced growth inhibition was much smaller in magnitude than the variation in growth between subsequent passages of the cells. Despite these limitations, this study is potentially important in that it describes a direct effect of a magnetic field on the interaction of melatonin with cancer cells, although unlike most other studies in this area, the mechanism does not involve an effect on melatonin synthesis or secretion.

**Opioids.** It is possible to speculate that other polypeptides apart from melatonin could be affected by exposure to electromagnetic fields and possibly lead to an increase in carcinogenic risk. For example, there is good evidence to indicate that exposure to weak magnetic fields can inhibit the functioning of endogenous opioid systems and the actions of exogenous opiate agonists in mice (see Kavaliers and Ossenkopp, 1992). It is therefore of interest to note that endogenous opioids, particularly the pentapeptide [Met^1]-enkephalin, have been shown to inhibit the proliferation of murine neuroblastoma cell lines (Zagon and McLaughlin, 1989a) most likely by depressing DNA synthesis and mitosis. Importantly, blockade of the action of these opioids appears to accelerate tumorigenesis and increase cell proliferation (Zagon and McLaughlin, 1989b). It is therefore plausible to consider that exposure to magnetic fields could affect carcinogenic events via field-induced changes in the endogenous opioid systems although this has yet to be demonstrated.

**Tumour Progression**

ELF fields could affect tumour progression via changes in immune function. However, exposure of animals to magnetic fields does not appear to result in any significant inhibition of immune responsiveness (Putinas and Michaelson, 1990; Morris et al, 1990). Exposure for 7 days to a 50 Hz magnetic field at 20 mT did not affect haematocrit values or either total or differential white blood cell counts in mice (Lorimore et al, 1990). In addition, assays of bone marrow stem cells and myelomonocytic progenitor cells failed to reveal significant effects on cell population dynamics, although subtle effects could not be ruled out. McLean et al (1991) examined the effects of exposing mice to a 2 mT magnetic field for 6 h/day after treatment with DMBA. No effects were observed on mononuclear cell counts, and natural killer cell activities in spleen and blood, or on spleen size, and it was suggested that magnetic fields did not affect the immune response. However, in animals also treated with the tumour promoter TPA exposure to the field resulted in both a greater number of enlarged spleens, and a greater number of mononuclear cells per spleen. Moreover, although mononuclear blood cell count was not significantly increased as a group, it was high in three out of ten animals. The authors interpreted their results as suggesting that magnetic field exposure compromised the animals' immune surveillance thereby reducing its effectiveness in combating tumour growth.

Possible effects on tumour progression have been examined directly. The incidence of leukaemia in a strain of leukaemia-prone mice was unaffected by periodic exposure to a 6 mT magnetic field pulsed at either 12 or 460 Hz (Bellossi, 1991). The mice were exposed to the magnetic field for 30 minutes twice a week, and leukaemia incidence was studied over five generations of the mice.
RADIOFREQUENCY FIELDS ABOVE 100 kHz (INCLUDING MICROWAVES)

Initiation

A series of reports from one group in particular have indicated that exposure of either Chinese hamster fibroblasts (Garaj-Vrhovac et al, 1990; 1991) or human lymphocytes (Garaj-Vrhovac et al, 1992) to 7.7 GHz radiation at 0.5 - 30 mW cm\(^{-2}\) for up to 60 minutes produced a statistically significant increase in the number of chromosome aberrations. It should, however, be noted that temperature was not actively controlled, and small temperature rises at the surface of the samples were reported. It would appear likely that temperatures within the cultures may have been elevated. Long-term irradiation of mice with 2.45 GHz at an SAR of about 1.2 W kg\(^{-1}\) was reported to induce gross DNA rearrangements (Sarkar et al, 1994). In contrast to these results, exposure of Chinese hamster ovary cells to 2.45 GHz radiation at an SAR of 33.8 W kg\(^{-1}\) for 2 hours did not produce a nonthermal increase in the number of chromosome aberrations, even in the presence of genotoxic chemicals (Kerbacher et al, 1990).

Promotion

Membrane Effects. Ion fluxes through the membrane constitute important signalling mechanisms and rely on ion gradients built up by the action of transmembrane ion pumps. A number of reports have suggested that radiofrequency (RF) radiation may be capable of affecting the activity of such pumps. It has been reported, for example, that the human erythrocyte Na\(^+\),K\(^+\)-ATPase can be induced to pump Na\(^+\) ion by application of 1 MHz electric fields at 2 kV m\(^{-1}\) (Liu et al, 1990). Another report has suggested that irradiation of a Na\(^+\),K\(^+\)-ATPase with 9.14 GHz RF at an SAR of 20 W kg\(^{-1}\) generally elevated its activity, although the activity was depressed by irradiation at 25°C (Brown and Chattopadhyay, 1991). This observation is consistent with earlier observations that RF irradiation may depress the activity of Na\(^+\),K\(^+\)-ATPase at temperatures which produced conformational changes in the active site.

Gene Expression. The possible effects of RF and microwave radiation on gene expression have not been thoroughly investigated. One recent study found that exposure of glioma cells to continuous wave (CW) radiation of either 27 MHz or 2.45 GHz resulted in a dose dependent athermal change in gross transcription as measured by incorporation of \(^{3}H\)-uridine (Cleary et al, 1990a). Transcription was elevated at an SAR of 25 W kg\(^{-1}\), but appeared to be unchanged or even depressed at higher SARs. No effect of frequency was obvious, and the reported effects appeared to persist over a relatively long time. It should be noted, however, that RF radiation at 2.55 GHz has been reported to alter gene expression in a bacterial system by a mechanism which was apparently thermal in nature, despite a gross temperature rise of only 0.1°C (Saffer and Profenno, 1992). Hence any reports of altered transcription following exposure at high SARs should be treated with caution.

Proliferation. Athermal effects on the rate of DNA synthesis have been reported following exposure of glioma cells (Cleary et al, 1990a) or human lymphocytes (Cleary et al, 1990b) to either 27 MHz or 2.45 GHz at SARs of 25 W kg\(^{-1}\). However, higher SARs resulted in a smaller response, and in some cases actually depressed DNA synthesis. In contrast, athermal exposure to 2.45 GHz radiation has been found not to affect transformation of human lymphocytes in vitro, although thermal exposure enhanced transformation to the
same extent as conventional heating (Czerska et al, 1992). In the same system, exposure to pulsed fields significantly increased transformation under both thermal and athermal conditions.

Krause et al (1990) have reported that exposure of mouse fibroblasts to 915 MHz amplitude modulated microwaves at an SAR of 3 W kg\(^{-1}\) increased ODC activity by 2- to 3-fold. To put this in perspective it should, however, be noted that phorbol ester treatment and serum stimulation both produced increases of over 20-fold under the same conditions.

**Tumour Progression**

It has been suggested that microwaves may affect tumour progression, and one mechanism for this could involve impairment of the immune system which normally plays a role in preventing tumour development. Veyret et al (1991) reported changes in the antibody responses of mice following repeated exposure to very low level pulsed 9.4 GHz microwaves, amplitude-modulated at 14-41 MHz, at an SAR of 0.015 W kg\(^{-1}\). The direction of the observed effect appeared to be dependent on the frequency of amplitude modulation.

A recent preliminary report suggested that daily exposure of groups of 44 Fisher 344 rats to either CW, or pulse modulated 915 MHz microwaves did not enhance the growth of brain tumours induced by direct injection of RG2 tumour cells (Salford et al, 1992). Exposures which were for 7 h/day and 5 days/week started 5 days after inoculation of tumour cells into the head of the right caudate nucleus. Rectal temperatures were monitored before, during and after exposure using an optical temperature device, and were not altered by exposure. Animals were sacrificed after 3 weeks and brains were examined histopathologically.

**SUMMARY**

There is no convincing evidence that ELF electric or magnetic fields cause genetic damage and it is therefore extremely unlikely that they could have any effect on the initiation of cancer. It is generally accepted that if these fields do affect carcinogenesis it is likely to be at the level of promotion. This possibility has been investigated at the cellular and subcellular levels, principally by exploring the possibility that cell signalling pathways may be affected leading to increased cellular proliferation. Many of these studies have centred on changes in calcium signalling, whilst others have followed the signalling pathways to the nucleus and examined the expression of proto-oncogenes known to be involved in regulating cellular behaviour. Possible effects on cellular proliferation have been assessed directly and animal carcinogenesis models have been employed to assess the potential for electromagnetic fields to act as promoting agents. Overall, the available experimental evidence remains contradictory and does not provide a clear indication that electromagnetic fields affect tumour promotion. Further studies are required to clarify the situation with respect to putative effects on cellular processes.

There is limited recent evidence suggesting that ELF fields may act as co-promoters, essentially enhancing the effects of chemical tumour promoters, possibly via an effect on cell-cell communication. These studies are, however, at best preliminary, and will need to be both repeated and extended before any firm conclusions can be drawn.

Exposure to electric or magnetic fields has, under some circumstances, been reported to inhibit the night-time synthesis of melatonin, which may have effects on the growth of certain tumours, and this has therefore been suggested as a route by which electromagnetic fields could influence tumour promotion. However, this effect has not always been successfully replicated, and it is possible that the reported changes in melatonin are more attributable
to the way the samples are collected, stored or analyzed than exposure to electromagnetic fields. Overall, the link between exposure to electromagnetic fields and depression of melatonin levels must remain tentative. Effects mediated by endogenous opioids have been postulated, but have not been demonstrated.

It has been suggested that ELF magnetic fields could affect tumour progression via a suppression of the immune system. There is, however, very little evidence for effects on the immune system and the positive results which have been reported are tentative and do not appear to have been independently replicated.

There is no convincing new evidence that athermal exposure to RF induces genetic damage and thus exposure is unlikely to initiate carcinogenesis. The evidence for effects on ion pumps or cell proliferation, which could constitute a mechanism for influencing tumour promotion, is extremely limited. There is no new convincing evidence that RF irradiation can affect tumour progression.

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