Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.fw001

Electromagnetic Fields

In Electromagnetic Fields; Blank, M.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1995.

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ADVANCES IN CHEMISTRY SERIES 250

Electromagnetic Fields

Biological Interactions and Mechanisms

Martin Blank, EDITOR
Columbia University

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.fw001

American Chemical Society, Washington, DC 1995

Electromagnetic fields

Electromagnetic fields: biological interactions and mechanisms/
Martin Blank, editor.

p. cm.—(Advances in chemistry series, ISSN 0065–2393, 250)

Includes bibliographical references and Index.

ISBN 0–8412–3135–4

1. Electromagnetic fields—Physiological effect. 2. Electromagnetic fields—Health aspects.

I. Blank, Martin, 1933– II. Series.

QD1.A355 no. 250
[QP82.2.E43]
540 s—dc20
[574.19'17]

95–41570
CIP

The paper used in this publication meets the minimum requirements of American National Standard for Information Sciences—Permanence of Paper for Printed Library Materials, ANSI Z39.48–1984. ♾

PRINTED IN THE UNITED STATES OF AMERICA

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.fw001

1995 Advisory Board

Advances in Chemistry Series

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.fw001

Foreword

The ADVANCES IN CHEMISTRY SERIES was founded in 1949 by the American Chemical Society as an outlet for symposia and collections of data in special areas of topical interest that could not be accommodated in the Society's journals. It provides a medium for symposia that would otherwise be fragmented because their papers would be distributed among several journals or not published at all.

Papers are reviewed critically according to ACS editorial standards and receive the careful attention and processing characteristic of ACS publications. Volumes in the ADVANCES IN CHEMISTRY SERIES maintain the integrity of the symposia on which they are based; however, verbatim reproductions of previously published papers are not accepted. Papers may include reports of research as well as reviews, because symposia may embrace both types of presentation.

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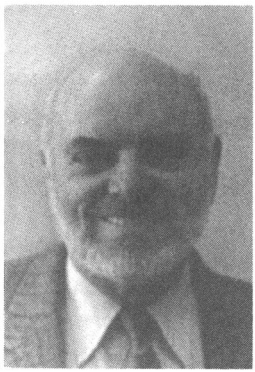
About the Editor



MARTIN BLANK received a B.S. in chemistry from City College of New York, a Ph.D. in physical chemistry from Columbia University, and a Ph.D. in colloid science from Cambridge University, England. In England, he developed a strong interest in the physical chemistry of biological membranes and biopolymers, and he has pursued this interest for more than 35 years in the Department of Physiology and Cellular Biophysics at Columbia University, as well as in other academic, industrial, and U.S. government settings. He has had appointments at Cambridge University (England), Weizmann Institute (Israel), University of California—Berkeley, Hebrew University (Israel), Monash University (Australia), Frumkin Institute of Electrochemistry (Russia), University of Warsaw (Poland), Tata Institute for Fundamental Research (India), Ben-Gurion University of the Negev (Israel), and University of Victoria (Canada). Industrial research experience has included California Research Corporation, Esso Research and Engineering, and Unilever Research laboratories in England and the Netherlands. He has worked for the U.S. Office of Naval Research (ONR) in London and in Arlington, Virginia, where he developed a research program on biomembrane electrochemistry. He has also consulted for many agencies, including the National Institutes of Health, the National Science Foundation, ONR, and the Electric Power Research Institute, on research programs.

His research has focused on biophysics of membrane transport and electrochemistry of proteins, and he has developed theoretical models of ion transport, hemoglobin equilibria, and ion-channel function during electrical excitation of nerves. Recently, he has been using this background to study the effects of low-frequency electromagnetic fields on protein synthesis in cells and on the function of the "ion pump" transport enzyme. His publications include about 200 papers and reviews, as well as 11 edited books on electrical properties of biological systems. In the past two years, he edited the *Proceedings of the First World Congress on Electricity and Magnetism in Biology and Medicine*, and books on the ONR program he developed, *Biomembrane Electrochemistry*, and the 4th Erice (Italy) course on *Nerve–Muscle Function* that he organized.

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Over the years, he has organized many meetings, including the Gordon Research Conference on Bioelectrochemistry, and has been active in the American Chemical Society, Electrochemical Society (Chair, Organic and Biological Division), Bioelectrochemical Society (President), and Bioelectromagnetics Society. He has also served on editorial boards of several journals, including *Journal of the Electrochemical Society* (Divisional Editor for Biology) and *Bioelectrochemistry and Bioenergetics* (North American Editor).

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Preface

ELECTROMAGNETIC FIELDS (EMFs) IN THE ENVIRONMENT come from sources that are both natural (e.g., UV, IR, and visible light) and man-made (e.g., electric power and appliances), and they cover a wide frequency range. Because the energy of a field depends on the frequency, the effects on living systems vary with the frequency range. Highly energetic fields at frequencies greater than about 10^{16} Hz are called ionizing radiation because the energy is sufficient to break chemical bonds. The lower energy EMFs in the microwave range (3×10^9 to 3×10^{11} Hz) cannot break bonds but do cause rapid heating of tissue as energy is absorbed. At the still lower power frequencies (50–60 Hz), even thermal effects of weak fields are negligible, but evidence of important biological effects is growing.

In recent years, interest has focused on the 50–60-Hz power-frequency range, largely because epidemiological studies have shown an increased risk of leukemia in children living close to electric power distribution lines. In a parallel but related development, electric devices based on low-frequency EMFs have been approved for therapeutic use to accelerate healing of bone fractures. The stimulation of biosynthesis suggested by both epidemiological and clinical studies has been observed in laboratory studies of cells in vitro. The data from changes in biosynthesis and other cellular processes after exposure to EMFs have recently provided important clues for understanding biological mechanisms. Especially relevant is the fact that the changes in protein synthesis caused by EMFs are similar to the "stress response" to noxious stimuli normally found in all cells.

As awareness of EMFs in the environment has grown, along with the sensitivity of the public to possible health risks, the need for sound scientific information has also increased. The Bioelectromagnetics Society was asked by the American Chemical Society's Division of Environmental Chemistry, Inc., to organize a symposium and tutorial on this subject for the spring 1992 ACS meeting in Denver, Colorado. As chair of the Intersociety Relations Committee, I organized a half-day program of tutorial lectures highlighting important facets (e.g., epidemiology, exposure, and biological mechanisms) of the problem. The program was approved by the Bioelectromagnetics Society Board and was well received at the ACS meeting. After a request from ACS Books to expand the symposium and tutorial into a book, we used the opportunity of a broader

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format to achieve more effective coverage, especially on the biochemical and biophysical mechanisms of interest to scientists. This book is the result. We have combined the knowledge of many experts on various aspects of the environmental EMF problem to provide an introductory survey at a scientifically sophisticated level. We trust the book will be informative and transmit the excitement and challenge of this field.

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Biological Effects of Environmental Electromagnetic Fields: An Overview

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This overview introduces the multidisciplinary problem of environmental electromagnetic (EM) fields. The different topics are presented in approximately the same order as the chapters in the book. The physical characteristics of electric and magnetic fields in the environment, as well as in the bodies of developing and mature biological systems, are considered first. Subsequent chapters discuss exposure and risk, and therapeutic applications of EM fields. A major section of the book deals with recent attempts to establish biological plausibility as well as the mechanism of interaction between EM fields and biological systems at both the molecular (physical transduction) and cellular (biochemical pathways) levels.

A WELL-KNOWN STORY tells of a successful politician who urgently needed some technical advice and approached a close adviser with the following request:

"Get me an appointment with a one-handed scientist!"

"What do you mean, a one-handed scientist?" was the reply.

"I need a one-handed scientist because every time I ask a normal scientist a question, he or she gives me an answer and then quickly adds '*but on the other hand...*'; I need a definite answer."

As this story suggests, many of the urgent questions that have been raised in connection with the environmental electromagnetic (EM) fields issue have no

0065–2393/95/0250–0001$12.00/0

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definitive answers at the moment, and definitive answers should not be expected from this overview or book. On the other hand, much has been learned about EM fields in the past few years. Two-handed scientists are really the only scientists, and those who have contributed to this volume, as well as the others whose work is reviewed here, provide the best available answers to the practical questions about EM fields that confront society today.

Spectrum of EM Waves

EM waves are periodic changes in electric and magnetic fields that propagate through space away from their source, which is an oscillating charge. At high frequencies the electric and magnetic fields move together, but at very low frequencies the fields can be effectively separated. The frequency determines the energy of the waves, and the biological effects vary with the frequency. To indicate the variety of effects, three different ranges in the frequency spectrum will be considered: the most energetic (ionizing radiation), an intermediate (communication) range, and the weakest (power-frequency) range.

The most energetic waves, which propagate at the highest frequencies, can dislodge electrons when they hit matter and are therefore called "ionizing radiation". Cosmic rays and X-rays (10^{18}–10^{22} Hz) damage cells, and as a result, people take precautions to minimize exposure. Even at lower frequencies near the visible range, overexposure to the ultraviolet (10^{16} Hz) waves in sunlight can damage the skin. The connection between exposure to sunlight and skin cancer has caused concern about recently documented changes in the ozone layer and the consequent decreases in the natural shielding against ultraviolet light.

Lower-frequency, less-energetic waves are nonionizing, but the microwaves (10^{9}–10^{11} Hz) that are used to cook foods could obviously damage our bodies if there were no shielding. (Actually, the fields emitted from a microwave oven cover a wider spectrum, including the much weaker power frequencies.) A closely related region of the EM spectrum came to public attention recently because of concern over a possible link between cellular phones, which transmit at about 10^{9} Hz, and brain cancer. Publicity on the *Larry King Show* and articles in the *New York Times* and *Time* have caused many people to suspect that cellular phones may be dangerous. Because very few studies of biological effects have been done in this frequency range, no one knows if they are dangerous. People generally believe that danger cannot exist unless effects are felt. However, cellular phone frequencies or the communication frequencies of radio and television (10^{4}–10^{8} Hz) can heat in our bodies at high power levels.

In the range of power transmission frequencies (60 Hz in the United States and 50 Hz elsewhere), the consensus has been that these extremely low frequencies (ELF) are benign because of the low energy levels. Intensity is also extremely important, of course, because a large current at 60-Hz frequency can cause great damage in electrocution. Nevertheless, these frequencies have not been considered a significant environmental concern.

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However, the power-frequency range is very close to the frequencies of many natural processes in the body. This range includes the rates of physiological processes that involve ionic currents, and also the rates of biochemical reactions that involve charge transfers between molecules. For example, the durations of action potentials in excitable tissue (nerves and muscles) are in the 1- to 10-ms range (which corresponds to 100–1000 Hz), and the turnover numbers of many enzymes are in the 50-Hz frequency range. In vitro studies using externally applied power-frequency-range EM fields have shown changes in the activity of enzymes as well as stimulation of biosynthetic processes that involve RNA polymerase (*see* chapters in this volume by MacGinitie, Bassett, and Sisken). The mechanisms of these effects of EM signals on physiological and biochemical processes may be directly linked to the power-frequency range. Streaming potentials due to the flow of blood and other fluids, and piezoelectric potentials due to the stressing of long bones, generally occur at lower frequencies.

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Electric Power Distribution

To understand the level of concern that has developed about possible health effects of EM fields, one must realize that electric power is widely distributed in our society at many different field strengths and frequencies. Although people generally do not sense EM fields, biological effects have been reported at frequencies and field strengths that are present in the environment.

Power is usually generated at around 20 kV, but step-up transformers raise the voltage to much higher levels to make the current lower and the power cheaper to transmit over long distances. Some transmission lines generate such large electric fields that a person could light an unconnected fluorescent bulb simply by holding it in the field under these lines. This example dramatically illustrates that large fields, capable of causing significant reactions, exist in the environment without being felt. The power goes through a step-down transformer to the distribution lines in neighborhoods at 5–35 kV, and another step-down transformer to 110–220 V for use in homes.

Although most of the discussion about risks of exposure has focused on electric power transmission and distribution lines, many additional sources of EM fields exist in the home and workplace. The role of grounding currents has been clarified as a substantial contributor to exposure. Some of the EM fields surrounding electric appliances in the home and standard office equipment are often higher than those near power lines. Modern society relies so heavily on electric power that finding places in the living and working environment that are free from EM fields is difficult.

Epidemiological Studies

Although controlled laboratory studies on living cells appear to be the way to obtain data about the biological effects of EM fields, human disease cannot be

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easily studied in the laboratory. This limitation has forced a reliance upon epidemiology and its studies of the factors that contribute to the incidence of disease in human populations. Epidemiological studies have their own methodological problems, and findings could be subject to confounding factors such as traffic patterns and socioeconomic class that often correlate with EM fields. The different kinds of information derived from epidemiology and scientific laboratory investigation are complementary in the study of human disease patterns. Epidemiology can find correlations, but establishing biological plausibility and the real testing of hypotheses about mechanism must come from laboratory investigation, in which variables can be controlled.

In the area of EM fields, public interest and concern has been driven by epidemiological studies. The first study to suggest that a risk may be associated with exposure to low-frequency EM fields was conducted in Denver in 1979 by Wertheimer and Leeper (*1*), who found that children living close to power lines had almost a 3 times higher chance of acquiring leukemia. Although the study was attacked for its limitations, it brought the potential problem to public attention. Subsequent studies have supported these original findings, including a repeat study in Denver conducted by the New York State Power Commission (*2*) and a study in Los Angeles (*3*). A published long-term study in Sweden (*4*) took advantage of detailed records of power usage and found that the effect depended upon a calculated 12-month average magnetic field level. In houses with a magnetic field above 2 mG, the relative risk of acquiring pediatric leukemia was 2.7, and in houses with a magnetic field above 3 mG, the relative risk increased to 3.8.

Often epidemiologists study special population subsets that have extraordinarily high exposures (e.g., power-line workers) in order to examine the effects of a particular factor. Such epidemiological studies generally have not found elevated risk in the adult populations, but Floderus et al. (*5*) recently found increased chronic lymphocytic leukemia for adults in electrical occupations. Other studies, using smaller populations, have reported (*6, 7*) a higher incidence of breast cancer in male telephone line workers. This finding has aroused much attention because of the rarity of breast cancer in men.

If EM fields do contribute to human disease, then determining the extent to which a particular disease is apt to be a factor in increased mortality is important. Pediatric leukemia is a relatively small contribution to human disease, and EM fields could account for relatively few extra deaths per year in the United States, perhaps on the order of 1000. However, if EM fields were a factor in breast cancer in women or prostate cancer in men, the range of extra deaths would be much higher. Obviously, many questions remain to be answered about the severity of the potential problem. The epidemiological studies suggest that EM fields are a factor in human disease, but how large a factor is still not known.

In addition to the evidence from epidemiological studies about possible detrimental health effects of exposure, medically related studies show (*8, 9*) that EM fields can affect biological processes in ways that are beneficial. Since the invention of the first electric devices in the 1700s, electricity has been used to

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treat medical conditions. Many well-known figures, including Benjamin Franklin, played roles in the medical history of electric devices. More recently, electric and EM fields have been used to accelerate the rate of bone healing, growth, biosynthesis, and repair.

To establish plausibility for health-related (therapeutic or malignant) biological phenomena due to electromagnetic fields, one must show an appropriate biological effect in a scientific context. Goodman and Henderson (*10, 11*) were the first to demonstrate changes in the transcription and translation stages of protein synthesis following exposure to weak EM fields. Effects were observed at the low-level fields linked by epidemiology to pediatric leukemia and at the higher fields used in bone-healing therapy.

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Magnitude and Sources of EM Fields in Everyday Life

All of the aforementioned studies are concerned with AC (alternating current) fields, that is, fields that oscillate at particular frequencies. DC (direct current or constant magnitude) electric and magnetic fields also exist in the natural environment, and their magnitudes are much greater than the AC fields usually studied. The electric field at the surface of the earth is around 130 V/m, and the magnetic field in the United States is around 500 mG. (The Earth is a giant bar magnet with magnetic poles very close to the geographic poles.) Although bacteria, birds, and migrating animals do take advantage of the Earth's magnetic field for orientation and navigation, these DC fields are not detected by humans.

Environmental AC fields are generally much lower than DC fields. In homes around the country, electric fields generated are usually between 0 and 10 V/m. Electric fields do not penetrate the body effectively, and thus a field of 1 V/m outside the body would measure 10^{-7} V/m beneath the skin. For magnetic fields, average values in the home are on the order of 0–2 mG. (One cutoff level in the Swedish leukemia studies mentioned was 2 mG.) Although most houses have very low levels of EM fields on average, the fields around many appliances can be very much higher.

Unlike electric fields, magnetic fields pass through the body unattenuated, and therefore the outside magnetic field would have the same value inside our bodies. A person standing under a high-voltage transmission line would experience a magnetic field of approximately 1 G (1000 mG) inside the body, and the field from transmission lines can often extend up to 0.5 km. For a distribution line, the fields would be around 100 mG and extend for shorter distances. These values are much higher than the average magnetic field background in the home.

Fields given off by certain common household appliances, such as an electric blanket, electric shaver, or electric hair blower, are sometimes higher than near a power line and are concentrated near the body. Many home and office appliances give off magnetic fields that are comparable to fields under transmission and distribution lines. Television receivers, computer monitors, projectors, and kitchen appliances are sources of magnetic fields that people are exposed to much of the time. Unlike the fields under transmission lines, fields

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generated by household and office appliances decrease very rapidly with distance. At about 1 m from most appliances, EM fields are effectively zero. The problem is that many appliances are often used very close to the body.

Scientific Issues

Signal Transduction. Experimental sciences have an important role to play in the debate about environmental effects of EM fields. At the very least, scientific studies must demonstrate that the correlations found using epidemiological methods are biologically plausible by showing that effects predicted from those studies can be obtained in a controlled laboratory investigation. A fuller understanding and eventually an ability to control effects will come from an increase in reliable information and from the formulation and testing of hypotheses about mechanism at both initiation (i.e., signal transduction) and cellular (i.e., biochemical pathways) levels.

Several physical mechanisms have been under consideration (*12*) to account for EM signal transduction at the cellular level. Among the popular proposals were resonance models, in which AC fields interact with the Earth's DC field, and the charge-to-mass ratio of the chemicals involved is important (*13, 14*). These ideas do not appear to have been successful in overcoming theoretical objections or in explaining observations. Cytoplasmic calcium ion changes in cells, as well as changes in enzyme activity, indicate EM field effects on ion binding and electron flow in molecular reactions. Free-radical reactions, which involve unpaired electrons, are also affected by magnetic fields, generally at much higher (>10 G) field intensities.

One of the ongoing discussions in considering mechanisms has been the "*kT* problem", where *k* is the Boltzmann constant and *T* is the absolute temperature (*15*). The energy level of EM fields that cause biological effects should be greater than *kT,* the energy associated with normal thermal motion or background thermal noise. Yet many biological studies show effects at very low field strengths. For example, certain cells in elasmobranch fish respond to low-level electric fields that are well below the *kT* level. Also, low-level EM fields can change the rate of transcription in certain organisms, as well as affect the activity of the Na^+/K^+-adenosinetriphosphatase (Na^+/K^+-ATPase) pump.

While biologists have measured and described effects, physicists have published calculations showing that low-level EM field exposure cannot affect biological processes. Biology is often much more complex than the models on which the calculations are based. The calculations may be correct, but the models are probably inadequate. Two classic examples follow, in which physicists were correct in their calculations but wrong in their conclusions.

In the early 1800s, geologists suggested that the Earth was billions of years old. Lord Kelvin, a physicist, asserted that if the Earth were this old, the heat at the core of the Earth would have diffused away long ago and the Earth would have cooled into a solid mass. Because the Earth had a molten core, the

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age estimated by the geologists had to be wrong. Kelvin thus rejected the geologists' theory. His calculations were correct, but his answer was wrong. In the late 1800s radioactivity was discovered, and radioactive decay generates enough heat to keep the core molten.

Another physicist, H. Jeffreys, thought that the theory of continental drift was impossible on the basis of the viscosity of slabs of rock flowing over one another. However, Jeffreys did not know of the spreading forces generated by the flow of molten rock from the core into the crust, and the once rejected old theory is currently accepted under the name "plate tectonics". Scientists know today that long ago one giant land mass broke apart into the continents that have been drifting ever since, largely from the spreading ocean floor.

These two stories illustrate that theoretical predictions based on simple physical models may be wrong when applied to complicated systems that are not completely understood. The same situation might hold with regard to the effects of EM fields on living cells and organisms. Effects on the disease process are even more complicated because the mechanisms of disease are less well understood.

Experimental studies on cells (*16*) have helped to define limits in the interaction of EM fields with biological systems. Cells exposed to 60-Hz fields showed a doubling in the activity of the enzyme ornithine decarboxylase (ODC), and the same change in ODC activity was also noted when the cells were exposed to 50-Hz fields. If the 50- and 60-Hz fields were alternated, each being on for a time period (called the coherence time), the same twofold increase in activity was obtained only if the coherence time was >10 s. If the time decreased to 10 s or below, the increase in ODC activity vanished. Apparently a coherent signal at a given frequency and amplitude, administered for longer than the coherence time, is needed before the effect will be translated by the biological system.

In another experiment, ODC activity was increased when cells were exposed to 60-Hz signals. When low-frequency noise composed of EM waves (30–300 Hz) was added to the system, the ODC activity decreased as the noise increased to the level of the signal (*17*). Introducing noise when a signal is causing some change in a cell had the effect of eliminating that signal. The level of the noise needed reflects the level of the signal that is causing the change.

The Na^+/K^+-ATPase membrane enzyme has been studied (*18, 19*) as a model for interactions of proteins with electromagnetic fields. Low-frequency electric fields appear to increase binding of activating ions on the Na^+/K^+-ATPase surfaces, whereas low-frequency magnetic fields appear to increase charge movements within the protein that coordinate the surfaces. The frequency optimum of magnetic field effects is very close to the turnover number of the enzyme, a condition suggesting that the magnetic field couples to charge movements during the chemical reaction. The frequency optimum for magnetic field effects on RNA polymerase (*11*) is also close to the reaction rate. These results suggest that one of the ways in which EM fields interact with cells can be by coupling directly to biochemical reactions.

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Cellular Mechanisms. Regardless of the ways in which signals from EM fields are physically transduced, linking these changes to cellular mechanisms that produce a biological response is necessary. Several plausible mechanisms have been suggested (*20*). These mechanisms include changes in biosynthesis (*10, 11*), G-protein-linked membrane receptors (*21*), and melatonin-linked mechanisms (*22*). The stimulation of biosynthesis when cells are exposed to EM fields has been demonstrated in many laboratories. Transcription and translation are both affected, and the two separate lines of experimental evidence support each other. Furthermore, the changes in biosynthesis tie in with pathways that are linked with oncogenic mechanisms, and this finding lends biological credibility to the epidemiological results.

With transcription, both magnetic and electric fields have been shown (*11, 23*) to stimulate the same transcripts, with similar patterns of amplitude, frequency, and time dependence on transcription. The only major difference is that the energy needed to induce transcription with a magnetic field is about 1000 times lower than the energy needed with an electric field.

Changes in protein synthesis, stimulated by low-frequency EM fields, are similar to those following the cellular stress called heat shock (*24*). The variations in the distribution of proteins with the strength of the magnetic field and with increasing temperature of heat shock are similar, as are the changes in many individual proteins. Further evidence of a connection has come from experiments showing transcripts for the heat shock protein hsp70 in all cases of EM stimulation. The energy needed to induce transcription from magnetic fields is many orders of magnitude lower than the energy needed for both electric and thermal stimulation.

The stress–response system is a well-defined pathway that is present in all cells. EM fields appear to stimulate a natural pathway similar to the one used by cells in response to heat and other physical stresses, except that the cells respond to EM fields at very low energy densities.

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Status Report

This brief survey of the EM field problem provides a unifying perspective on the major areas covered in this volume. The presence of significant biological effects due to EM fields is generally acknowledged, but we cannot be sure if there is a health risk associated with exposure. The molecular research suggests that fundamental reaction rates can be affected in the body by weak EM fields. Strong evidence on the cellular level indicates that weak magnetic fields are stimulating the stress–response pathway. Activation of this pathway helps to control damage due to a physical stimulus. However, if the system is compromised or the body is overexposed, the stress response may not be able to compensate. The research findings appear to justify caution regarding exposure to EM fields but show no clear links to disease.

Although definitive answers to the scientific and medical questions are not yet available, the Office of Technology Assessment (*25*) counsels a simple strat-

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egy: prudent avoidance. The use of as many EM field sources as possible should be avoided or minimized. In the home, this caution applies especially to electric blankets, shavers, and hair dryers that are used very close to the body. Because of the rapid falloff with distance exhibited by the EM fields of small appliances, exposure can be significantly minimized by increasing separation from the source. The electric alarm clock should be moved away from one's bed (and thus one's head), and one should not sit close to the screen while watching television. Children should be kept out of the kitchen when food processors or other machines that have motors with large currents flowing through them are being used.

Outside the home, people experience many levels of exposure throughout the day as they go about their daily routines. Rapid-transit trains and office machines (computers and copiers) are good examples of sources of repeated exposure. The transport problem may get worse with introduction of maglev (magnetic levitation) trains, although the magnetic fields do not appear to be that much higher than the ones in many rapid-transit systems in use today.

This book focuses on scientific questions concerning the biological effects of EM fields but does not directly consider many potential practical implications of this research, such as the enormous costs associated with changes in power distribution and in appliances if such changes prove advisable. Business and government interest in restructuring power transmission, communications industries, and public transport introduces a political and economic dimension to the problem that is difficult to estimate. Certainly, the development of sound approaches to those practical problems will require a firm scientific foundation upon which to build. This book is intended to contribute to that goal by providing an efficient scientific introduction to a multidisciplinary environmental problem and also by transmitting the excitement inherent in the search for mechanism.

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RECEIVED for review March 8, 1994. ACCEPTED revised manuscript December 22, 1994.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch002

2

Spectrum and Intensity of Environmental Electromagnetic Fields from Natural and Man-Made Sources

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This chapter presents a review of the numerous sources of nonionizing electromagnetic fields (EMF) over the frequency range from 0 Hz (static fields) to the ionizing radiation boundary at approximately 10^{15} Hz in the UV spectral region. The wide range of natural and man-made sources of EMF is described for static electric and magnetic fields, fields at extremely low and very low frequencies (up to 30 kHz), radio-frequency (RF) fields (30 kHz–300 GHz), and optical radiations in the IR, visible, and UV regions of the spectrum. A description is given of exposure to natural environmental electric and magnetic fields. The frequency spectrum and intensities of man-made sources of EMF are reviewed for power generation and distribution systems, industrial equipment, residential appliances, public transportation, communication systems, and medical devices.

THE CHARACTERIZATION OF HUMAN EXPOSURE to nonionizing electromagnetic fields (EMFs) in the workplace, in homes, and in public places has become a major effort over the past 15 years because of concerns about the potential health effects of these fields. The nonionizing portion of the electromagnetic spectrum extends from static fields at 0 Hz to a frequency of approximately 10^{15} Hz in the UV radiation band. This maximum frequency corresponds to a photon energy of approximately 5 eV (electron volts), which is roughly the lower limit of energy sufficient to disrupt a chemical bond. Electro-

0065–2393/95/0250–0013$12.75/0

magnetic fields at frequencies higher than 10^{15} Hz are the ionizing portion of the electromagnetic spectrum, which includes the vacuum UV, X-ray, and gamma-ray regions.

An enormous number of processes use nonionizing electromagnetic fields, as illustrated in Figure 1. Of particular interest in recent research have been the biological interactions and possible health effects of fields in the extremely low frequency (ELF) band, which incorporates the power frequencies of 50 and 60 Hz that are used worldwide. Because of widespread concern about a possible elevation in cancer risk associated with residential or occupational exposure to power-frequency fields, the primary focus of this chapter will be on characterizing human exposure to ELF fields.

Environmental Fields of Natural Origin

Naturally occurring static and time-varying electric and magnetic fields observed at the surface of the Earth originate from the properties of the Earth's core, electrical discharges in the atmosphere, and solar and lunar influences on ion currents in the upper atmosphere. Both static electric and magnetic fields, of entirely different origins, are present at the Earth's surface. The static electric field results from the differing electric charges of the Earth's surface (negative) and the upper atmosphere (positive). This field has a strength of approximately 130 V/m in fair weather at the Earth's surface, which decreases to 45 V/m at an altitude of 1 km, and to less than 1 V/m at an altitude of 20 km (*1, 2*).

The Earth's static magnetic field (the geomagnetic field) originates from electric current flow in the Earth's core. The vertical component of this field is a maximum at the magnetic poles, with a flux density of about 70 μT [1 T (tesla) = 10^4 G (gauss)], and is zero at the magnetic equator. The horizontal component of the geomagnetic field is a maximum at the magnetic equator, with a flux density of about 30 μT, and is zero at the magnetic poles.

The time-varying electric fields in the Earth's atmosphere have small amplitudes and low frequencies (<30 Hz), and arise from thunderstorm activity and pulsations in the geomagnetic field that produce currents within the Earth (telluric fields). Local fields in the vicinity of lightning strikes, however, can reach very high intensities on the order of thousands of volts per meter. The naturally occurring electric field in the atmosphere is only 0.1 mV/m at power frequencies (50–60 Hz), and decreases rapidly at higher frequencies (*1*).

Large, intermittent time-varying magnetic fields are produced in the atmosphere as a result of intense solar activity and thunderstorms, and can reach levels up to 0.5 μT during magnetic storms (*3*). Diurnally varying magnetic fields with flux densities of about 30 nT are produced as a result of solar and lunar influences on ion currents in the upper atmosphere.

Superimposed on the slowly time-varying fields associated with irregular atmospheric events are weak fields that are generated by lightning discharges and propagate in the resonant spherical cavity formed by the Earth's surface and the lower boundary of the ionosphere (*4*). This effect has been named the Schu-

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch002

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch002

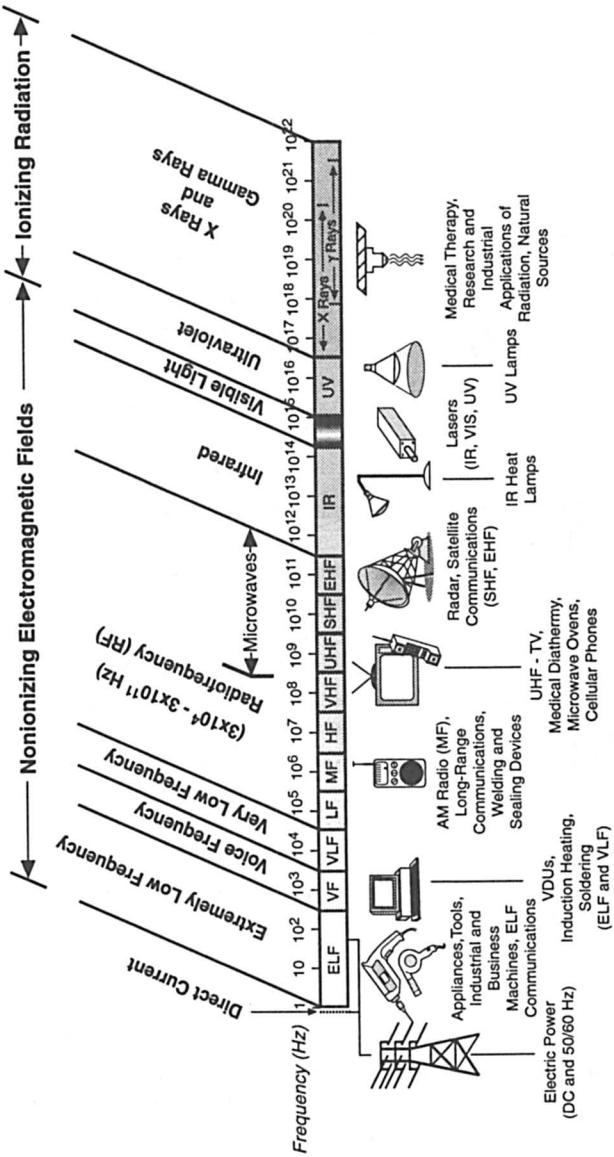

Figure 1. Depiction of the electromagnetic spectrum, showing the nonionizing and ionizing regions and various applications of electromagnetic fields (EMFs) in medicine, industry, communications, electric power transmission, and personal appliances. The seven frequency bands within the RF region are LF (low frequency, 30–300 kHz), MF (medium frequency, 300 kHz–3 MHz), HF (high frequency, 3–30 MHz), VHF (very high frequency, 30–300 MHz), UHF (ultrahigh frequency, 300 MHz–3 GHz), SHF (superhigh frequency, 3–30 GHz), and EHF (extremely high frequency, 30–300 Ghz).

mann resonance phenomenon, and the fields have a periodic frequency dependence with the lowest five resonances observed at 8, 14.1, 20.3, 26.4 and 32.5 Hz. At 8 Hz the measured values of the horizontal magnetic field and the vertical electric field associated with the Schumann effect are, per unit frequency bandwidth, 0.6 $nT/Hz^{1/2}$ and 100 $(\mu V/m)/Hz^{1/2}$, respectively (*4*).

Static Electric and Magnetic Fields

The major man-made sources of strong static electric fields are high-voltage, direct-current (DC) transmission lines. The values of the static electric and magnetic fields measured at ground level under a 500-kV DC transmission line carrying a 2-kA current load are 21 kV/m and 22 μT, respectively (*5*). Ion charge densities measured at ground level beneath lines of this voltage have been found under typical ambient conditions to be on the order of 10 nC/m^3. However, ozone levels are generally low, on the order of a few parts per billion (*6*).

Strong static magnetic fields are used in numerous technologies, including various energy systems, research facilities, industrial processes, transportation, and medicine. In the area of energy technologies, several of the large thermonuclear fusion reactors that have been designed as prototype models during the past two decades use fields as high as 9–12 T for confinement of an ignited plasma, with fringe fields up to 50 mT in regions accessible to personnel (*7*). Large magnetohydrodynamic power generators produce fringe fields >10 mT at operator locations (*8*). Various designs of superconducting magnetic energy storage systems have been proposed (*8–10*), most of which involve fields on the order of 4–5 T. These systems can expose personnel to fringe fields that reach levels as high as 0.7 T (*10*). Superconducting generators and transmission lines using fields of 6–7 T have also been proposed (*7*). These systems, however, have relatively small fringe fields, on the order of 0.1 mT, at locations where operators or other individuals are likely to be exposed.

Strong magnetic fields are also present in several industrial processes that use high static electric fields for chemical separations. For example, in aluminum production plants the operators changing anodes on prebake cells are in fields of up to 57 mT for 10–20 min (*7*). Exposures of workers in chloroalkali plants to fields as high as 39 mT have been recorded at several different facilities, and routine exposures fall in the range of 7–14 mT (*11, 12*).

A source of potentially high public exposures to static magnetic fields involves the use of magnetically levitated vehicles for high-speed transportation (*7, 13*). Field levels up to 50 mT in the passenger compartment were calculated for various designs of magnetically levitated vehicles, and flux densities approaching this value were measured in experimental test vehicles (*13*).

Several applications of high-intensity static magnetic fields, including magnetic resonance imaging, are now being widely used in medicine. These technologies are described in a later section of this chapter.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch002

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Advances in Chemistry; American Chemical Society: Washington, DC, 1995.

Sub-Radio-Frequency Electric and Magnetic Fields

The frequency range from 0 Hz (static fields) to 30 kHz [the lower boundary of the radio-frequency (RF) spectrum] is denoted here as the sub-RF region and is composed of three frequency bands: (1) ELF fields with frequencies below 300 Hz, (2) voice frequency (VF) fields from 300 to 3000 Hz, and (3) very low frequency (VLF) fields from 3 to 30 kHz. By far the most common exposure to sub-RF fields in residential, occupational, and public settings is to ELF fields, which include the 50- and 60-Hz power frequencies at which electricity is generated, transmitted, and used throughout the world. In this chapter, special attention is given to human exposures to power-frequency fields from a variety of sources. These sources include electric power transmission and distribution lines, long-range military submarine communication systems, electric transportation systems, and a wide variety of appliances and machines used in the home and workplace (*1–3, 13*).

Until approximately 1980 the major concern about the health effects of ELF fields was centered on the power-frequency fields near high-voltage transmission lines. However, during the past 15 years a number of reports have suggested that adverse health effects may result from routine exposures to ELF fields in occupational and residential settings (*3*). Stimulated by these reports, dozens of studies have been conducted since the early 1980s to characterize the electromagnetic exposure environment in homes, public areas, and the workplace. These studies focused primarily on ELF fields, although attention was given to other sources in the sub-RF frequency range such as VLF fields from video display systems (*3*).

Electric Power Transmission and Distribution Lines. Electric power transmission lines carry 60-Hz current loads ranging from several hundred amperes to 2 kA with operating voltages up to 765 kV in the United States and even higher levels in other countries. The electric and magnetic field profiles as a function of distance from the high-voltage conductors on transmission lines with various configurations have been extensively characterized (*14–16*). For three-phase lines operated at 765 kV and carrying a load current of 2 kA, the maximum 60-Hz electric and magnetic fields at 1 m above the ground surface near the center of the right-of-way are 12.9 kV/m and 33 μT, respectively. These field levels decrease to 8.8 kV/m and 28 μT, respectively, for a 500-kV line with a load current of 1.5 kA, and to 5.3 kV/m and 19 μT, respectively, for a 345-kV line with a load current of 1.0 kA.

Transmission lines generally terminate at substations, where the voltage is stepped down to levels of less than 35 kV for local power distribution on primary lines that are typically less than a few kilometers long. At locations that are close to the consumers, the voltage is again stepped down by transformers to levels of 110–480 V and carried over short distances on secondary distribution lines. Because of their lower voltages and load currents, the electric and mag-

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch002

netic fields at ground level in the vicinity of distribution lines are substantially weaker than those near high-voltage transmission lines. Typical values of the power-frequency electric and magnetic fields below power distribution lines are less than 50 V/m and 2 μT, respectively. However, as recently pointed out by Kaune (*17*), these fields generally decrease rather slowly as a function of the horizontal distance from a distribution line. This characteristic of distribution lines results from the fact that they usually have unequal currents in their separate conductors because of ground currents, and hence the algebraic sum of the conductor currents is nonzero. The existence of a net current on a distribution line gives rise to a magnetic field that decreases only as the inverse of the distance from the line. In contrast, if the conductor currents are completely balanced and the net current is zero (as it is in a perfectly balanced three-phase transmission line), the magnetic field decreases approximately as the inverse square of the distance from the line. Simple models of power distribution lines that neglect the influence of unbalanced currents can therefore give rise to erroneous estimates of the magnetic fields in nearby structures.

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Residential Exposures to ELF Fields. The four basic sources of exposure to 50–60-Hz fields in the home are (*18*): (1) fields from neighboring transmission and distribution lines, (2) ground currents in the home and between homes and nearby sources of electric current, (3) home wiring, and (4) household appliances. In the United States the primary source of power-line residential fields is distribution lines, whereas in some other countries the primary source is transmission lines because homes are built in close proximity to them [e.g., in Sweden (*19*)]. The electric fields from power lines are well shielded by the walls and windows of homes, but 50–60-Hz magnetic fields penetrate these structures with little attenuation. Ground currents that are not compensated by an equal return current are present in nearly all residences that ground the neutral conductor on a metal water pipe. These unbalanced currents can pass under and through a home, and even between homes.

Household wiring is generally not a large source of magnetic fields because the supply and return wires carrying current to lights and appliances are usually closely spaced and carry nearly equal currents in opposite directions (*17*). However, measurable fields can result from unbalanced currents that arise from the use of three-way switches and two or more circuit breaker panels within the home. The older style of knob-and-tube wiring that is still present in many residences can also contribute to the ambient magnetic field.

Probably the largest single source of human exposure in the home is from the magnetic fields generated by many common household appliances and tools. As shown by Gauger (*20*), the fields near the surfaces of some appliances reach levels greater than 1 mT. Although these fields drop off rapidly with distance because of the localized nature and configuration of the source, the residual fields at typical distances from the body during appliance use are still large compared to other sources of magnetic fields in the home (Table I). These exposures, however, are usually of short duration (minutes per day), and the field levels are large only in regions of the body closest to the appliance (*21, 22*).

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Table I. Magnetic Fields from Various Appliances and Electric Tools

Appliance or Electric Tool	*15 cm*	*30 cm*
Can opener	160	27
Electric saw	120	25
Vacuum cleaner	75	20
Electric shaver	65	10
Mixer	61	11
Hair dryer	50	7
Electric drill	20	3
Portable heater	15	4
Fluorescent light fixture	13	4
Fan (range hood)	9	3
Television	7	2

NOTE: All values are the maximum flux density (60 Hz, μT rms) at a distance of either 15 or 30 cm from the source.
SOURCE: Adapted from reference 20.

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One example of a prolonged residential exposure to a relatively high 50–60-Hz magnetic field is provided by the older style of electric blankets, in which the fields at the surface typically reach levels of 1–2 μT (*23*). An interesting aspect of many personal appliances, especially in older models, is the production of harmonic fields that extend into the megahertz frequency range (*24*). These higher frequency fields can induce significant body currents and have not been adequately characterized as a source of human exposure to EMF.

The characterization of residential exposures to power-frequency fields has become a major effort during the past decade because of evidence obtained in several epidemiological studies suggesting that fields from residential power lines may be linked in some undefined manner to an elevated risk of various types of childhood cancer, primarily leukemia and nervous tissue tumors (*3*). This apparent association is statistically significant only when exposures are estimated from the proximity and current-carrying capacity of the power distribution lines. The actual field levels in homes determined by direct measurements over short periods of time (minutes to days) have only a weak, and statistically insignificant, association with childhood cancer risk.

The possibility of a link between childhood cancer risk and exposure to fields from residential power lines was first suggested in 1979 on the basis of an epidemiological study in the Denver, CO, region conducted by Wertheimer and Leeper (*25*). These investigators developed a "wire code" that characterized the magnetic fields produced by residential power lines on the basis of the distance of a power line from a residence and its current-carrying capacity (e.g., thick

primary wires were assumed to carry more electric current than thin secondary-span wires). The so-called "high-current configurations" of power distribution lines were more frequently associated with residences of childhood cancer cases than were the "low-current configurations" of power lines.

Later studies on childhood cancer by Savitz et al. (*26*) in the Denver, CO, area, and by London et al. (*27*) in the Los Angeles, CA, area, led to results consistent with those of the original Wertheimer and Leeper (*25*) study. However, in the Savitz and London studies direct measurements of 60-Hz fields in the homes of cancer cases and matched controls did not show a statistically significant correlation between magnetic field levels and cancer risk. Various modifications and simplifications of the original Wertheimer and Leeper wire code have been proposed (*28, 29*), but none of these has weakened the association between the proximity of homes to high-current configurations of power lines and an elevated risk of childhood cancer. With the exception of one study by Wertheimer and Leeper published in 1982 (*30*), several attempts to correlate adult cancer risk with either high-current power-line configurations or direct measurements of fields in the home have led to negative results (*31*).

The apparent association between wire codes as a surrogate measure of residential exposure to power-frequency magnetic fields and the risk of childhood cancer has led to a large number of other epidemiological surveys during the past several years. Nearly all of these studies involved both direct measurements of residential magnetic fields and the use of alternative measures of exposure that could be assessed without entering the homes of the subjects. These alternative measures have included the Wertheimer–Leeper wire codes in their original or modified forms (*28, 29*), estimates of the distances of high-voltage power lines from residences (*32, 33*), measurements of fields at the location of the front door of each residence (*34*), and the use of models that attempt to relate directly measured residential fields to various characteristics of neighboring power lines such as their proximity and power loads (*19, 35, 36*). The last of these approaches was also used to estimate residential exposure to power-frequency magnetic fields prior to cancer diagnosis in the subjects under study (*19, 35, 36*).

Stevens (*37*) hypothesized that a metric based only on the distance of a residence to the nearest power line may provide a reasonable estimate of exposure. Feychting and Ahlbom (*38*) argued, however, that this method gives a less reliable prediction of exposure than that obtained from a more complex model in which power loads are taken into account. Nevertheless, the possible role of distance as a surrogate exposure metric was supported by a recent meta-analysis of 13 epidemiological studies on childhood cancer (*39*). This analysis demonstrated that a statistically significant association exists between the distance of electricity transmission and distribution systems from residences and the risk of childhood leukemia and nervous tissue tumors.

Achieving a resolution of the issue of why surrogate measures of exposure of children in the home environment to 50–60-Hz fields from residential power lines is correlated with an elevated risk of cancer, whereas the field levels di-

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rectly measured in the home are uncorrelated, remains a major challenge. Significant difficulties in resolving this issue are posed by several unique features of the electromagnetic environment within the home. First, the total range of average 50–60-Hz fields within homes is small; the temporal average of fields monitored over periods of hours to days varies by only a factor of 2–5 (e.g., about 0.1 μT in the "low-exposure" homes to about 0.2–0.5 μT in the "high-exposure" homes). A further complication is introduced by the fact that power lines account for only 17–21% of the variance in residential field levels (*40, 41*). This finding suggests that less than 0.1 μT of the difference in the average residential exposure of children living near "high-current" versus "low-current" configurations of power lines can be accounted for by the fields from the power lines per se.

A second difficulty is associated with the large spatial and temporal variability of fields within the home. As already discussed, the use of personal appliances can introduce exposures for brief periods that exceed the average 24-h magnetic field levels in the home by 2–4 orders of magnitude. Although large temporal variations in ambient magnetic field levels also occur within the home, present evidence indicates that these variations do not negate the value of spot measurements of fields within homes as valid estimates of time-weighted-average exposures (*17*).

Nevertheless, efforts have significantly increased over the past few years to characterize the continuous exposure histories of residents within homes during periods of several hours to several days using personal magnetic field dosimeters (*19, 41, 42*). To date these extensive measurements have not provided new insights into which factors, if any, in the electromagnetic environment of the home are responsible for an elevated risk of childhood cancer. However, both the time resolution and the bandwidth of personal exposure monitors used in these studies are limited. As a result, brief exposures to high-amplitude magnetic field transients and to high-frequency harmonic fields have not been captured in the personal exposure histories recorded with these dosimeters. Several research programs are currently in progress to characterize these potentially important aspects of the residential exposure environment.

A third major difficulty in resolving the question of whether power-line fields are, in fact, associated with an elevated risk of childhood cancer is the role of confounding variables. Many of the major epidemiological studies conducted over the past several years examined the confounding role of chemical exposures and socioeconomic variables that could conceivably be linked to an elevated risk of childhood cancer and simultaneously be correlated with residential proximity to high-current power-line configurations. Although exposures to various household chemicals and airborne pollutants are associated with an elevated childhood cancer risk (*26, 27, 43*), introducing corrections to account for the influence of these confounding variables has had little effect on the estimate of the risk of childhood cancer based on residential proximity to high-current power lines.

Occupational Exposures to ELF Fields. A number of professions involve occupational exposures to relatively high levels of power-frequency

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fields. These professions include various classes of electrical workers, operators of induction heaters, and motor operators in electrical railroad systems. In the first major study on cancer risk in relation to occupational exposure to ELF fields, Milham (*44*) reported in 1982 that electrical workers had an elevated risk of leukemia relative to the entire population of adult males in the State of Washington. During the following 12 years, more than 40 publications appeared on the subject of cancer risk among workers with electrical job titles (*31, 45*). Although these studies have been marked by considerable variability in the types of cancers and the relative risk factors for workers in different electrical occupations, the total body of evidence collected to date suggests that electrical workers have an elevated risk of leukemia and brain tumors (*31, 45–47*). Some limited evidence (*48–52*) also suggests that the incidence of breast cancer may be increased among electrical workers, although an elevated risk for this type of cancer was not observed in one of the largest studies on electrical workers conducted to date (*53*).

Many of the early studies on cancer risk among electrical workers used only job titles as a surrogate measure of exposure. However, several studies (*53–61*) demonstrated convincingly that many classes of electrical occupations involve exposures to time-weighted-average 50–60-Hz magnetic field levels that are significantly greater than those encountered in residences and other nonoccupational settings. Average exposures during the workday ranging from 0.5 to 2.5 μT have been measured with personal dosimeters for several classes of electrical workers. Some of the highest levels of exposure have been recorded for workers at electric power stations, live-line workers, electricians, cable splicers, and welders. For all of these classes of workers, mean exposure levels for 50–60-Hz magnetic fields in the range of 1–2.5 μT have been recorded in several studies.

Despite the extensive database on exposures of workers in electrical occupations and other jobs that involve the frequent use of electrical equipment (e.g., machinists and construction workers), the large number of epidemiological studies on cancer risk have not as yet provided a consistent picture. Within the same job categories studied in different epidemiological surveys, large variations have been observed in the relative incidence and mortality from specific types of hematopoietic and nervous system tumors. Attempts to correlate confounding variables such as exposure to potentially carcinogenic chemicals with cancer risk among electrical workers have not satisfactorily explained the apparent elevation in the risk of leukemia and brain tumors. As with residential exposures, the question has been raised of whether time-average measurements of fields obtained from recordings in the workplace or from personal exposure monitors worn by the workers are capturing the biologically relevant exposure parameters. For this reason, efforts are under way to characterize more precisely the high-intensity transients and intermittent exposures to high-amplitude fields experienced by electrical workers in various job categories.

In addition to work that directly involves electrical equipment and power transmission and distribution systems, numerous other occupations involve ex-

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posure to ELF fields. As demonstrated by Breysse et al. (*62*), the average power-frequency magnetic field level in a typical office environment is high relative to that commonly found in homes, and several devices such as photocopiers and microfilm readers produce local fields in excess of 1 μT. The range of ambient field levels observed by these investigators ranged from 0.1 to 0.65 μT, with a mean value of 0.32 μT. The growing use of visual display units (VDUs) in offices and homes is providing an increasingly common source of exposure. These devices produce fields at operator locations that range in frequency from 50–60 Hz to several tens of kilohertz, for which a more detailed description is given in a subsequent section of this chapter.

Several specialized occupations involve relatively high exposures to power-frequency magnetic fields. For example, in a Swedish study (*63*), induction heaters operating at 50 Hz were found to produce magnetic fields as high as 6 mT at operator-accessible locations. High field levels near induction heaters operating at 180 Hz and above were also observed in a Canadian study (*64*). The electrosteel production process commonly used in northern Europe also involves high exposures to workers; 50-Hz field levels near ladle furnaces can reach levels up to 8 mT (*63*). Large power-frequency fields have been measured in the proximity of specialized equipment such as welding machines and demagnetizers (*65, 66*), and at worker locations the field levels are typically in the range 0.1–1.0 mT.

A source of exposure for both workers and the general public that has been a subject of several studies during the past few years is electrified urban transit systems (*67–70*). The fundamental ELF operating frequencies used for these systems in the United States, Europe, and other locations worldwide are typically 16.67, 25, 50, or 60 Hz. However, significant harmonic fields extending through the ELF and VF ranges have been measured for seven different electrified rail systems. The strength of the ELF fields, which are the dominant source of exposure, reaches levels of 10 μT in some sections of the operator's compartment, and typical levels are 1–5 μT at locations where the operators are seated in the various types of trains.

ELF fields of a similar magnitude have been measured in the passenger compartments of various electrified rail systems; the highest values of both the ambient electric and magnetic fields over the frequency range of 5–45 Hz were observed for the Washington, DC, Metro and the New Jersey Amtrak trains. The maximum magnetic and electric fields measured in this frequency range were 60 μT and 700 V/m, respectively. Although this level of electric field is small compared to values found in transmission line corridors, it is an order of magnitude greater than fields at ground level under a typical urban 12-kV distribution line operated at 60 Hz.

Both for train operators and long-distance commuters, these levels of ELF fields are significant compared to the fields from nearly any other routine source of exposure. Because of the extensive period of time that operators of electric trains are exposed to high levels of ELF fields, several efforts are under way to provide a more detailed characterization of their occupational exposure envi-

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ronment. Studies have also been undertaken to assess the relative cancer risk among these workers. In a recent Swedish study (*52*) on the health records of electric railway workers during 1961–1979, the risk of leukemia and brain tumors was not found to be elevated relative to the general population, but an excess of breast cancers and endocrine tumors was observed.

Sub-Radio-Frequency VF and VLF Fields

Sources of human exposure to fields in the VF and VLF bands from 300 Hz to 30 kHz include various induction heating devices, electronic surveillance systems, radionavigation systems, and VDUs (*13, 71*). The highest levels of exposure to fields in this frequency range are generally experienced by operators of induction heating equipment, including electric furnaces, operating at frequencies below 10 kHz (*63, 64*). Security devices such as metal detectors typically have magnetic flux densities of less than 0.1 mT (*13*).

The most common human exposures to fields at sub-RF frequencies are from the widespread use of VDUs in both the home and workplace (*72*). As already discussed, these devices emit a broad spectrum of nonionizing fields associated with the formation of an image on the VDU screen using cathode ray tube (CRT) technology. Many of the newer devices use liquid-crystal and thin-film transistor technologies, with a significant reduction of operator exposures. However, a majority of the VDUs worldwide still use the CRT technology involving the application of electromagnetic fields for horizontal and vertical deflection of the electron beam that forms the image on a phosphor screen.

The electromagnetic emissions from conventional VDUs have been extensively characterized (*73–80*) at typical operator locations ranging from 30 to 60 cm in front of the screen. At a distance of 30 cm, the ranges of electric and magnetic field levels are the following: (1) ELF electric and magnetic fields from the vertical beam deflection system have values that are less than 65 V/m and 1.0 μT, respectively, with frequencies in the range of 50–80 Hz; (2) VLF and RF fields used for the horizontal beam deflection system have fundamental frequencies ranging from 15 to 50 kHz, with harmonics extending up to 200 kHz; the electric and magnetic field levels of these emissions are less than 50 V/m and 0.5 μT, respectively; and (3) static electric fields that are built up on the VDU screen have amplitudes that are usually less than 10 kV/m, although higher values can occur under conditions of low ambient relative humidity.

A variety of techniques can reduce the static electric charge on VDU screens and attenuate the ELF, VLF, and RF emissions to levels that are within existing guidelines (*72*). Higher frequency fields in the UV and X-ray bands do not penetrate the VDU casing and therefore do not pose a health risk.

RF Electromagnetic Fields

RF fields in the broad frequency range from 30 kHz to 300 GHz are widely used in industrial processes, communication systems, radar, and a variety of medical

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and domestic devices. The spectral region from 300 MHz to 300 GHz is commonly referred to as the microwave band. Human exposures to RF fields from communication systems, from industrial equipment, and from devices used in the home and in public places are described in this section. Medical applications of RF fields are described in a later section.

Communication Systems. RF fields in the environment are primarily produced by broadcasting systems (*81*). Although radio and television transmitters operate over frequencies ranging from about 10^5 to 10^9 Hz, the primary sources of public exposures are from AM radio (535–1610 kHz in the United States), FM radio (88–108 MHz), and television signals transmitted in the very high frequency (VHF: 30–300 MHz) and ultrahigh-frequency (UHF: 300–3000 MHz) bands. In several European countries the AM radio band is 150–175 kHz, and in the former Soviet Union it extends from 150 to 2000 kHz. There are also a number of specialized communication systems that emit RF fields, such as the Long-Range Navigation (LORAN) system along the coastlines of the United States, which transmits pulse trains on a 150–170-kHz carrier wave.

A survey of ambient RF field levels in 15 large cities in the United States showed (*82*) that the median exposure level to ambient RF fields was 50 $\mu W/m^2$. For the small fraction of the population living near broadcast towers (less than 1%), considerably higher exposures were recorded, and in some "hot spots" that were observed, the ambient RF power density exceeded 10 mW/m^2. In general, military and commercial radar systems, such as those used for aircraft navigation, contribute a negligible amount to the average public exposure to RF fields.

A source of exposure to RF fields that is becoming increasingly common involves the use of portable radiotelephones. These devices, which were first introduced on the commercial market in the mid-1980s, are generally referred to as cellular (or mobile) telephones. The present generation of these devices consists of analog radiotelephone systems, with fixed-base transmitters that operate at frequencies centered around 900 MHz. The base station transmitting antennas that are currently used consist of vertical arrays of dipole antennas mounted on platforms that are typically 25–50 m high. The radiotelephones are compact transceivers that contain either a monopole antenna or a sleeve-type dipole antenna mounted in a durable metal case.

Under the geometric conditions in which cellular telephones are used, namely, with the transceiver antenna at a distance of only a few centimeters from the head, exposures occur in the reactive near-field zone, and the absorption of RF power is highly anisotropic (*83, 84*). Accordingly, compliance with recommended limits of human exposure is generally determined by calculations of the specific energy absorption rate (SAR) in units of watts per kilogram of tissue within detailed anatomic models of the head. Measurements of the SAR in phantoms constructed from tissue-equivalent materials have also been used for this purpose. These techniques (*85–87*) demonstrated that a cellular telephone operating at 800–900 MHz and emitting 7 W of power could introduce energy

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absorption into the head that exceeds the current SAR limits established by the Institute of Electrical and Electronic Engineers (*88*) for low-power devices used in a "controlled environment" (e.g., an occupational setting) or an "uncontrolled environment" (e.g., a residence or public area). However, under normal conditions of operation involving less than 1 W of radiated power, the SAR limit would usually not be exceeded.

A new generation of digital radiotelephone systems with adaptive power control is in an advanced stage of development and will soon surpass the original analog devices on the worldwide market. The new systems will use a frequency band close to that of the current systems, with communications centered at approximately twice the frequency of the analog radiotelephone systems. These digital devices will emit low-power RF signals with ELF pulse modulation.

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Occupational Exposures. Potentially high occupational exposures to RF fields can occur during work at radio and television broadcast towers and in locations near radar antennas. However, the field intensity decreases rapidly as a function of distance from such facilities, and routine exposure of workers is generally less than 0.8 W/m^2 (*71, 89*).

Extensive use of devices involving induction heating or RF dielectric heating procedures is common in industries worldwide. Induction heating is generally used in arc welding and for processing metals and semiconductors, including forging, annealing, tempering, and brazing procedures. Fields used for these purposes range in frequency from tens of kilohertz to a few megahertz, and the field levels to which workers are exposed are generally much higher than those from other occupational electromagnetic field exposures (*13, 66, 90, 91*).

Dielectric heating involves the direct deposition of RF power into materials that are to be sealed or dried. The most common use of this procedure is in sealing plastic materials using machines operating with high RF power output at frequencies up to about 30 MHz (*92*). Although operator exposures can be large in the vicinity of these devices, a number of protective measures such as shielding can be implemented to substantially reduce the exposure level (*93*).

Two other common sources of occupational exposure to RF fields involve the use of portable communication equipment and police radar units. The RF emissions from mobile radiocommunication devices were described earlier. Police radar systems are low-power devices that operate in the 10–35-GHz frequency range, with an output power density at the aperture that is less than 3 mW/cm^2 (*94, 95*). At the high frequencies and low RF intensities used in police radar units, the extent of penetration of their RF emissions into the eye or other potentially sensitive tissues is very small.

Residential and Public Exposures. The most common residential application of RF fields is in cooking with microwave ovens. Power levels of 300–1000 W at a frequency of 2.45 GHz are used in various consumer models. The leakage of RF fields through the door seals and the viewing screen of a mi-

crowave oven is very small, and at typical user distances from the oven the power density is only a few microwatts per square centimeter (*96, 97*).

Other sources of human exposure to RF fields in the home and in public places include mobile radiotelephones (discussed earlier) and various types of RF anti-intrusion alarms. These alarms are low-power devices that emit RF fields with power densities on the order of 10 $\mu W/cm^2$ at locations where people could be exposed (*71*). Broadly speaking, typical exposures to ambient RF fields in the home or in public places are well within existing exposure guidelines for the general population (*88*).

Optical Radiation

Electromagnetic fields at frequencies above 3×10^{11} Hz are commonly referred to as optical radiation, and encompass the IR, visible, and UV regions of the spectrum (*98*). The most common man-made sources of optical radiation are lasers, electrical discharges in gases or vapors, and solid materials that are heated to a temperature at which they emit photons (incandescence). Various types of lasers emit monochromatic photons at high intensities and at specific frequencies across much of the optical radiation spectral region. The major types of lasers are the semiconductor lasers (laser diodes), gas and vapor lasers, and liquid dye lasers. The range of applications of these devices in industry and medicine is extremely large and includes holography, material processing, optical radar, optical fiber communication, surgical and dermatological applications, and photocoagulation (*99*). Various guidelines for the protection of humans from visual or other forms of damage from laser light have been formulated (*100–102*).

In the IR spectral region, the major concerns for human health relate to surface heating and burns. Strong absorption of IR radiation by water and other constituents of superficial body tissues is the primary source of damage. In the visible and UV spectral regions, damage to living tissues can result from both thermal and photochemical mechanisms (*98*). The latter process varies strongly with wavelength, and depends upon the absorption properties of specific molecular components of tissue. Of particular concern are visual and dermal interactions of UV and visible radiation from the sun (*103*). Although the UV-B (wavelength 280–315 nm) and UV-C (wavelength 100–280 nm) spectral bands account for only about 2% of the total solar irradiance at the Earth's surface (*104*), exposure to these wavelengths can result in severe corneal damage and skin erythema unless adequate protective measures are taken.

Medical Applications

Both static magnetic fields and electromagnetic fields are extensively used in medical procedures. Several of the major uses of these fields include fastening dentures and other prosthetic devices in the head region, the facilitation of bone fracture reunion, blood flow measurements, electrostimulation, RF hyperthermia procedures, and magnetic resonance imaging.

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Small permanent magnets with surface fields as high as 0.5 T have been used in Japan and other countries to fix dentures in place (*105, 106*), and to secure various prosthetic devices within the head and neck regions (*107*). Although the local magnetic fields decrease rapidly with distance from the surface of the magnet, small tissue regions are exposed continuously to static fields in excess of 0.1 T (*13*).

For more than two decades, pulsed and sinusoidal magnetic fields (*108, 109*) and low-frequency electric fields (*110, 111*) have been used extensively in the treatment of nonrejoining bone fractures and arthroses. By using a pair of coils placed around the limb containing a bone fracture, both ELF sinusoidal magnetic fields and pulsed magnetic fields with repetition frequencies from 1.5 to 77 Hz have been used to inductively couple electric fields and currents into the region of the fracture. Some of the complex waveforms used for treating bone fractures have a significant energy content at frequencies of several kilohertz. Both pulsed and sinusoidal magnetic field waveforms are effective in promoting bone cell proliferation and fracture repair (*112*). A capacitatively coupled 60-kHz electric field signal also is effective (*110*). A range of signal strengths has been used to treat bone fractures; the majority of procedures induce electric fields on the order of 0.1–1.0 V/m in the fracture region (*113*). However, induced electric fields as low as 10^{-4} V/m were reported (*112*) to facilitate the repair of recalcitrant bone fractures.

A widespread use of ELF magnetic fields in medicine involves the measurement of blood flow rates with electromagnetic flowmeters during prolonged surgical procedures. In this technique, which involves the detection of a magnetically induced voltage that is proportional to blood flow rate, a small cuff that contains both the magnet coil and the pickup electrodes is placed around the blood vessel perimeter. With this method, blood flow rates in major vessels such as the portal vein and coronary artery and in large regions of an organ such as the pancreas can be measured with a precision of 1–2% for prolonged periods (*113*).

A variety of devices are used clinically to treat tumors by the technique of RF-induced hyperthermia (*114–116*). These devices apply the RF field either external to the body for heating superficial tumors or inside the body for interstitial hyperthermia. Although large RF fields are applied locally to the patient, the field levels at the location of medical personnel are generally quite small unless there is excessive leakage of RF fields from the applicator, the generator, or the connecting cables (*117*).

The most widely used medical procedure that involves exposure of both patients and medical personnel to magnetic and electromagnetic fields is the magnetic resonance imaging (MRI) technique (*118, 119*). This diagnostic imaging procedure involves the combined use of (1) static magnetic fields with flux densities up to 2 T for alignment of magnetic nuclei such as protons, (2) RF fields with frequencies up to 100 MHz to selectively excite resonant transitions in these nuclei, and (3) magnetic field gradients on the order of 1–2 mT/m to

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define the tissue location of the magnetic resonance signals (*120*). The gradient fields are switched systematically across the tissue volume of interest at frequencies in the ELF range in order to generate the complete magnetic resonance image. The waveforms of the rapidly switched gradient fields are complex and contain spectral components that extend from the ELF region to tens of kilohertz.

Although the patient undergoing an MRI procedure is subjected to a variety of magnetic and electromagnetic fields at relatively high intensities, surveys of adjacent areas near MRI units where medical personnel are located have shown the exposure levels for these workers to be low. For example, recent measurements on a 1.5-T MRI system showed the static magnetic field to be 1.5 mT and the amplitude of the time-varying gradient magnetic fields to be 0.1 μT at the operator's console (*121*).

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Concluding Remarks

This review demonstrates that a great deal of research has been conducted to characterize the complex electromagnetic exposures to which humans are continuously subjected in the workplace, in homes, and in public locations. Relatively high levels of electric, magnetic, and electromagnetic fields are frequently involved in electric power generation and transmission, in a large number of industrial processes, and in several commonly used medical procedures. However, with the possible exception of certain types of exposure to power-frequency fields and to high-intensity RF and optical radiation, human exposures to electromagnetic fields generally fall within the guidelines that have been established by a number of national and international organizations on the basis of well understood health risks.

One of the challenges currently facing the scientific community is to gain a better understanding of the possible health implications of occupational and residential exposures to relatively low levels of power-frequency fields. Another major challenge is to develop cost-effective methods for mitigating human exposures to electromagnetic fields, both at power frequencies and at higher frequencies in the RF and optical regions of the spectrum. Considerable progress toward this goal has been made in recent years, but further advances are technically possible and can be achieved with continuing research and development efforts over the coming decade.

Acknowledgments

Research support is received from the U.S. Department of Energy under Contract DE–AC06–76RLO 1830 with the Pacific Northwest Laboratory. The Pacific Northwest Laboratory is operated for the U.S. Department of Energy by the Battelle Memorial Institute.

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RECEIVED for review January 30, 1995. ACCEPTED revised manuscript May 3, 1995.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch002

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch003

3

Typical Electric and Magnetic Field Exposures at Power-Line Frequencies and Their Coupling to Biological Systems

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In this chapter we review five major sources of electric and magnetic fields. We then present some typical exposures as measured with personal dosimeters. The major sources of electric and magnetic fields to be discussed include power lines, electric railways, appliances, ground currents, and naturally generated fields, both by the body and the Earth's atmosphere. We also review the coupling of these fields to the body in order to estimate how the externally generated fields compare with those that are generated by the body itself.

PART OF THE PROBLEM OF DETERMINING if the fields generated by the distribution of AC (alternating current) power constitute a safety hazard requires an understanding of what the sources of the fields are and how they are coupled to the body. A knowledge of the sources aids in estimating the exposure that humans receive under a variety of conditions near the sources.

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Naturally Generated Fields

The electromagnetic power radiated from natural sources as a function of frequency is shown in Figure 1. These fields can be thought of as being generated in a spherical capacitor between the surface of the Earth and the ionosphere, which begins at about 50 km above the surface. This capacitor is charged by about 100 lightning strokes per second from thunderstorms worldwide. At DC (direct current) the average value of the electric field at the surface of the Earth is about 130 V/m, and it can range up to 3000 V/m near an active thunderstorm (*1*). These fields are not uniform, and local values can vary widely with temperature and humidity. The polarity of the fields can reverse in minutes. Thunderstorms as far as 50 km away can affect the local field values. The magnitude of the field generated by the atmosphere decreases rapidly as the frequency in-

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch003

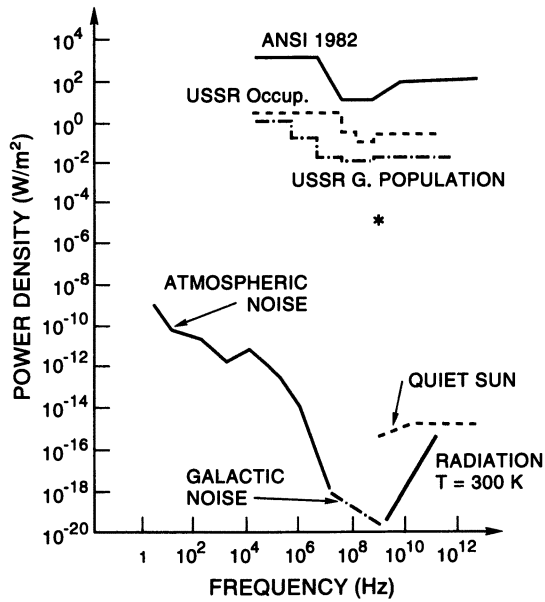

Figure 1. Power emitted from natural sources as a function of frequency and standard exposure limits. The average atmospheric noise power density incident on the Earth (reproduced with permission from reference 27. Copyright 1982 Institute of Electrical and Electronics Engineeres, Inc.) is compared with the 1982 American National Standards Institute (ANSI) and USSR exposure standards. USSR Occup refers to the allowed occupational exposure levels; USSR G Population refers to the general population exposure levels. The average signal level for 15 U.S. cities is designated by the asterisk at 10^{-5} W/m^2 power density and 10^9 Hz frequency. (Reproduced with permission from reference 28. Copyright 1989 Williams and Wilkins.)

creases. At 60 Hz the average value of the electric field is reduced to about 10^{-3} V/m.

The corresponding average magnetic fields are about 5×10^{-5} T at DC, and $(5 \times 10^{-12}\ \text{T})/(\Delta f)^{1/2}$ at 60 Hz, where Δf is the bandwidth. These fields are generated by the Earth's rotation around a liquid core, by the currents flowing in the ionosphere, and (particularly above about 6 Hz) by lightning activity. The most spectacular manifestation of the ionospheric currents is the aurora borealis. Both these naturally generated fields at 60 Hz are small compared with the fields that are measured in a typical home or workplace.

A second major source of naturally generated fields is the body itself. The body uses electrochemical signals to control the movement of muscles and to transmit information from one part of the body to another. Typical electric signals for the heart, nerves, and muscles are shown in Figure 2 (*2*).

The signals shown in Figure 2 also serve as a source of electric and magnetic fields that propagate through the rest of the body. Two of the most useful applications of these fields are the electrocardiogram (ECG) and the electroen-

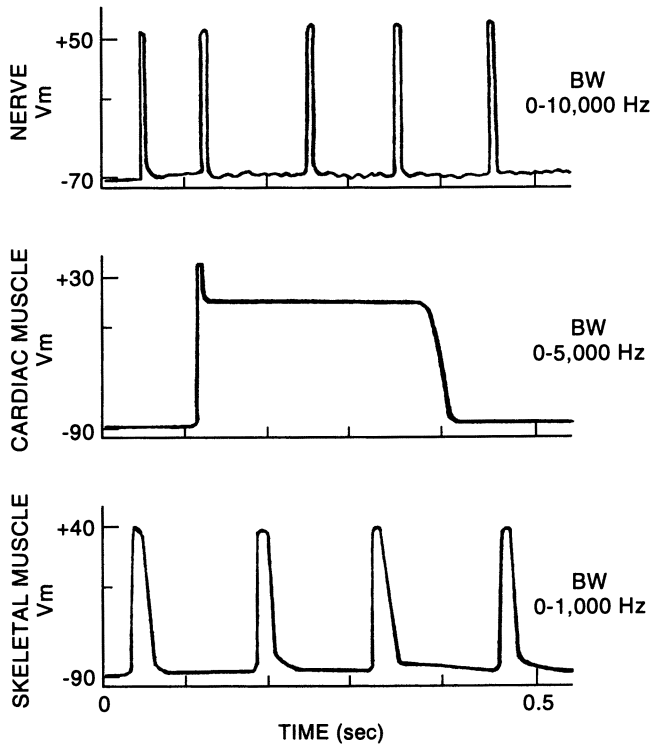

Figure 2. Transmembrane potentials (V_m) *in nerve and muscle. BW is bandwidth used for measuring the signal. (Reproduced with permission from reference 2. Copyright 1992.)*

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch003

cephalogram (EEG). The ECG is generated by the heart. Typical ECG signals have a peak value of about 1 mV with repetition rates from 45 to 150 beats per minute. The EEG signals are generated by the activity of brain cells. Typical EEG signals are about 30 μV between electrodes spaced a few centimeters apart with peak signals up to 50 μV at alpha rhythms around 10 Hz. Another source of electric signals is the stomach, which generates signals with frequency components of 2–10 cycles per minute. All of these signals are typically chaotic in nature, and this condition means that the beat-to-beat interval demonstrates substantial variations in time. The intervals between the beats change with the needs of the body and with changes in activity. Additionally, adaptive processes in the body lead to changes in the response of the same nerve fibers over time for repetitive activity (*3*). This variability is in contrast to power-line signals, which are very stable at either 50 or 60 Hz.

We will compare these naturally occurring signals with externally generated signals in order to estimate the likelihood that the external fields will have significant biological effect.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch003

Externally Generated Fields

Power Lines. The power lines that distribute most of our electrical energy are one of the most obvious sources of externally generated electric and magnetic fields. This distribution system usually consists of transmission lines and distribution lines. The transmission lines are the high-voltage lines that are used to transport power over long distances. They operate at voltages in the range from 100 to 700 kV. The currents in these lines range from 100 to 1000 A and typically average from 300 to 400 A. A variety of configurations are used for transmission lines. Among the most common are three-wire and six-wire configurations. A typical electric field distribution for a transmission line is shown in Figure 3 (*4*). In Figure 3, the electric field (*E*) is measured at ground

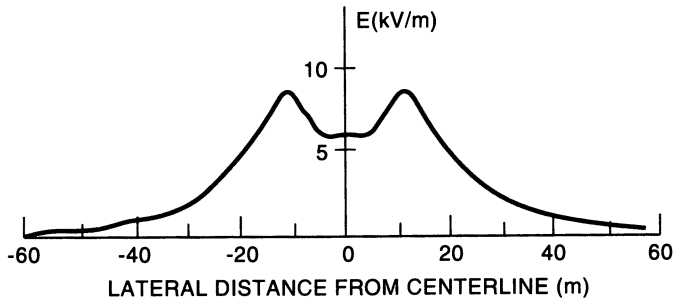

Figure 3. Electric fields (E) *as a function of distance from 525-kV transmission at ground level. (Reproduced with permission from reference 29. Copyright 1987 Williams and Wilkins.)*

Table I. Maximum Electric Field Intensities at Midspan Under Various Configurations and Voltages of Electric Power Transmission Lines

Highest System Voltage (kV)	*Electric Field Strength Under Line at Midspan (kV/m)*
123	1–2
245	2–3
420	5–6
800	10–12
1200	15–17

SOURCE: Reproduced with permission from reference 33. Copyright 1982.

level under a 525-kV transmission line. The three electric phases (i. e., conductors) of the line were located in a horizontal configuration 10 m above the ground and with a phase-to-phase spacing of 10 m. The drawing gives electric field data as a function of lateral distance measured from the centerline of the transmission line. The maximum electric fields at midspan for a number of line configurations are given in Table I (*4*). In most areas the maximum electric fields are limited to <10 kV/m. In Montana the limit is 1 kV/m at the edge of a right-of-way.

The distribution for magnetic fields from transmission lines as a function of distance is shown in Figure 4 (*4*). Again, the magnetic fields from these lines

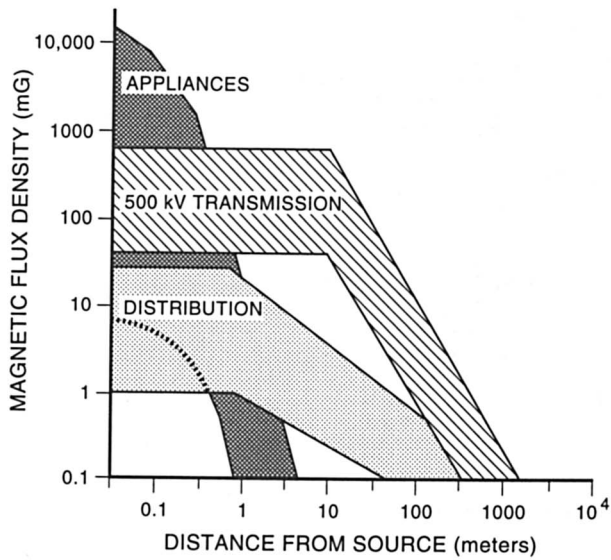

Figure 4. Magnetic flux intensity as a function of distance from various sources. (Reproduced from reference 30.)

vary with the current, the geometry, and the phasing of the wires. A number of computer programs make it relatively straightforward to calculate these fields (*5*). Zaffanella and Kaune (*6, 7*) analyzed a number of power-line configurations. The fields can be significantly reduced by using a split-phase, six-wire configuration over a three-wire system (*6, 7*). We showed (*8*) that the fields may be reduced even further by going to a multiwire coaxial configuration and, when the sum of the currents on all the wires is zero, both the electric and magnetic fields will be zero outside the cable. In general, the more symmetrical the wire geometry and the current distribution, the lower the fields will be (*8*). The fields from transmission lines affect a relatively small number of people because most lines travel through sparsely populated regions and the right-of-ways are relatively wide.

Distribution lines are much more pervasive. They are the lines running from the substations to feed the transformers that supply power to homes and businesses. In Colorado the primary lines in this system employ 13.5 kV. The secondary lines employ 240 V phase to phase and 120 V to ground. The peak electric fields are typically in the vicinity of 100 V/m, as shown in Figure 5. The electric field contribution from the distribution lines to the fields in a house is typically on the order of 5 V/m and is usually smaller than those generated by sources in the house.

The range of magnetic fields for distribution lines as a function of distance is shown in Figure 4. The contribution of these fields to the fields measured in a home or a business is quite variable. It depends not only on the distance from the

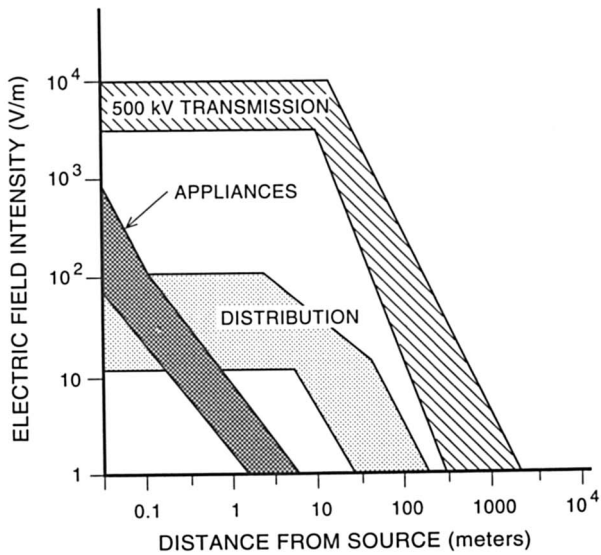

Figure 5. Electric fields at 60 Hz as a function of distance from a number of sources. (Reproduced from reference 30.)

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wires and the spacing between the wires but also on how much load is downstream. Wires at the end of the line carry less current than those closer to the substations. The fields from underground wires decrease more rapidly than those from overhead lines because the underground wires are usually closer together.

Electric Railways. Electric railways may provide a second source of relatively large externally generated magnetic fields to which a substantial number of people may be exposed for extended periods of time. The fields in these vehicles are generated by several sources. The first source is the feed system, which may come either from an overhead catenary or a third rail that feeds power to the locomotive or the drive motors in the coaches. The catenary current is returned through the rails and thus forms a loop enclosing the interior of the train. These fields are largely horizontal and relatively uniform with height. In the three-rail system, all three rails are at approximately the same height, and the fields tend to be higher near the floor than near the ceiling. The fields generated by the currents in these feed systems vary with the load on the locomotion system and are typically highest during start-up and at high speed or during a hill climb. A typical field pattern for a catenary feed system on an Amtrak train is shown in Figure 6 (*9*). Drive motors, if located under the floor of the coaches, are the second major source of fields in these vehicles. The third source of fields in the coaches comes from the lighting and related passenger services.

The frequency of the power feeds for the drive system varies from 16 to 60 Hz, and significant harmonic content has been measured to 2560 Hz. Much of the harmonic content is generated by semiconductor choppers that are used to control the power delivered to the traction motors. The power spectral distributions for a number of train systems are shown in Figure 7 (*9*). A comparison of the peak and average values measured in the passenger compartments of the

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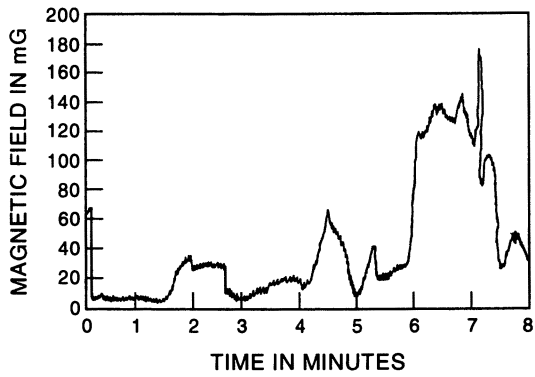

Figure 6. Typical example of the variability of magnetic fields caused by catenary current over time in an Amtrak train. (Reproduced from reference 9.)

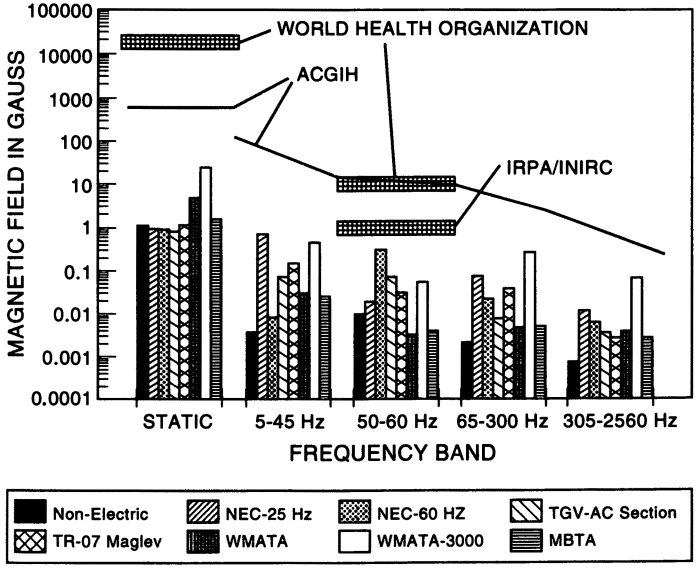

Figure 7. Electrified rail maximum magnetic field levels within low-frequency sub-bands compared to minimum threshold limit values of known guidelines. Maglev is magnetic levitation; ACGIH is American Conference of Governmental Industrial Hygienists; IRPA/INIRC is International Non-Ionizing Radiation Committee of the International Radiation Protection Association; TR-07 Maglev is German Transrapid TR-07; NEC is Amtrak Northeast Corridor (Washington, DC–Boston, MA); NJT is New Jersey Transit, New Jersey Coast Line (Long Branch) section; TGV is French Train a Grande Vitesse, Paris–Tours, France; WMATA is Washington, DC Metrorail, Gaithersburg, MD–Washington, DC; WMATA 3000 is Washington, DC Metrorail 3000 series cars; MBTA is Boston, MA Metropolitan Area Transit System. (Reproduced from reference 9.)

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trains is shown in Figure 8 (*9*). Somewhat surprisingly, the fields for a magnetic levitation (maglev) train are not substantially larger than those of other electrically driven trains. They do, however, generate rather complicated time-varying fields.

Ground Currents. An important source of magnetic fields in many homes is generated by the currents flowing in the plumbing. The electric feed system to most homes in North America is single phase at 240 V with a center tap to ground. If the current loads on the two halves of the transformer to ground are different, then the difference current will return to the power system through the ground. If there is a good contact and a low resistance between the house and power pole via the ground wire, then the difference current will return to the power system through the ground wire. If, however, this path has a higher resis-

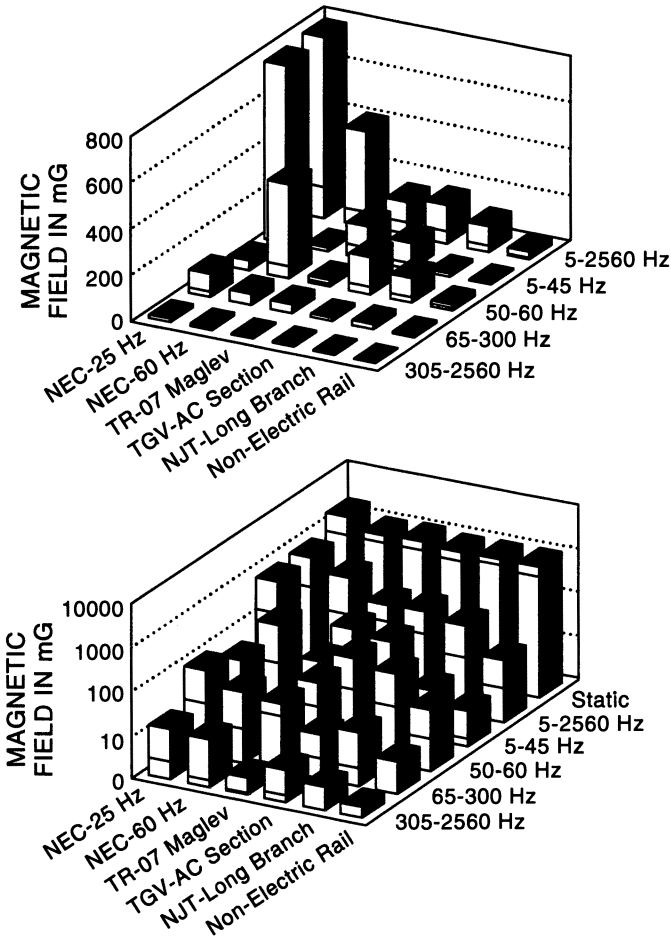

Figure 8. Maximum (bar top) and average (horizontal line) magnetic fields in passenger compartments on inter-city electrified systems as a function of low frequency sub-bands. Top: time-varying magnetic fields plotted on a linear scale. Bottom: time-varying and static magnetic fields plotted on a log scale. (Reproduced from reference 9.)

tance than the path through the plumbing to the water main down the street and back through a neighbor's ground wire, then much of this current will take the path of least resistance. Currents of a few amperes in the plumbing are fairly common. The magnetic fields from these currents decay as $1/r$ rather than as the multiwire system value $1/r^n$, where r is the distance from the current to the point of interest and n is related to the number of wires and their phasing. In the two-wire case, at distances much larger than the spacing between the wires, n is equal to 3. Thus, fields from plumbing may be the dominant source of magnetic fields

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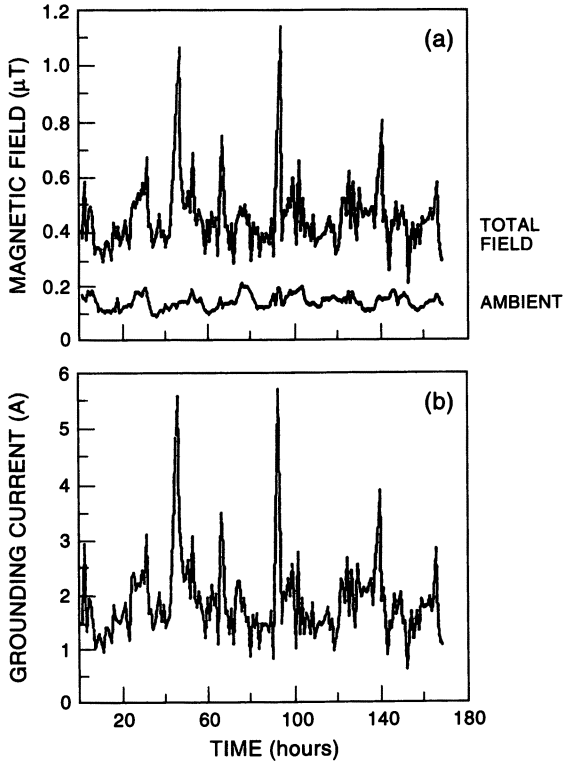

Figure 9. Hourly averages of data collected in house over a 1-week period. a: Total magnetic field and ambient field. b: Grounding current measured on the water-service pipe. (The data points are connected by computer graphics lines, which may show jobs between the points.) (Reproduced with permission from reference 10. Copyright 1990.)

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in a house, as the fields from the wire pairs carrying the current to lights and appliances fall off rapidly with distance. An example of a case in which the ground current in the plumbing was the dominant source of the magnetic field is shown in Figure 9 (*10*). The rapid variation of the ground currents with changes in the load current leads to the rapid fluctuations of the field with time.

Appliances. Typical values for the electric fields generated from household appliances are given in Table II (*4*). The fields from most appliances decrease rapidly with distance. People usually work close to them for only short periods of time. Exceptions to this situation are electric blankets and computer monitors. Typical magnetic field values for appliances are shown in Table III (*4*). Again, most of these fields decrease rapidly with distance, and exposures

Table II. Typical 60-Hz Electric Field Levels at 30 cm from 115-V Home Appliances

Appliance	*Electric Field Level (V/m)*
Electric blanket	250
Broiler	130
Stereo	90
Refrigerator	60
Electric iron	60
Hand mixer	50
Toaster	40
Hair dryer	40
Color television	30
Coffee pot	30
Vacuum cleaner	16
Incandescent bulb	2

SOURCE: Reproduced with permission from reference 34. Copyright 1992.

Table III. Magnetic Flux Densities (mT) at 60 Hz Near Various Appliances

Appliance	*z = 3 cm*	*z = 30 cm*	*z = 1 m*
Electric ranges (>10 kW)	6–200	0.35–4	0.01–0.1
Electric ovens	1–50	0.15–0.5	0.01–0.04
Microwave ovens	75–200	4–8	0.25–0.06
Garbage disposals	80–250	1–2	0.03–0.1
Coffee makers	1.8–25	0.08–0.15	<0.01
Can openers	1000–2000	3.5–30	0.07–1
Vacuum cleaners	200–800	2–20	0.13–2
Hair dryers	6–2000	<0.01–7	<0.01–0.3
Electric shavers	15–1500	0.08–9	<0.01–0.3
Television	2.5–50	0.04–2	<0.01–0.15
Fluorescent fixtures	15–200	0.02–4	0.01–0.3
Saber and circular saws	250–1000	1–25	0.01–1

NOTE: Values are ranges for three to five models, measured at distance *z* from appliance.
SOURCE: Reproduced with permission from reference 35. Copyright 1984.

Table IV. Electric Field Levels at 60 Hz at Center of Various Rooms in a Typical U.S. Home

Location	*Electric Field Level (V/m)*
Laundry Room	0.80
Dining Room	0.90
Bathroom	1.2–1.5
Kitchen	2.60
Bedroom	2.4–7.8
Living room	3.30
Hallway	13.00

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occur over relatively short periods of time. The major exceptions are, once again, electric blankets, computer monitors, and, occasionally, electric motors.

With computer monitors, the fields are asymmetrical and decrease rapidly with distance. For example, on one monitor, fields as high as 100 mG may be measured at the top surface of the case, but at 6 inches from the front of the screen the fields were only 1 mG. Electric motors, such as those that drive small fans in computer printers or duplicating machines, may generate fields as high as 100 mG at the surface of the machine that decrease rapidly with distance to values like 10 mG at 6 inches from the machine. The relatively high magnetic field exposures from a hair dryer or an electric razor are orders of magnitude higher than the average field exposures. Typical exposure times are short, however. The significance of these exposures, if any, is yet to be demonstrated.

Typical Exposures

The typical exposure received by a human during the course of a single day fluctuates over several orders of magnitude. Average values of the 60-Hz electric fields in various rooms of typical U.S. homes are given in Table IV. These values are somewhat smaller than those we measured in Denver, Colorado, which averaged about 7 V/m and ranged as high as 200 V/m (*11*). We found the electric fields to vary rapidly with position in a typical room. This variation occurs because objects like metal floor lamps or metal chair frames short-circuit the electric fields. Additionally, the body is a relatively good conductor; thus, as one moves around, one changes the electric fields in the vicinity.

The average magnetic fields as measured in a number of epidemiological studies are shown in Figure 10 (*12*). Typical values for fields to which an individual might be exposed during the course of a day are given in Figure 11 (*12*). Again, the fields to which one is typically exposed during the course of a day

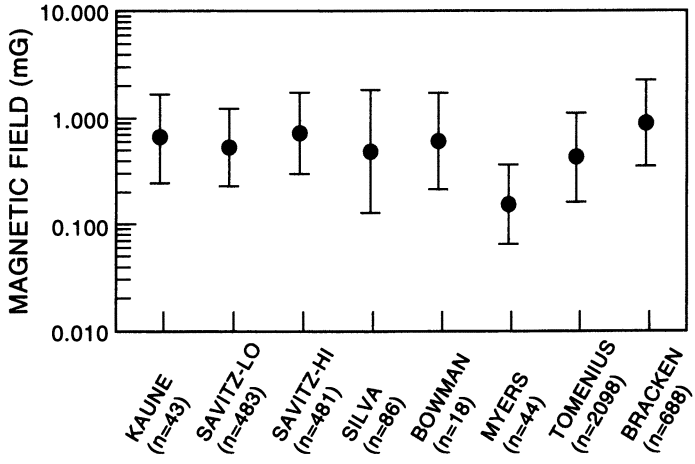

Figure 10. Residential magnetic field measurements with geometric standard deviations. (Reproduced with permission from reference 12. Copyright 1992.)

vary greatly, and peak fields may be >100 times the average fields. With the variation in these fields and a lack of knowledge with respect to the mechanisms by which the fields affect biological systems, it is difficult to know how the fields should be characterized. To date, most of the fields have been characterized in terms of the average of the root mean square (rms). However, some evidence (*13*) suggests that the horizontal component is more important than the vertical component. Additionally, the stability of the field or its coherence could be important in allowing the biological system to separate an external signal from a naturally generated chaotic signal.

Coupling of the Fields to Biological Systems

The coupling of electric and magnetic fields to structures in the body is quite different. By comparison to air, the body is a relatively good conductor, and thus only a small fraction of the electric field penetrates more than a few millimeters below the skin. Matching the boundary conditions for the perpendicular component of the electric field (E) at 60 Hz for a plane surface gives

$$E_{\text{internal}} \approx (\sigma/j\omega\varepsilon)E_{\text{external}} \approx 4 \times 10^{-8} E_{\text{external}}$$

where σ is the conductivity, ω is the angular frequency, ε is the dielectric con-

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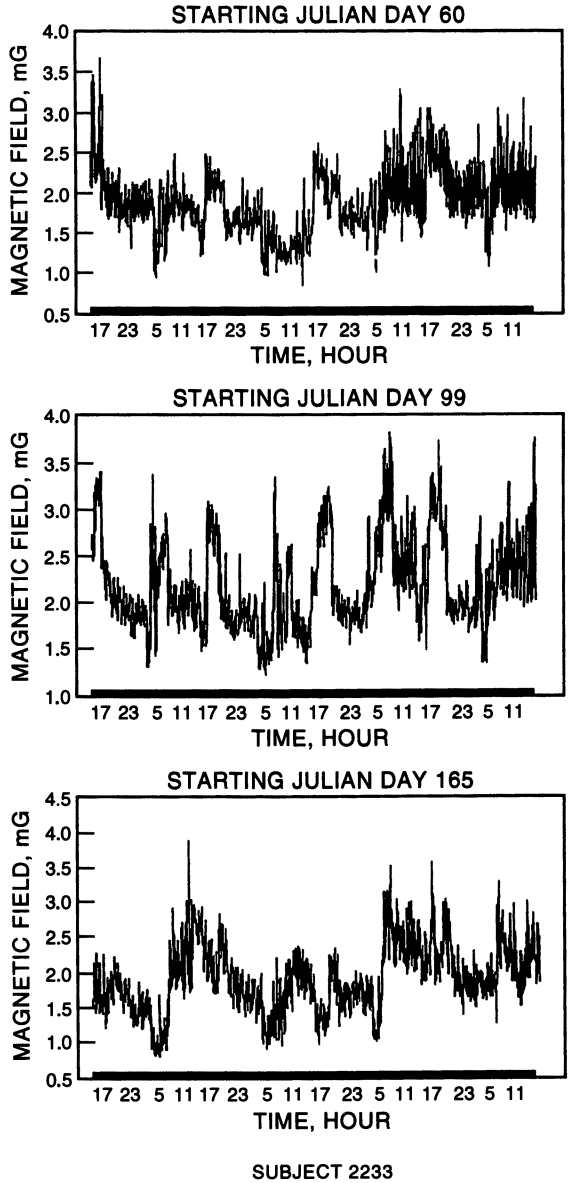

Figure 11. Measured magnetic field exposure by typical individual over the course of the day. (Reproduced with permission from reference 31. Copyright 1992.)

stant for tissue, and $j = \sqrt{-1}$. For an external field of 200 V/m at 60 Hz, this equation gives an internal electric field of about 8×10^{-6} V/m. A much more complete job of modeling the effects of externally applied fields was carried out by Kaune and Phillips (*14*). The resulting induced current densities, *J*, as calculated from a model study, are shown in Figure 12. This figure shows axial current densities and surface rms electric-field intensities for a grounded human, pig, and rat exposed to a vertical 60-Hz, 10-kV/m electric field. Relative body sizes are not to scale. All values of the current density are in units of nanoamperes per square centimeter. The internal current densities were calculated along an axis parallel to the long axis of the body and averaged over the cross-sectional areas shown as dashed lines. The calculated current densities shown at the surfaces of the human and the pig are perpendicular to the body surface.

Some evidence indicates at least two important ways by which electric and magnetic fields can affect biological systems. The first is through the currents that are induced in the body. The second is through a mechanism that involves both the AC and DC magnetic fields. Induced currents at levels of $\geq 10^{-2}$ A/m^2 are biologically active in such functions as bone growth (*15*) and Ca^{2+} transport across membranes (*16*). With an applied field of 200 V/m, the peak induced current would be expected to be about 40 nA/cm^2 or 4×10^{-4} A/m^2. This value is less than current densities that have been shown to be important in experiments on bone growth and the injection locking of pacemaker cells. However, the modeling that has been done thus far does not take into account the full complexities of the variations in the resistivity between fat, muscle, bone, and blood vessels. If the blood in the vessels is assumed to have a low resistance, then the vessels can work like antennae to concentrate potential differences into a small region of space. At 10 V/m the total voltage drop across a person 2 m tall

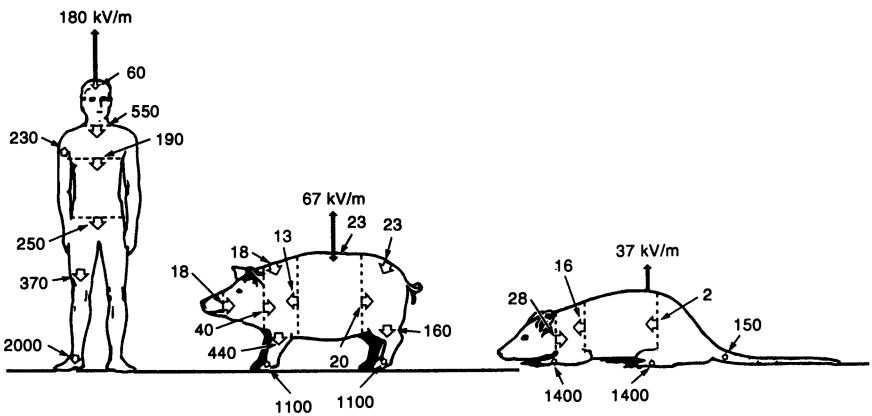

Figure 12. Induced current densities for a vertical 60-Hz 10-kV/m electric field. (Reproduced with permission from reference 29. Copyright 1987 Williams and Wilkins.)

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch003

is about 2 V. If as much as a few millivolts is concentrated across a cell membrane, then the biological effects could be significant. Voltages of a few millivolts can change the firing rate of pacemaker cells. Sharks can detect electric fields in the range of 5×10^{-7} V/m using long channels that concentrate the fields across a relatively small number of membranes (*17*).

Faraday's law for induction enables the calculation of the induced currents generated by a time-varying magnetic field. This calculation has been done for simple ellipsoidal models in a uniform field and for homogeneous materials, as illustrated in Figure 13. Again, induced current densities for fields of 1 μT calculated on the basis of average conductivity are 2 or 3 orders of magnitude smaller than are known to be important. However, the calculations have not yet been attempted for complex models, in which the variations in the resistivity between fat, muscle, bone, and blood vessels are included along with their complex geometry. For an extreme case in which the blood vessels are short circuits and concentrate all the induced voltage across a single piece of tissue or membrane, as much as 2 mV might be generated across the membrane from a time-varying magnetic field of 1 μT at 60 Hz, which is just large enough to be significant for exposures on the order of 1 s in changing the firing rate of pacemaker cells.

With respect to the second mechanism involving both the AC and DC magnetic fields, the evidence is much less definitive. Small magnetite particles have been discovered in leukocytes and other human tissue (*18*). These particles in a time-varying magnetic field could apply torque directly to membranes, affecting the opening and closing of channels, and, thus, the behavior of cells. The

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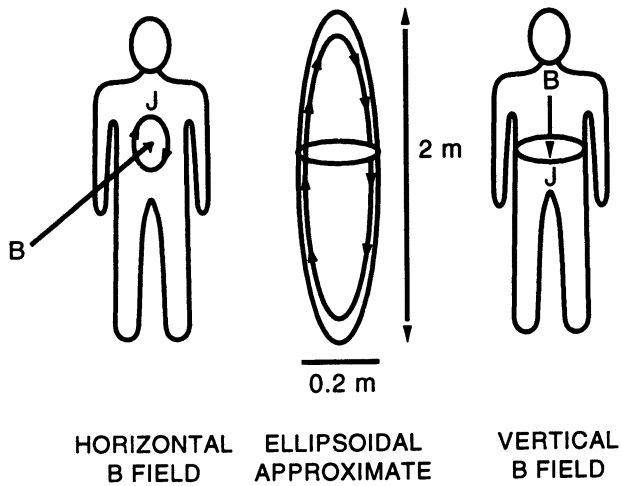

Figure 13. Induced current directions in ellipsoidal proximation to a human. J *is current density. (Reproduced with permission from reference 32. Copyright 1990 Institute of Electrical and Electronics Engineers, Inc.)*

force (F) on a magnetic particle with a dipole moment m in a magnetic field gradient (∇B) is given by

$$F = m \cdot \nabla B$$

The saturation magnetization for these particles, which are 20–30 nm on a side, leads to magnetic moments that range from 10^{-15} to 5×10^{-13} A·m^2. If these particles are arranged into chains in which the moments of each crystal are strongly coupled to each other, then the torque (T) on the resulting magnetic dipole is given by

$$T = |m||B| \sin\phi$$

where ϕ is the angle between the dipole and the applied flux density, B. Also, some evidence suggests (*19–26*) that these fields at the cyclotron or Larmor frequencies of important ions can be biologically important, if these signals are applied in a coherent manner for periods of time on the order of minutes or longer.

Thus, the future is likely to show that we will need to characterize both the AC and DC magnetic fields, the angle between them, and the length of exposure in order to be able to assess the importance of the exposure to magnetic fields for a given biological system.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch003

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RECEIVED for review March 8, 1994. ACCEPTED revised manuscript February 2, 1995.

4

Bioelectromagnetic Dosimetry

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Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch004

This chapter describes bioelectromagnetic dosimetry, which deals with the definition and measurement of the physical quantities that must be specified in evaluating interaction of electric and magnetic fields with living organisms. The essential aspects of bioelectromagnetic dosimetry are discussed in some detail for extremely low frequencies and are summarized briefly for radio-frequency fields, including microwaves. Basic equations are cited to show the importance of relative orientation of field vectors and object surfaces, object and source size, object-to-source distance, and frequency or pulse shape and duration. The role of environmental static fields is discussed. Some reference to recent research is made.

FOR THE DOSIMETRY OF DRUGS it is generally sufficient to clearly characterize the agent by giving its chemical composition and then to specify the quantity administered and the manner of administration (e.g., in solid form as a pill, dissolved in liquid, or intravenously). Dosimetry of electric and magnetic fields is in many ways more complex because it requires specification of many more parameters. First, at low frequencies (as opposed to microwave frequencies), electric and magnetic fields can to a high degree of approximation be applied separately, and therefore specifying whether the applied field is electric or magnetic is essential. Second, because electric and magnetic fields are vectors, it is essential to specify not only magnitudes but also field directions with respect to directions characteristic for the culture vessel (e.g., axial or transverse) or the

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exposed animal (e.g., dorsal–ventral, head–tail, etc.). For electric fields the shape of the exposed object greatly affects induced currents, and for magnetic fields, cross-sectional dimensions of the culture vessel or animal also must be specified. In addition, for both electric and magnetic fields one must know whether the exposed object was electrically insulated or electrically grounded.

The wave shape or frequency content of the applied signal appears to influence the observed biological effects significantly. If the signal is purely sinusoidal and is applied continuously, only its frequency and the duration of exposure need to be specified. However, if pulsed fields are employed, then completely specifying the rise time, decay time, duration of individual pulses, and pulse repetition rate is essential. Furthermore, one must know whether the given pulse characteristics are those of the applied magnetic field or were measured with a pick-up coil that detects an induced electric field. In general, wave shapes of the applied magnetic and induced electric pulses are radically different.

Finally, a description of electromagnetic field exposure necessarily includes measured values of continuous or intermittent background fields. These background fields are

- the usually but not always insignificantly small power-frequency electric fields present in home and laboratory environments
- power-frequency magnetic fields, which frequently can be of significant magnitude inside incubators or near devices such as centrifuges or shakers that contain electric motors
- the geoelectric field, which is essentially a static field (about 100 V/m in fair weather)
- the "static" geomagnetic field

Several experiments have shown (*1–3*) that some biological effects depend upon synergism between static and time-varying magnetic fields; both magnitudes and relative directions are apparently important. In a steel-frame building or inside a laboratory incubator, the "static" magnetic field may differ substantially in both magnitude and direction from the field in free space and may also vary from point to point. Specifying the characteristics of the background field at the exposure location is therefore essential, at least when biological effects of weak (gauss or milligauss) magnetic fields are investigated. Electric fields, applied or existing in air, are greatly reduced upon penetration into any electrically conducting medium, such as cell culture medium or the animal body; they are also reduced by high-loss dielectrics such as building walls.

The preceding discussion applies primarily to low-frequency fields, including those at power and audio frequencies, and to pulsed signals whose essential frequency content (discussed in the next section) is restricted to less than about 100 kHz. Different considerations apply to radio-frequency and microwave fields, which will be discussed only very briefly at the end of the chapter.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch004

Frequency, Pulse Shape, and Repetition Rate

Purely sinusoidal signals are generally encountered only in carefully controlled laboratory environments. Signals from commercial power lines and distribution systems may contain harmonics (i.e., integral multiples of the fundamental power frequency of significant magnitude up to the fifth) and transient spikes of milli- or microsecond duration. Instruments have been developed to measure these characteristics of the man-made electromagnetic environment (*4*). Specialized industrial devices, notably variable-speed motors and their control systems, as used for example for pumps or railroad engines, may also draw currents of very complex wave shapes and thereby cause the magnetic field from nominally sinusoidal sources to have a significant energy content above the fifth harmonic (*5*).

Of particular interest is the frequency content of therapeutic pulsed field devices because they were frequently used in the past either to produce or to investigate biological effects. Unfortunately, description of these signals in research reports is frequently very incomplete or even misleading. For example, the signal illustrated by Figure 1, which is similar to that of some therapeutic devices used to accelerate healing of bone fracture nonunions, would be correctly described as having a pulse-repetition frequency of 20 Hz. However, it should not simply be called a "20-Hz signal" or an "extremely low frequency (ELF) signal" because very little of its energy is at 20 Hz or even below 1000 Hz. Fourier integral analysis of such a pulse burst (*6, 7*) gives

$$F_1(\omega) = 2\pi \sum_{n=-\infty,\neq 0}^{n=\infty} BT_2 \left[\frac{\sin(\omega - \omega_1)T_2}{(\omega - \omega_1)T_2} + \frac{\sin(\omega + \omega_1)T_2}{(\omega + \omega_1)T_2} \right] \delta(\omega - n\omega_o) \qquad (1)$$

where the delta function $\delta(\omega - n\omega_o)$ indicates discrete spectral lines at multiples

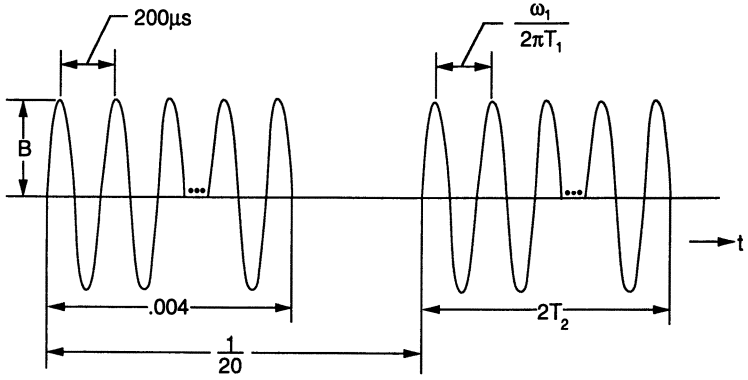

Figure 1. Biphasic pulse burst repeated at frequency ($\omega_o/2\pi$) = 20 Hz.

of the pulse-repetition frequency ($\omega_o/2\pi$), which is 20 Hz in the selected example. The amplitudes of the spectral lines are given by the expression in brackets in eq 1. The spectral peak for positive frequencies occurs, as illustrated in Figure 2, where $[\sin(\omega - \omega_1)T_2]/[(\omega - \omega_1)T_2]$ is equal to 1, which is the case when $\omega = \omega_1$ or at the frequency of the sine wave within each individual burst. In the present example this frequency is $10^6/200$ Hz (=5 kHz)! The duration $2T_2$ of the pulse burst determines the widths of the spectral lobes as indicated by eq 1 and illustrated on Figure 2.

The spectrum of a unidirectional [direct current (DC)] pulse (Figure 3a) is shown on Figure 3b. Only if such a pulse is combined with the biphasic signal of Figure 1 to give the signal of Figure 4 will the Fourier spectrum have significant energy content at low frequencies.

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Low-Frequency Electric Field Dosimetry

In Vitro Dosimetry for Cell and Tissue Cultures. Culture media generally have an electric conductivity on the order of 1 S/m (*8*), similar to that of fluid-saturated living tissue (*9*). A material is considered to be an electric conductor, as opposed to an insulator or dielectric, if the ratio of conduction current density, σE, to displacement current density, $\varepsilon(\partial E/\partial t)$, is much greater than one, where σ is the electric conductivity, E is the electric field, and ε is the dielectric permittivity. For a field varying sinusoidally in time at frequency $\omega/2\pi$, this relation can be written as

$$\text{conductor when } \frac{\sigma}{\omega\epsilon} >> 1 \qquad (2)$$

The dielectric permittivity is given by $\varepsilon = \varepsilon_r\varepsilon_0$, where ε_0 is the permittivity of free space, which is equal to 8.84×10^{-12} F/m, and ε_r is the relative dielectric constant. Below 100 Hz ε_r of living tissue can be as large as 10^6 (*9*); nevertheless, eq 2 still gives a value of 180 at 100 Hz with $\sigma = 1$ S/m and $\varepsilon_r = 10^6$. Thus at ELF, culture media can be considered to be electrically conducting fluids. Electric fields are therefore most easily introduced to cell cultures by making

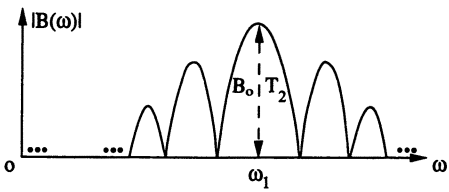

Figure 2. Spectral envelope of the signal of Figure 1. If a single burst is replaced by bursts repeated at frequency f_o*, the continuous spectrum becomes the envelope of spectral lines spaced at intervals* f_o*.* B_o *is the peak amplitude of the applied magnetic flux density.*

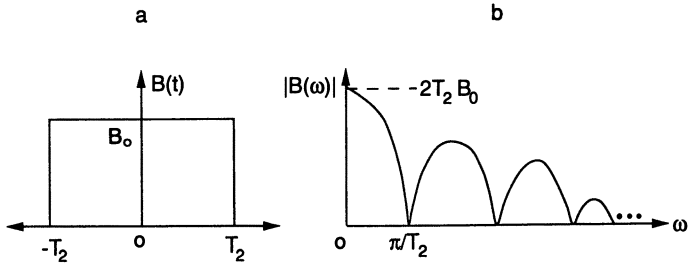

Figure 3. Direct current pulse (a) and Fourier spectrum (b).

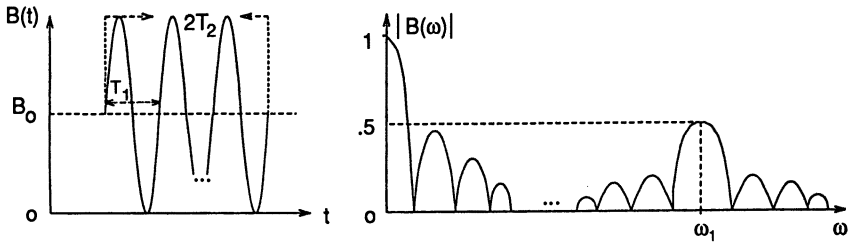

Figure 4. Unidirectional pulse and spectral envelope. If burst is repeated at frequency f_o, the spectrum consists of discrete spectral lines at f_o intervals. The illustrated continuous spectrum then becomes the envelope indicating relative amplitudes of the spectral lines.

conducting contact and measuring the series current, *I*. If the electrodes are large enough and their shape is relatively simple, or if the field is calculated at a point in the field at a distance that is relatively large in comparison with the electrode size, then the electric current density, *J*, is given by *I/A*, where *A* is the cross-sectional area of rectangular electrodes or of rectangular vessels. The electric field can then be calculated from

$$J = \sigma E \tag{3}$$

if the conductivity is measured.

If the current is introduced into the medium by point or rod electrodes, the computation of the current density becomes more complicated, and appropriate literature (*10, 11*) must be consulted. All such calculations assume that the culture medium, including cells and tissue cultures, is electrically homogeneous. This approximation is reasonable for freely floating cells at relatively low density. If cells are plated at the bottom of a culture vessel and become confluent, or nearly so, the culture fluid and cell system must at least be considered as a two-layer medium, in which the bottom (cell) layer may have a vastly different (and at ELF usually much lower) electric conductivity than the fluid. Current density and field evaluation then require more complex calculations, such as are used for

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch004

ground conductivity evaluation in geophysics (*11*), or possibly numerical evaluation using available two- or three-dimensional electrostatics programs (*12*).

The use of electrodes for introducing electric current into biological fluids requires caution to avoid chemical reactions at the electrode surfaces and consequent contamination of the fluid. The most successful method for solving this problem consists of using Agar bridges (*13*).

For freely floating cells at relatively low density in a conducting medium, calculating the electric fields at the surface and within the cell membrane and the cell cytoplasm is of interest. If one assumes as a first, very rough approximation that the cell can be represented by a conducting sphere (the cytoplasm) of radius a, surrounded by a thin, low-conductivity membrane of outer radius $r = b$ (and thickness $\Delta = b - a$), these fields can be computed in closed form using the quasi-static approximation. For a field E_o applied at $r >> a$ in a conducting medium, electric fields $\bar{E}$ at the surface and within the cell membrane and cytoplasm are given in the following series of equations.

$$r >> b \qquad \bar{E} \approx \hat{r}E_o \cos\theta - \hat{\theta}\, E_o \sin\theta \approx \hat{z}E_o$$

$$r = b^+ \qquad \bar{E} \approx \hat{r}\,\frac{3}{2}E_o\frac{Ya}{\sigma\Delta}\cos\theta - \hat{\theta}\,\frac{3}{2}E_o\sin\theta$$

$$r = b^- \qquad \bar{E} \approx \hat{r}\,\frac{3}{2}E_o\frac{a}{\Delta}\cos\theta - \hat{\theta}\,\frac{3}{2}E_o\sin\theta$$

$$r = a^+ \qquad \bar{E} \approx \hat{r}\,\frac{3}{2}E_o\frac{a}{\Delta}\cos\theta - \hat{\theta}\,\frac{3}{2}E_o\frac{Ya}{\sigma\Delta}\sin\theta$$

$$r = a^- \qquad \bar{E} \approx \hat{r}\,\frac{3}{2}E_o\frac{Ya}{\sigma\Delta}\cos\theta - \hat{\theta}\,\frac{3}{2}E_o\frac{Ya}{\sigma\Delta}\sin\theta$$

$$\frac{a}{\Delta} \approx \frac{10^4}{6} \qquad \left|\frac{Ya}{\sigma\Delta}\right| \approx 4 \times 10^{-5} \text{ at } 60 \text{ Hz}$$

In these equations, the conductivities of culture fluid and cytoplasm, designated by σ, are considered equal; the specific admittance of the membrane is given by $Y = \sigma_2 + j\omega\varepsilon_2$, where σ_2 is membrane conductivity, ε_2 is membrane dielectric permittivity, and $j = \sqrt{-1}$; θ is the colatitude measured from the direction of field E_o; $\Delta << a$; b^+ is defined by $r = b + \delta$ where $\delta << b$; and correspondingly, $b^- = b - \delta$, $a^+ = a + \delta$, and $a^- = a - \delta$. Unit vectors are $\hat{z}$ in the direction of the applied external field and $\hat{\theta}$ and $\hat{r}$, respectively, in the colatitudinal and radial directions.

Another method (*14*) for introducing an electric field into a culture medium is illustrated by Figure 5. It is suitable only if small values of the electric field within the culture medium are desired. Continuity of current (or conserva-

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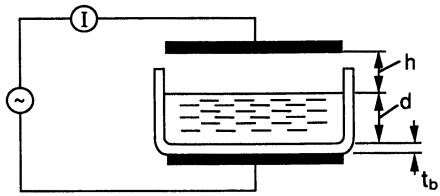

Figure 5. Method for introducing a small electric field into a conducting fluid without conductive contact.

tion of charge) at a plane, infinite boundary between a "perfect" (i.e., zero-conductivity) dielectric medium with permittivity ε and a conducting medium of conductivity σ requires that the ratio between the electric field outside (in the dielectric; E_o) and the electric field inside (the conductor; E_i) be given by

$$\frac{E_i}{E_o} = \frac{\omega\epsilon_0}{\sigma} \tag{4}$$

Using this relation, one obtains for E_i in terms of the applied voltage V between electrodes

$$|E_i| = \left| \frac{V}{-j\dfrac{\sigma}{\omega\epsilon_0}(h + t_b/\epsilon_r) + d} \right| \approx \frac{V\omega\epsilon_0}{\sigma h} \tag{5}$$

where h is the height of the upper electrode above the fluid surface, t_b is the bottom thickness of the containment vessel, ε_r is the relative dielectric constant of the vessel material, d is the depth of fluid in the vessel, σ is the conductivity of the fluid, and ω is the radian frequency, which equals $2\pi f$. At ELF a typical value of $\omega\varepsilon_0/\sigma$ might be 10^{-8}. If h equals 1 cm, V would have to be 1000 V to obtain for E_i the value of 1 mV/m. To check the value of E_i obtained by eq 5, one can measure the series current I; it is related to the cross-sectional area A of the vessel by

$$I \approx \sigma E_i A \tag{6}$$

and might be of the order of microamperes if A is several square centimeters.

Dosimetry for Animal or Human Exposure. Equation 4 also provides a first approximation for the current inside and outside an animal body subjected to an external electric field. Here, of course, one does not deal with infinite plane boundaries, but shape effects come into play. An electric field that is initially uniform becomes distorted in the immediate vicinity of the animal

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such that it is oriented everywhere perpendicular to the conducting body. Whether the animal is electrically grounded or is located on an insulating platform will significantly affect the field distribution. Finally, when considering animal or human exposure, one is often primarily interested not in an average electric field within the body but rather in field and current distribution within different body parts. The electric inhomogeneity (e.g., bone vs. muscle conductivity) and variation in cross section of limbs and trunk then determine the exact current distribution. Extensive model calculations as well as measurements on "phantoms" (saline body models) have been performed (*15–17*). The results are that in a human standing on an electrically conducting ground in a 10-kV/m, 60-Hz electric field, maximum current densities of 0.03 A/m^2 are estimated in the leg and 0.004 A/m^2 in the neck. The larger value corresponds to an E_o/E_i ratio of 3.33×10^5 when a body conductivity of 1 S/m is assumed.

Shape, body size, and body orientation effects also require substantial corrections when equal internal electric fields inside different animals are to be obtained by applying external electric fields. Thus, applying a 30-kV/m, vertical, 60-Hz electric field in a rat cage is necessary to obtain roughly the same current densities within the rat body as inside a human standing upright in a 10-kV/m, 60-Hz field (*17*).

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch004

Low-Frequency Magnetic Field Dosimetry

General Considerations. To a very high degree of approximation, the ELF magnetic flux density $B(t)$ inside living tissues or cell cultures is equal to that applied externally. This relationship is a consequence of two conditions. First, the magnetic permeability of tissue and cells is, to within a fraction of 1%, equal to that of free space (except in the immediate vicinity of microscopic magnetosomes, to be discussed later); second, the relatively low electric conductivity (at most on the order of 1 S/m) of living matter, in comparison with that of metallic structures ($\approx 10^7$ S/m), guarantees that the magnitude of the secondary magnetic field produced by the induced eddy currents is negligible (*18*). Therefore the applied magnetic field can be measured externally or calculated from known external current distributions (such as in power-frequency transmission or distribution lines, or in specially designed coil configurations) without need to correct for any modification due to the insertion of an animal or tissue preparation.

A time-varying magnetic field vector **B** induces an electric field vector **E** according to Faraday's law such that

$$\oint \mathbf{E} \cdot \mathbf{dl} = -\iint \frac{\partial \mathbf{B}}{\partial t} \cdot \mathbf{ds} \tag{7}$$

where **dl** is a vector element of length and **ds** is a vector element of area.

In Electromagnetic Fields; Blank, M.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1995.

When a magnetic flux density B is applied parallel to the axis of an infinitely long, electrically homogeneous circular cylindrical body, eq 7 reduces to

$$E_\phi = \pi f B r \tag{8}$$

where E_ϕ is the circumferentially directed electric field, f is frequency, and r is the radial distance from the center of the cylinder. This last relation is frequently employed in estimating induced electric fields in animal bodies and cell cultures but will give incorrect results when media are neither cylindrical nor electrically homogeneous. Equation 8 nevertheless indicates roughly that one needs a 15-μT magnetic flux density B, perpendicular to the horizontal cross section of a circular culture well of 1-cm radius, to induce in it an electric field equal to that produced in a human by a 1-μT magnetic flux density oriented parallel to the head–foot axis.

A key question as yet unsolved in magnetic field dosimetry is whether observed biological effects at low frequencies are due to the induced electric field or to the time-varying magnetic field itself. The first answer has been generally assumed in the past and is probably correct when the induced electric fields are relatively large, that is, at least 10^{-3} V/m. Some experiments on cells in cultures that give average induced electric fields of this order of magnitude have shown (*19*) clear electric field effects. On the other hand, experiments leading to smaller induced electric fields have clearly been shown (*20*) to be independent of electric field magnitudes.

If biological effects are due to the induced electric field, an obvious consequence of eq 7 is that the orientation of a culture dish, or any other object, within the magnetic field will have major consequences. Because only the component of the magnetic field that is perpendicular to a surface contributes to the induced electric field in the plane of that surface, the two culture vessel arrangements illustrated by Figure 6 will give entirely different induced electric

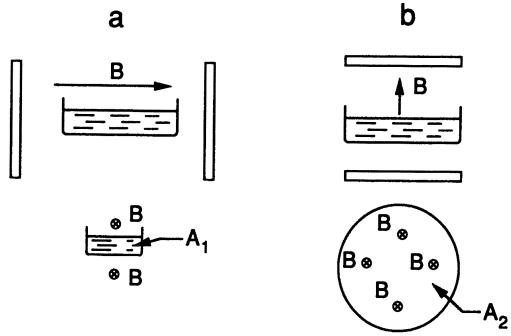

Figure 6. Orientation of culture vessel affects magnetically induced electric field. a: Magnetic field parallel to fluid surface. b: Magnetic field perpendicular to fluid surface.

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field magnitudes and distributions. If the fluid in the circular dish is homogeneous, the electric field in the arrangement of Figure 6b can be calculated by eq 8 at various radii; the surface area perpendicular to B is A_2. For the arrangement of Figure 6a the cross-sectional area A_1 is that of a rectangular, vertical plane through the culture dish, and the field computation becomes much more complex although a solution in mathematically closed form is available (*21*).

Method for Deciding If an Induced Electric Field Produces a Biological Effect. Misakian and Kaune (*22*) suggested the use of concentric-ring culture dishes to decide whether a particular in vitro effect is due to the induced electric field or is caused directly by the magnetic field. According to eq 8, cells in rings of different radii will experience different electric fields but are subjected to the same magnetic field that is perpendicular and of the same amplitude over the entire dish surface. If a time-varying magnetic field is generated over a sufficiently large cross-sectional area, the experiment can also be performed with a number of separate culture plates of different diameters. In either case the experiment is not as straightforward as it might appear at first sight. If cells during culture settle to the bottom of the vessel (even if they do not plate), results from different rings or wells with different diameters are comparable only if cells per unit volume of culture medium and cells per unit surface area are both the same in all rings or separate wells. The required uniform surface distribution is achieved only if all wells or rings are initially filled with the cell-culture medium mixture exactly to the same height and if cell density per unit volume is carefully maintained.

A further limitation of the method is that eq 8 is only valid for a homogeneous medium. Therefore it can be applied only when the cell density in the culture medium is relatively small. Induced field distributions obtained for cylindrical vessels by numerical methods do not follow eq 8 when the cell density is high (*23, 24*).

Scaling from In Vitro to In Vivo or Between Different Organisms Assuming Effects Due to Induced Electric Fields. A first-order approximation, based on eq 7 or eq 8, would indicate that the magnetic flux density B would have to be varied in inverse proportion to the radius r (in a plane perpendicular to the direction of B) if the same induced electric field is to be obtained in different preparations or organisms. Thus one might expect (assuming similarity of a particular physiological system in mouse and human) that a 10-μT, horizontal, 60-Hz magnetic field in a mouse of 2.5-cm diameter, and a 1-μT, vertical, 60-Hz magnetic field in a human of 25-cm mean body diameter would produce similar effects.

The electric fields (E_i) and current densities (J) induced by a time-varying magnetic field $B(t)$ will follow paths different from those produced in tissue by an externally applied electric field (E_a). This fact is illustrated by the lumped-circuit approximation, shown in Figure 7, of a rectangular body in which dis-

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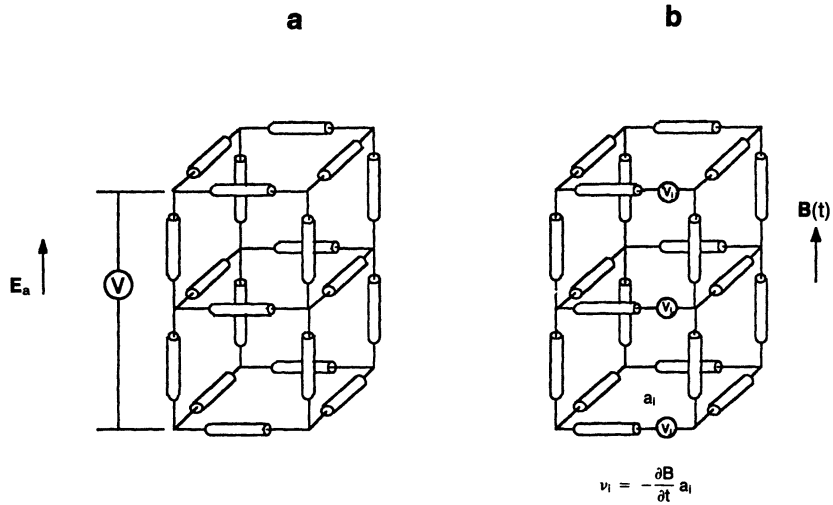

Figure 7. Externally applied and magnetically induced electric fields produce different current distributions: (a) applied electric field; (b) applied time-varying magnetic field. (Reproduced with permission from reference 28. Copyright 1992 Wiley.)

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crete circuit elements (principally resistive and capacitive) have been substituted for the continuous distribution of different cells, intercellular matrix, and fluid that make up a real biological entity. For an applied electric field E_a, which is much smaller inside than outside the body according to eq 4, the generator or generators are arranged linearly as indicated in Figure 7a. Furthermore, because E_a at ELF is quasi-static (*25*), the electric field distributions inside the body over any closed path must be such that

$$\oint \mathbf{E} \cdot d\mathbf{l} = 0 \tag{9}$$

even in the presence of inhomogeneities or anisotropic structures. If the body of Figure 7a is electrically conductive, currents will primarily flow in the direction of the applied field. Internal structures that are electrically insulated, for example by membranes, will simply be bypassed (for details of such situations *see* references 17 and 26). On the other hand, $B(t)$ will produce circulating currents in electrically isolated structures that are proportional to their electric conductivity (σ), their cross-sectional area (a_i) perpendicular to $B(t)$, and the time rate of change of $B(t)$, as suggested by eq 7. For the lumped circuits in Figure 7b, eq 7 gives for each loop i ($i = 1, 2, \ldots$) of area a_i

$$V_i = \oint \mathbf{E} \cdot d\mathbf{l} \approx -\frac{\partial \mathbf{B}}{\partial t} a_i \tag{10}$$

Because electric properties of biological substances can change substantially over a scale of nanometers (for example when a membrane is present), eq 7 does not predict the electric field at every point. Any numerical computation based on it (*27*) will give correct answers on a micro scale only when selected areas, or the corresponding mesh sizes, as in Figure 7, are sufficiently small to correctly represent inhomogeneities on a micro scale. Because the induced fields depend upon the lengths of current paths within electrically insulated structures, the size, for example of organs contained within insulated membranes, becomes important. Groups of cells connected by gap junctions also may present relatively large cross-sectional areas perpendicular to $B(t)$ and allow induction of correspondingly larger electric fields in the cell interior (for numerical estimates, *see* reference 28).

These considerations, which are essentially all based on the difference between eqs 7 and 9, indicate that the electric fields inside the human or animal body induced by time-varying ELF magnetic fields cannot be expected to produce the same biological effects as internal electric fields due to externally applied ELF electric fields. Scaling between different organisms or from in vitro to in vivo also is very different for application of external electric and magnetic fields, even if the magnetic fields exert a physiological effect only through induced electric fields or current densities $J = \sigma E$. In the absence of detailed numerical micro-scale models one must still rely on eq 8 for first-order scaling.

Direct Effects of ELF Magnetic Fields and Role of Ambient (Geomagnetic) DC Field. Epidemiological findings that suggest health effects of 60-Hz fields implicate weak power frequency magnetic rather than electric fields. Biological effects of time-varying ELF magnetic fields exhibit frequency windows, that is, effects at some frequencies but not at others, but do not become more prevalent or stronger as the frequency increases. Such an increase with frequency might be expected, in view of eqs 7 and 8, if the cause were the induced electric fields (*29*). In vitro experiments also have shown (*20*), by using the multiring method already described, that some observed biological consequences of ELF magnetic field exposure were not due to the induced electric field. 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 v of radius a and permeability μ contains an energy of $\frac{1}{2}(B_m^2/\mu)(v)$, and a magnetic flux density B_e induces an electric field E so that $\frac{1}{2}(\varepsilon E^2)$ integrated over the same volume is equal to the energy corresponding to B_m, then using eq 8 gives the ratio

$$\frac{B_e}{B_m} = \frac{2}{a\pi f\sqrt{\mu_0 \epsilon}} \tag{11}$$

where μ_0 is the permeability of free space. For a radius a of 3 cm, one obtains, at 60 Hz, values for B_e/B_m of 10^8 or 10^5, depending upon whether one assumes for

the dielectric permittivity, ε, that of free space (ε_0) or that of muscle tissue at ELF ($\varepsilon \approx 10^6\varepsilon_0$). Thus the time-varying magnetic flux density (B_e) necessary to store a given quantity of energy in the electric field that it induces is many orders of magnitude larger than the flux density (B_m) that would store the same amount of magnetic energy! If biological experimentation shows that effects are produced by very weak time-varying magnetic fields, then clearly it is important to consider all possible mechanisms for direct magnetic field–tissue interaction without assuming a priori that biological effects can be due only to the induced electric fields.

When direct effects of magnetic fields are considered, the type of dosimetry needed depends to a large extent upon the nature of the field–biosystem interaction. One mechanism, which probably requires alternating magnetic flux densities of at least 100 μT (*30, 31*), involves free-radical chemical reactions. Two other mechanisms, however, are in principle applicable at lower ELF fields (down to 10 μT and possibly much lower). Kirschvink et al. (*32*) have discovered domain-size magnetite crystals in human brain tissue at very low volume concentration (4–110 parts per billion), but at 100 times higher concentration in a transformed (Jurkat) T-cell line (*33*). A very general scheme for interaction of such magnetic crystals with the surrounding tissue has been proposed (*34*), but not enough is known at the present time about the magnetite-to-membrane or magnetite-to-cytoplasm interface to permit experimentally verifiable predictions (*35, 36*).

The other model for direct magnetic field–biochemical interaction is that of ion parametric resonance. It was first proposed by Lednev (*37, 38*), and a modified form was recently published by Blanchard and Blackman (*39*). Some of its quantitative predictions agreed with experimental results obtained by Yost and Liburdy (*1*) considering Ca^{2+} influx in mitogen-treated lymphocytes. Very recently Blackman et al. (*3*), considering magnesium, vanadium, and other ions in neurite outgrowth from PC-12 cells, obtained data that appeared to support their version of the model. The model postulates Zeeman splitting of presumably infrared vibrational modes at an ion cyclotron frequency $f_o = qB_o/2\pi m$ in the ELF range, where q and m are, respectively, the ionic charge and mass, and B_o is an applied DC magnetic field in the magnitude range of the Earth's field. Frequency modulation of the Zeeman levels by an externally applied, parallel-oriented magnetic field at the frequency f_o, or a subharmonic f_o/n, changes the transition probability between infrared vibrational modes. The resulting changes in the unknown dynamic configuration of the ion and the protein to which it is bound are then assumed to affect a series of possibly very complex biological processes. The lifetime of the ion within the protein or protein complex must be relatively long, ~1 s.

Importantly, the model predicts a variation of the transition probability p between vibrational modes given by

$$p = K_1 + K_2(-1)^n J_n(nB_{AC}/B_o) \qquad (12)$$

where J_n is the Bessel function of order n. At the resonance frequency ($n = 1$) or

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch004

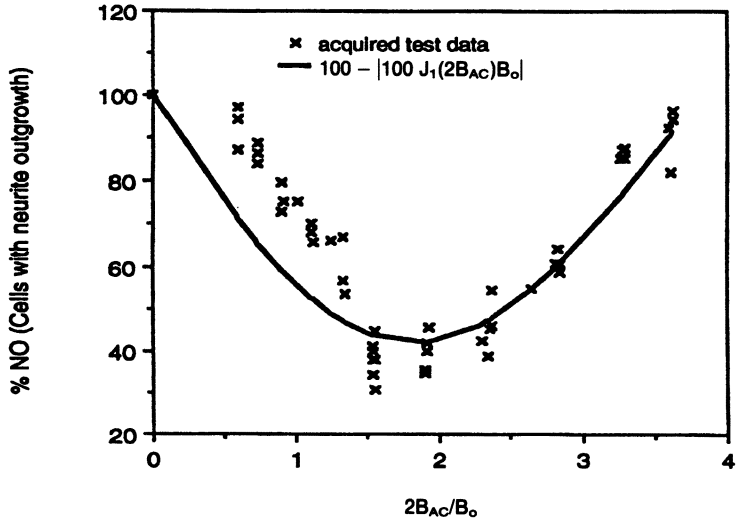

Figure 8. Fit of neurite outgrowth (NO) data to the ion parametric resonance (IPR) model for resonance frequencies of 25 Hz (B_0 = 20.3 μT) and 45 Hz (B_0 = 36.6 μT) using only n = *1 in eq 12. Possible ions are* Mg^{2+} *and* V^{4+}. *(Reproduced with permission from reference 3. Copyright 1994 Wiley.)*

its subharmonics ($n > 1$), the numerically normalized experimental results can then be compared with the predictions of eq 12 by adjusting the parameters K_1 and K_2 to obtain a best fit to experimental data.

In the Blanchard and Blackman (*39*) version of the model, eq 12 is modified by insertion of a factor of 2 in the argument of the Bessel function, which leads to different numerical results for *p*. Exposing PC-12 cells to appropriate DC and AC magnetic field amplitudes at a single resonance frequency, Blackman et al. (*3*) obtained a decrease in the number of cells with neurite outgrowths in comparison with unexposed cells. That decrease appeared to follow the $J_n(2nB_{AC}/B_0)$ version of eq 12, as illustrated by Figures 8 and 9. However, the model was tested only over a range of $2B_{AC}/B_0$ between 0 and 3.8, corresponding to positive values of the first-order Bessel function. [It would have been useful to extend the range further to at least 5.3 because the negative peak, $J_1(5.3)$ = –0.346, is comparable in magnitude to the positive peak, $J_1(1.85)$ = 0.581]. In any case the factor of 2 in $J_n(2nB_{AC}/B_{DC})$ is incompatible with the basic assumptions of the model as formulated by Lednev (*see* reference 40). Much more detail was given by Blackman et al. (*3*), Lednev (*37, 38*), Polk (*40*), Adair (*41*), Bruckner-Lea et al. (*42*), and Markov et al. (*43*).

The experiments of Liburdy (*1*) and Blackman (*3*), without adequately confirming any particular theoretical model, and the earlier reports by Liboff (*44*), Smith (*45*), McLeod (*46*), and Reese et al. (*47*) clearly show the need for controlling, or at least reporting, the direction and amplitude of the DC magnetic field when biological effects of weak ELF magnetic fields are investigated. The

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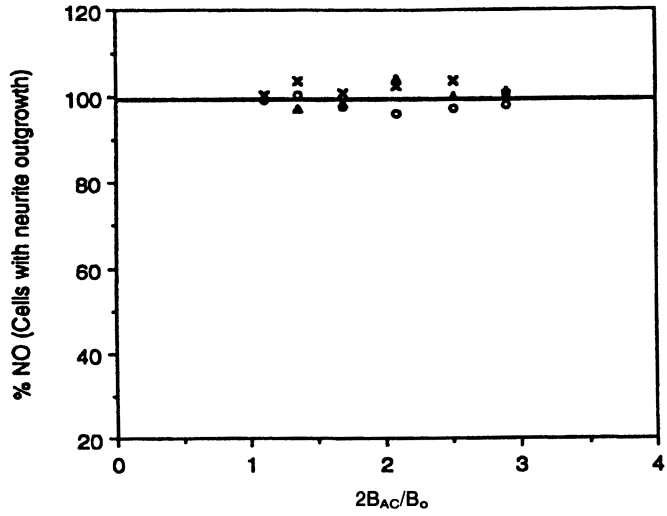

Figure 9. Neurite outgrowth (NO) data for off-resonance conditions (45 Hz, B_0 = 2 μT). (Reproduced with permission from reference 3. Copyright 1994 Wiley.)

model of Kirschvink (*34*) also would require interaction between DC and AC fields, although no predictive theory for critical frequencies or amplitude ratios is available. For reference the parametric resonance or ion cyclotron frequencies of some physiologically important ions are listed in Table I for a static magnetic flux density of 50 μT, corresponding to the magnitude of the geomagnetic field at many midlatitude locations; also listed are required flux densities for resonance at 60 Hz.

Exposure Systems and Magnetic Field Measurements. The best-known and simplest system for generating spatially uniform magnetic fields over a relatively large volume is the Helmholtz coil arrangement (*48*). Uniform fields over larger volumes can be obtained with multicoil arrangements (*49, 50*) or with long solenoids (*48*). Designs of these systems, as well as of parallel-plate

Table I. Ion Resonance Frequencies for B_0 = 50 μT and Required DC Magnetic Flux Densities for 60-Hz Resonance

Ion	*Charge/Mass*	f_0 *(Hz)*[a]	B_0 *(Hz)*[b]
Fe^{3+}	5.15	41.0	73.2
$^{40}Ca^{2+}$	4.78	38.0	78.9
Mg^{2+}	7.89	62.8	47.8
P^{3+}	9.28	73.8	40.6

[a]Ion resonance frequency values at B_0 = 50 μT.
[b]Magnetic flux density values required for f_0 = 60 Hz.

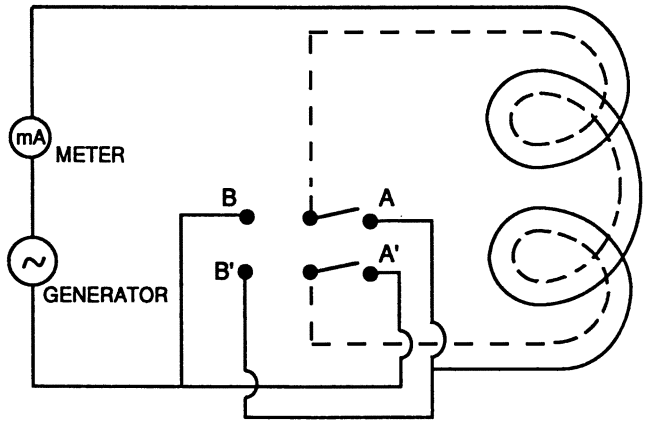

Figure 10. Double-wound coils with double-pole–double-throw (DPDT) switch for current reversal in adjacent conductors.

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exposure arrangements and exposure chambers, have been discussed in great detail by Misakian et al. (*51*).

Good experimental practice for biological experiments requires that both exposed and sham cultures and animals be subjected to exactly identical environments with the single exception of the exposure parameter, which in the present context is the applied magnetic AC or DC field or a combination of fields. Performing data analysis blind, that is, in such a manner that the person who records the data does not know if a particular sample was exposed, is also desirable. These requirements can be achieved most easily for field exposure by using double-wound coils (*50*), as illustrated by Figure 10. With one position (AA') of the double-pole–double-throw (DPDT) switch, currents in both parallel wires flow in the same direction and a magnetic field is produced in the desired exposure volume; when the DPDT switch is in the other position (BB'), currents in parallel wires flow in opposite directions, cancelling the magnetic field in the exposure volume. A blind experimental procedure is achieved if the position of the switch is concealed from the person who performs the data analysis. The double winding can be most easily produced by employing standard two-conductor speaker cable (*52*).

An additional advantage of using double-wound coils is that heat generation by ohmic losses in the windings is the same for both exposed and sham setups. However, the double-wound coils do not ensure the same kind of vibrational excitation, if it is significant or measurable, of both exposed and sham cultures. With currents flowing in different directions through adjacent wires, different vibrational modes can be set up by the attractive or repulsive forces between wires. Particularly when large fields and correspondingly large currents and forces are involved, it is always desirable to check for vibrational (acoustic) excitation of sample platforms. This procedure requires microphones or acceler-

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ometers that are insensitive to magnetic fields, such as, for example, the capacitive accelerometer manufactured by Analog Devices (*53*).

Magnetic field amplitudes can be established either directly by field measurement or indirectly by measuring coil currents. Fields calculated from measured currents and known coil configurations should agree closely with directly measured amplitudes. Generally, employing both methods is desirable. For the measurement of AC magnetic fields either a calibrated induction coil, a Hall effect probe, or a flux-gate magnetometer can be used (*54, 55*). For DC magnetic fields either Hall effect or flux-gate instruments are suitable; however, at flux densities below 100 μT most Hall effect instruments are not very accurate or stable. The probes of flux-gate instruments themselves generate a small magnetic field in the kilohertz range and therefore should only be used for initial field measurements and should not be kept in operation during sample exposure. Some flux-gate magnetometers, even when equipped with filters so as to respond only to the DC field, will overload in the presence of a sufficiently large AC field. Static field measurements should therefore always be made with the AC field turned off.

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Survey of Radio-Frequency and Microwave Dosimetry

Detailed discussion of radio-frequency (RF), including microwave, dosimetry is beyond the scope of this chapter. In any case several excellent treatments of both experimental and theoretical aspects of RF dosimetry are available, for example those by Stuchly and Stuchly (*56*), Lin (*57*), Durney et al. (*58*), Gandhi (*59*), and Guy (*60*) . The following paragraphs will therefore be concerned only with the most fundamental aspects of RF dosimetry and will primarily emphasize the differences between low-frequency (particularly ELF) and RF dosimetry.

An electromagnetic structure, consisting of one or more current-carrying conductors, radiates a significant amount of energy only when its dimensions (L) are significant in comparison with the wavelength (λ)

$$\frac{L}{\lambda} > 0.1 \tag{13}$$

At 60 Hz, for example, the wavelength in free space is 5000 km, and therefore most man-made devices on the Earth are at most very inefficient radiators. Furthermore, electric current or charge systems, even if they change in time, store or circulate energy, within the electric and magnetic fields in their immediate vicinity, that is not radiated. This immediate field vicinity is the region of the quasi-electrostatic and induction fields. The distance d from an electrically short ($L << \lambda$) current-carrying conductor where the amplitudes of the radiation and induction fields are equal is given by

$$d = \frac{\lambda}{2\pi} \tag{14}$$

At 60 Hz this distance is 796 km. Therefore one encounters at ELF in the vicinity of all kinds of devices, including transmission lines, only quasi-electrostatic and induction fields, not radiation. *Non-ionizing radiation* is an inaccurate term when applied to fields from ELF sources encountered on the Earth.

At both low and high frequencies, electric fields produce currents, and these currents generate magnetic fields; similarly, time-varying magnetic fields generate electric fields. But in the quasi-electrostatic and induction fields, depending upon the nature of the source and the location of the observation point, the relative amplitudes of electric and magnetic fields can vary widely. Electric fields are associated with voltages, and magnetic fields depend upon currents; the ratio of electric to magnetic fields is not a constant (depending upon material properties) as it is for a plane wave in free space. For a radiated wave this ratio, the wave independence in a loss-free medium, is

$$Z_o = \sqrt{\frac{\mu}{\epsilon_0}} = \frac{E}{H} \tag{15}$$

where the magnetic field H is equal to B/μ. Thus for radiation, which becomes only important at sufficiently high frequencies as indicated by eqs 13 and 14 (because $f = c/\lambda$), calculation of E from H is possible, or vice versa. This relation is true at least for a plane wave if reflected waves are not present. The power per unit area in an electromagnetic wave is given by the Poynting vector

$$\mathbf{S} = \mathbf{E} \times \mathbf{H} \tag{16}$$

whose instantaneous magnitude can be computed in view of eq 15 from either E or H. However, even when sources and distances are large enough in comparison with λ for radiation and wave propagation to be important, one must differentiate between the near-field and far-field regions. Equation 15 is valid only in the far-field region. Depending upon the exact form of the radiating structure, the near field extends outward to a distance z given approximately by

$$z \approx \frac{L^2}{\lambda} \tag{17}$$

where L is the largest dimension of the radiator. Thus part of the human body can still be in the near-field region of a 900-MHz cellular phone antenna whose maximum dimension might be 10 cm, because $z = (0.1)^2/0.333 = 0.03$ m.

At RF the reflection of waves at boundaries between electrically dissimilar media becomes important, as does skin depth (δ) of electric conductors. The skin depth is the distance at which the penetrating part of the incident wave (i.e., the part that was not reflected at the boundary surface) has been attenuated by the factor $1/e$. As an example, the skin depth for muscle tissue as a function of frequency is shown in Figure 11. The generally used dosimetry measure at RF is

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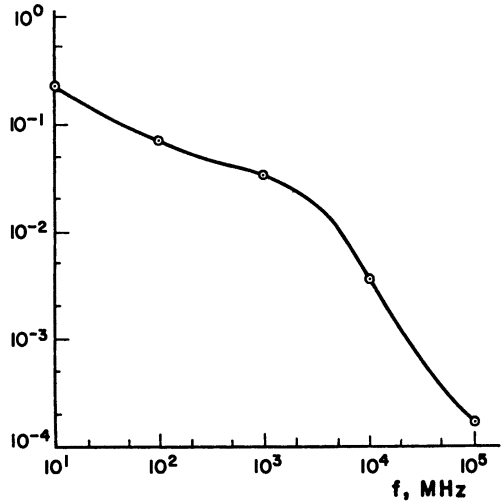

Figure 11. Electromagnetic skin depth (δ, given in meters) in muscle tissue for a plane wave. (Reproduced with permission from reference 63. Copyright 1986 CRC Press.)

the specific absorption rate (SAR), which is defined (*57*) as "the time (*t*) derivative of incremental energy (d*W*) absorbed by an incremental mass (d*m*) contained in a volume element (d*v*) of a given density ρ":

$$\text{SAR} = \frac{\mathrm{d}}{\mathrm{d}t}\left(\frac{\mathrm{d}W}{\mathrm{d}m}\right) = \frac{\mathrm{d}}{\mathrm{d}t}\left(\frac{\mathrm{d}W}{\rho \mathrm{d}v}\right) \tag{18}$$

where SAR is expressed in watts per kilogram. Sometimes total absorbed energy is of interest, particularly for short-period, pulsed exposure. This quantity is given as specific absorption (SA), defined as the time integral of SAR and expressed in joules per kilogram. The terms SAR and SA are applicable to both whole-body dosimetry and distributive dosimetry; the latter deals with individual body parts (*62*). The measurement of SAR and SA necessarily involves consideration of source characteristics (such as field distribution in a wave guide, and wave polarization), body or body-part orientation, reflection at boundary surfaces, and wave penetration. Information on such measurements and related computations is contained in references 56–62.

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RECEIVED for review April 6, 1994. ACCEPTED revised manuscript November 3, 1994.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch004

5

Issues Relating to Causality of Bioelectromagnetic Effects

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Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch005

Significant controversy exists about whether environmental electromagnetic fields (EMFs) can cause effects in biological systems. EMF interactions have essentially no biochemical specificity, so that competing changes associated with both biological phenomena and intrinsic fluctuations (noise) are extremely important. Only if this competition is successful can effects be attributed to environmental fields. One general approach to assessing causality is based on estimates of a generalized signal-to-noise ratio (S/N). Such estimates have usually considered physical criteria in which the signal is the energy change associated with the environmental field, the background is the energy change due to biologically generated fields, and noise consists of energy fluctuations. More recently the S/N approach has been extended by considering a more biological criterion: changes in the number of molecules in a biochemical pathway. Such criteria can be used to estimate approximate threshold conditions for expecting bioelectromagnetic effects and to identify candidate target tissues.

Nature of Reported Effects

Many different types of bioelectromagnetic effect studies have been reported, using both in vitro and in vivo conditions. Overall, these experiments are complex, in that the biological, chemical, and physical state of the system needs to

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be carefully controlled to determine if changes are actually due to electric and magnetic field exposures. Often the changes measured with respect to controls are relatively small (e.g., less than 50%). Moreover, the inferred field strengths at the cell and tissue level are often regarded as small. For this general reason, considerable skepticism exists about whether these effects are real. The complexity and controversial nature of much of bioelectromagnetic research are recognized, as can be seen in the review literature (*1–10*).

Seeking the Most Sensitive Biophysical Mechanism

Our general approach is to attempt to construct the most sensitive possible theoretical models that could represent the "primary target" of a biological system and to then incorporate all of the realistic influences with which electromagnetic fields (EMFs) must compete (*11–17*). By primary target we mean that part (or parts) of a biological system that interacts with electric or magnetic fields to produce a molecular change that is then amplified by one or more biochemical cascades so that a biological effect results. Any cascade is most sensitive to changes at its "input", that is, a site at which an external influence causes a molecular change. The downstream stages in a cascade may also be subject to an external influence, but the additional contribution to an effect at these sites should be much smaller. Thus, we consider primary targets that on physical grounds are likely to be preferential sites for EMFs to cause an initial molecular change that can be cascade-amplified. In this sense we focus on the biophysical mechanism, rather than on the biological mechanism that relates to the specific sequence of molecular events that are downstream in the cascade and that are involved in the ultimate, observed change in some constituent (e.g., intracellular Ca^{2+} level, radioactive thymidine incorporation, or ornithine decarboxylase activity) in a biological system.

Explicit inclusion of confounding influences within the biophysical model is also essential. Specificity is a critical concept in biology, as evidenced by the key role of specific enzyme catalysis in biochemical activity. Yet electric and magnetic fields are biochemically nonspecific. Essentially all biological molecules have some charge and are polarizable and will therefore experience an interaction with the local electric field. As is already well-established in bioelectromagnetics research, this property means that biological structures that concentrate the electric field are the most studied candidate interaction sites. Specifically, the cell membrane is widely viewed as the most likely site of interaction. In addition, however, nonmembrane macromolecules with significant charge (e.g., DNA) can be considered. In contrast, relatively few biological materials have distinguishing magnetic properties; a number of biological molecules have significant magnetic properties (e.g., ferritin), but biologically synthesized magnetite, which is present in the form of magnetosomes (bilayer membrane-enclosed magnetite; Fe_3O_4), stands out as having the largest magnetic moments by far. Thus, our approach is to consider cell membranes, highly charged mac-

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch005

romolecules, and biological magnetite as the starting point for the construction of theoretical models.

We also attempt to systematically include competing influences because the nonspecificity of extremely low frequency (ELF) fields warns us that any other source of changes of the same type of molecule is real competition. As discussed briefly herein, this competition has led to theoretical models based on signal-to-noise ratio (S/N) criteria. This approach corresponds to regarding the biological system as perfectly analogous to a measurement system: observing a biological effect is equivalent to EMF detection by the biological system. This approach leads naturally to the view that specificity, noise, background, interference, and modulating influences should all be considered in seeking to understand the conditions that result in biological effects due to EMFs.

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Changes Due to External Electric and Magnetic Fields As Signals

Measurements are fundamental to empirical science, as they provide the quantitative information regarding nature that allows hypotheses (theoretical models) to be tested. Briefly, measurement is a quantitative mapping of an experimental outcome into one of a series of "bins" (Figure 1). For example, if a parameter is measured with a resolution of 1%, this value means that the output is assigned to a particular single bin of 100 bins. If 0.1% resolution is obtained, 1000 bins are involved. In this view, the measurement resolution is described by the number of bins in some parameter space. Fluctuations [noise (N)] that blur the outcome

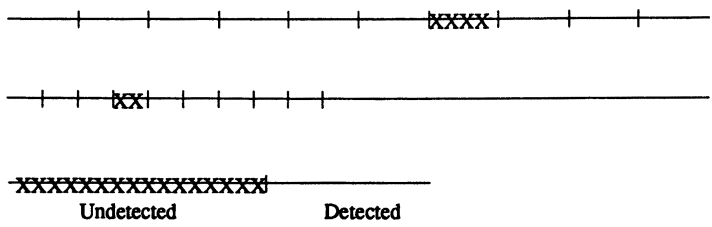

Figure 1: Schematic illustration of the measurement process, in which an experimental outcome parameter, X, is assigned to a single, particular "bin". Here a bin is a range of X values that cannot be distinguished (cannot be resolved). Three illustrations are given. Top: a low-resolution measurement in which X is assigned to bin 3 out of 10 bins, which illustrates a measurement with 1/10 (10%) resolution. Center: a higher-resolution measurement in which X is assigned to bin 42 out of 100 bins, representing a 1/100 (1%) resolution. Bottom: the coarsest possible measurement, in which X is assigned to bin 2 out of two bins. This minimal measurement is regarded here as equivalent to detection, with the left bin corresponding to "undetected" and the right bin to "detected". In this context, the measurement threshold separates these two bins, for which a S/(B+N) criterion can be sought as an estimate.

cause spreading over more bins and therefore reduce measurement resolution. Similarly, competing events that alter the outcome [background (B)] also spread the outcome values. Thus, measurement precision is fundamentally related to how many bins can be distinguished in the assignment of experimental outcomes. Measurement accuracy is a broader issue. An accurate measurement has both small systematic errors and high precision (resolution).

In this sense, "detection" is defined as the coarsest possible measurement, namely, the assignment of the experimental outcome to one of only two bins ("detected" or "not detected"). The analogy with bioelectromagnetic effects is that an effect is (or is not) truly caused by the external field. That is, a true bioelectromagnetic response is analogous to detection. For this reason, constraints on causality can be estimated by considering physical aspects of the interaction of a field with a portion of the biological system. To date, most electric field threshold estimates have focused on fundamental thermal fluctuations and field interactions with the cell membrane, with particular attention to interaction candidates that involve the transmembrane voltage (*11–14, 18–20*). Others have considered extracellular electric field interaction candidates (*1, 21–23*) or magnetic field interactions that involve strongly magnetic material, particularly magnetite (*19, 24–29*), or magnetically directed radical production (*30–32*). The so-called ion resonance phenomena have been strongly criticized from the thermal noise viewpoint (*33, 34*), as there appears to be no way to treat the ubiquitous molecular collisions and still preserve "resonance". More recently, the EMF-induced change in molecular number, $(\Delta n)_{EMF}$, has begun to be considered as the basis of a more biological S/N criterion for detection (*16*).

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Generalized S/N Approach

Physical Basis. Here a view is adopted that makes an analogy with experimental measurement (Table I) (*11–14*). Essentially all measurements have a physical basis that allows assignment of an experimental outcome to one of a number of bins. A generally accepted but approximate criterion for determining

Table I. Measurement Analogs for Bioelectromagnetic Interactions

Quantity	*Symbol*	*Significance*
Signal	S	Environmentally generated change
Background	B	Biologically generated change
Interference	I	Non-EMF competition
Noise	N	Change due to fundamental fluctuations

NOTE: The change associated with an interaction site is the basis for comparison of these three possible sources. An estimate based on a generalized signal-to-(background + noise) ratio, S/(B+I+N), can then be sought. EMF is electromagnetic field.

if a signal (S) is detectable is based on comparing the energy of the signal plus confounding energy changes to the energy change of the background and noise taken separately. A key ingredient in this approach is identifying the appropriate interaction energy, which usually means finding a portion of the biological system (e.g., the cell membrane) that (1) plausibly experiences energy changes due to field changes and (2) couples to biochemical processes. Only then can a biological system be altered.

This approach assumes that the background fields and the appropriate noise can be estimated, so that a corresponding energy change can be determined. First, both the background and noise are formulated in terms of the same parameter (e.g., equivalent electric field within the cell membrane, E_m). Then the associated background and noise powers are computed and time-integrated to obtain the energy. The background and noise will usually have different power spectra, as background is associated with biological processes such as neural activity, cardiac electric behavior, and electrokinetic phenomena associated with moving tissue and body fluids. For this reason, the power as a function of frequency should be separately sought for background and noise.

Interactions may occur predominantly within a narrow frequency band, Δf. Thus, if the interaction mechanism responds mainly within Δf, then only the contributions between frequencies f and $f + \Delta f$ are included. By estimating the energy of the "signal + background + noise" and comparing it to the energy of the "background + noise" alone, a quantitative criterion for detection is obtained. Typically this comparison is done by calculating a ratio of signal to (background + noise), designated S/(B+N). However, for an unambiguous response, S/(B+N) might, for example, be 1, 2, or 10. This latitude emphasizes that a S/(B+N) approach provides only an estimate, not a rigorously sharp threshold. In short, the idea is that the condition, S/(B+N) > 1, plausibly estimates that a true response could occur.

An illustration of the general approach has been previously described (*11–14, 19, 20*) that used the change in the membrane field energy associated with transmembrane voltage changes, $\Delta\psi$. When a transmembrane voltage-responding interaction such as channel gating is considered, the entire membrane is involved, so the quantity to consider is the electric field energy, W_m, within the entire membrane

$$W_m \approx \frac{1}{2} \varepsilon_m \int E_m^2 \, dV_m \tag{1}$$

where dV_m is an incremental membrane volume, ε_m is the permittivity of the membrane, and the integral is over the total membrane volume.

The signal is the change in the membrane field energy, ΔW_m, which is caused by a change, ΔE_e, in the external field, E_e. For simplicity, the cell membrane is represented by an inert membrane with zero time-average transmembrane voltage. In this case, the change in the membrane field energy caused by the external electric field is

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$$\Delta W_m \approx \frac{1}{2}\varepsilon_m \int (\Delta E_{m,S})^2 \, dV_m \quad \text{where} \quad \Delta E_{m,S} = f(\Delta E_e) \qquad (2)$$

where $\Delta E_{m,S}$ is the change in membrane electric field associated with the external field. The function $f(\Delta E_e)$ is introduced to describe how the membrane field changes at different sites over the membrane as the external field changes. For example, for a spherical membrane with radius r_{cell} and membrane thickness d, $\Delta E_m = (1.5 r_{cell} \Delta E_e \cos\theta)/d$, where θ is the angle between the applied field and the site of interest on the membrane. For simplicity, previous estimates have used the maximum value of this change, $\Delta E_{m,max} = 1.5 r_{cell} \Delta E_e / d$. Although the particular cell geometry controls the detailed response that gives $\psi(t)$ at different locations on the membrane, the change in transmembrane voltage is generally proportional to the electric field, E_e, within the aqueous extracellular medium. Importantly, E_e is the applied electric field within the aqueous medium external to a cell and is not an air field.

For in vivo conditions, the biologically generated background fields should also be considered. Although in general not yet understood quantitatively, such background fields could presumably be determined eventually; they could then be characterized by $E_{e,B}$ at the same site for which E_e is specified. This characterization means that the associated energy change created within the membrane can be estimated by using eq 2 with $\Delta E_{m,B}$ substituted for $\Delta E_{m,S}$. The dependence on frequency f gives the power spectrum of the background field. Moreover, fundamental noise results in randomly fluctuating changes in the membrane field energy, and this phenomenon is described consistently by again using the corresponding transmembrane noise field, $\Delta E_{m,N}$, in eq 2 to estimate the corresponding fluctuating energy changes within the membrane.

The external field change, ΔE_e, that is required to cause an effect can be estimated by considering the contributions of both ΔE_e and the confounding field changes, $\Delta E_{m,B} + \Delta E_{m,N}$ (background + noise contributions), to the total energy change. One way of making the comparison is through the ratio

$$\frac{(S + B + N)}{(B + N)} \equiv \frac{(\text{Energy})_{S+B+N}}{(\text{Energy})_{B+N}} \approx \frac{\Delta W_{m,S+B+N}}{\Delta W_{m,B+N}} \geq 2 \text{ (for detection)} \qquad (3)$$

Thus, for example, $\Delta W_{m,S+B+N}$ is the change in membrane field energy for the superposed equivalent signal, background, and noise fields. Equation 3 is equivalent to requiring the signal-to-(background + noise) ratio to exceed 1 for detection. That is, if S/(B+N) > 1, detection is expected. However, this approach does not yield a sharp threshold for causality, only an estimate.

Molecular Basis. A much more biological approach is based on the assertion that fields do not change biological systems, but rather molecules do. This assertion must be true because the biological effects of interest persist beyond the time of field exposure. To estimate thresholds a quantitative criterion

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based on molecular changes is needed. Our approach (*16, 17*) assumes that if a biological effect is to be interpreted as resulting from an EMF exposure, then an EMF-induced molecular change must be comparable to changes due to other, competing influences. With this in mind we focus our attention on the change in the number of accumulated molecules in a biochemical pathway. Rates themselves could be considered, but molecular (or ionic) rates are subject to greater fluctuations than the cumulative number of molecules or ions. Thus, emphasizing molecular accumulation should lead to the most electrically sensitive possibilities, that is, the smallest thresholds.

The "granularity" of molecular and ionic systems is critical to understanding fundamental limits for molecular changes. Biochemical reaction rates and transport are stochastic at the molecular level, and Poisson statistics governs their fluctuations. These fundamental fluctuations are not readily apparent for two reasons. First, macroscopic systems such as common laboratory solutions and cell suspensions contain a large number of molecules. Second, biochemical assays involve long times (seconds or longer) compared to molecular collision times ($\approx 10^{-12}$ s). Fluctuations at the molecular level are therefore usually not apparent.

Nevertheless, fundamental random fluctuations in molecular numbers (n) provide an inescapable limit for the changes that can be regarded as causal. Specifically, Poisson statistics applied to molecular processes leads to the familiar error: $(\Delta n)_{shot} = \sqrt{n}$ ("shot noise"). Generally, if a biochemical reaction or transport process is characterized by its average net rate, $\bar{J}$, this average can be regarded as due to the difference of a forward rate, J_+, and backward rate, J_-. In this case, the random fluctuation in molecular number for an exposure time t_{exp} is $(\Delta n)_{shot} = \sqrt{(J_+ - J_-)t_{exp}} \approx \sqrt{2\bar{J}t_{exp}}$. To be regarded as the cause of a biological effect, an EMF-induced change must at least be comparable to $\sqrt{2\bar{J}t_{exp}}$. Otherwise the "effect" could not be distinguished from changes that occur randomly, that is, without an external cause. This constraint on causality is equivalent to a S/N criterion in which the signal is the field-induced molecular change, $(\Delta n)_{EMF}$, and the noise is $(\Delta n)_{shot}$. Of course, still larger molecular changes may well be needed to have biological significance; this problem is more difficult and to our knowledge has not been thoroughly addressed, even in chemical toxicology, in which similar issues of potential human health hazards are routinely considered.

Background in this case consists of naturally occurring fluctuations in endogenous levels of the specific molecules. These fluctuations immediately point to a major difficulty: if an EMF alteration in molecular number is to be regarded as causal, then we need to know the frequency of occurrence of naturally occurring changes. Sometimes their frequency is known (or can be observed, e.g., normal Ca^{2+} fluctuations or oscillations), but if a relatively distant downstream event is what is actually measured, then the level of background must be inferred. In either case a first approach can be sensibly based on using fundamental

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random fluctuations in molecular number (shot noise). If this approach leads logically to a predicted threshold that is much higher than found experimentally, then this candidate mechanism must be eliminated as a possible explanation of the biological effect.

Interference (I) is the change in molecular number due to non-EMF influences and is another source of competition. It is considered in analogy with interference in measurements and is critically important if an interaction is not biochemically specific. This source of competition is widely appreciated in biochemical measurement science. For example, specific ion electrodes generally produce significant responses for many ions other than the one for which a response is intended. With biological effects due to EMFs, mechanical perturbations of cells and tissues can result in molecular changes, and the change in molecular number due to this general, non-EMF influence is a very relevant example of interference (*17*).

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Implications for Design of In Vitro Experiments. One generalized S/N approach is based on physical criteria and incorporates biologically generated background electric fields (*13*). This S/(B+N) approach also suggests how exposures might be provided in experiments. Noise is fundamental, so for this reason in vitro cell culture involves noise. Thus, fundamental membrane noise (thermal noise, $1/f$ noise, shot noise-associated ion transport, and channel noise) (*18, 35–37*) is expected. Fundamental noise, however, is primarily associated with microscopic systems (e.g., the volume of the cell membrane). Macroscopic imposition of a spatially coherent electric field with random time variation is not equivalent true noise, as noise fluctuations are uncorrelated between individual (noncontacting) cells in a culture.

However, in vitro studies generally do not attempt to simulate biological background fields. Presumably this absence is partly due to our quantitative ignorance of the total fields associated with time-varying biopotentials and electrokinetic phenomena associated with moving fluids and tissues. Background fields should be predominantly electric fields, as magnetic fields induced by electric currents of biological origin are extremely small, and those associated with magnetite should be highly localized, that is, within a few micrometers of magnetosomes (*24–26*). Nevertheless, the naturally occurring fields comprise an inescapable source of energy changes that should compete with any changes caused by external fields.

As suggested previously (*13*), the design of in vitro experiments can include the simulation of background fields, once they have been fully determined for in vivo conditions. For background electric fields associated with distant sources, a recorded background field could be superposed upon the applied external field whose effect is to be studied. If, however, the background source is nearby under in vivo conditions, then the spatial gradients of the background field may be important, and they could be simulated by a partial reconstruction of the in vivo anatomy. A systematic addition of simulated background fields to in vitro cell culture exposures could thus be sought. A progressive study begin-

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Advances in Chemistry; American Chemical Society: Washington, DC, 1995.

ning with conventional in vitro cell culture conditions (noise only) could proceed to systematically add simulated (recorded) background fields (background + noise). This simulation would in principle provide a systematic approach to investigating the causal thresholds for bioelectromagnetic effects due to external fields. The first steps have already been taken in studies (*38–40*) in which spatially coherent but temporally random electric fields were imposed during in vitro conditions, and the extinction of effects was then observed.

Combined Chemical and Field Exposures: An Expanded Hypothesis

The main thrust of established bioelectromagnetic research considers biological systems that are exposed to various electric and magnetic fields but generally does not explicitly consider the much more complex possibility that the biological system has also been exposed to foreign molecules. Clearly, increased complexity without compelling motivations should be avoided in hypothesis generation. Moreover, a hypothesis should not be taken seriously unless it is testable. As outlined in this section, the question of causality provides one such motivation, whereas the potential association of rare human disease with environmental fields provides another. Moreover, a specific version of this very general hypothesis also appears to be testable.

The most general form of the hypothesis is that combined chemical and field exposures might together alter biological systems. If so, one implication is that the energy needed to cause biological changes, for example, alteration of DNA, need not come entirely from electric or magnetic field energy. Instead, changes in chemical free energy can in principle contribute. A much more specific subhypothesis that makes this viewpoint clear is briefly presented now, along with its motivations (*41*).

Field-Stimulated Uptake. This specific subhypothesis assumes that transient magnetic fields cause stimulated uptake of otherwise cell-membrane-impermeant molecules because of magnetite-mediated pore creation (*17*), and these normally excluded foreign molecules interact with intracellular molecules (e.g., DNA) to cause alteration of the cell. Why should this hypothesis be considered? Two reasons are that at present this hypothesis is qualitatively consistent with what is associated with possible human health hazards, and it also may be able to satisfy conditions of causality.

The concern about, and study of, human exposure to toxic chemicals is well-established. It has an established scientific basis and is the focus of considerable research and regulatory activity. Here the expanded general hypothesis is that the toxic effects of some environmental chemicals are enhanced because of simultaneous or subsequent exposure to electric and magnetic fields. Although a direct field modulation of local chemical reaction rates could be considered, a much larger effect is likely if fields act so as to provide access of foreign chemicals to intracellular reaction sites.

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The underlying hypothesis for most bioelectromagnetic research is that direct interaction of fields with normal biological systems is capable of causing effects, some of which are deleterious. But a persistent difficulty is that the interaction mechanisms and associated threshold conditions are not well established for weak field conditions. Moreover, much of the research has been directed (*1, 2, 4, 8*) to steady power-distribution conditions (50- and 60-Hz sinusoidal fields).

Motivations. Nonsteady environmental fields include occasionally encountered local sources (e.g., domestic appliances and industrial equipment) and power-distribution transients, but such fields have not received much attention. The specific hypothesis considered here is that transient electric and magnetic fields may occasionally be large enough to satisfy a generalized S/N condition and also to cause uptake of foreign molecules that are present because of a previous chemical exposure. These foreign molecules may not be the usual toxic molecules, as most recognized toxic molecules are lipophilic and therefore passively cross the cell membrane. Instead, water-soluble molecules, particularly charged molecules, may be involved. The stimulated uptake hypothesis is therefore motivated by the following considerations.

1. Alteration of a very small subpopulation of cells, N_{min}, can have significant biological consequences (*see* next section). With mutations, alteration of only one cell can be significant. Chemical-induced mutations are well-known: their required energy originates from chemical free energy. Thus, if a transient magnetic field can cause molecular uptake of an external mutagen, then a significant biological effect is achieved, but the energy involved in mutation comes mainly from chemical free energy, not field energy.
2. Residential magnetic field transients of many different types have been found recently (*42, 43*), some of which have relatively large magnetic fields (B) and/or time rates of change (dB/dt). Still larger values may occur, but far less often, and could cause uptake even if they are rare.
3. Rare human diseases are considered in epidemiological studies that involve environmental electric and magnetic fields (*2, 4, 6, 7*). A requirement that both a prior chemical exposure and a magnetic field exposure occur will decrease the frequency of occurrence of any effects, with only chemically exposed individuals susceptible.
4. Rare but large transients could exceed endogenous fields (fundamental noise plus biologically generated fields) (*13, 44, 45*) within the human body and thereby overcome a fundamental objection that endogenous fields are usually believed to be much larger than those caused by environmental fields. However, a more severe constraint appears to originate in "mechanical interference", which is the normally occurring uptake into the cells of some tissues because of normal tissue strain. Considera-

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch005

tion of this form of competition has led to the hypothesis that mechanically protected tissue surrounded by bone (bone marrow or brain tissue) should therefore be further considered (*17*).

5. As suggested in the following paragraph, transient magnetic fields may cause stimulated uptake by cells. Candidate processes are magnetite-mediated pore creation, "rare electroporation" or alteration of metabolically driven transport pathways. Present understanding of field-stimulated uptake does not extend to conditions involving multiple exposures (pulses) with weaker field strengths and has also not quantitatively considered the role of mechanical stress at the cell membrane level, even though purely mechanical stresses operating alone have been reported to cause uptake of large foreign molecules. Thus, stimulated uptake by a small cell subpopulation cannot yet be ruled in or out.

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Some qualitative expectations can be stated. Transient magnetic fields (1) could interact with magnetic material, particularly biological magnetite (*26, 27*), and result in direct magnetic field effects, and (2) could induce electric fields and cause altered transmembrane voltages. In direct magnetic field interactions for very large B, large rotational displacements of magnetite may cause membrane openings due to mechanical perturbation of cell membranes (*17*). This process would represent an extension of normally occurring mechanically stimulated uptake (*46, 47*). In indirect magnetic field interactions, two known possibilities for electric-field-stimulated uptake of impermeant molecules are (1) electroporation and (2) modulation of active cell membrane transport processes. In the first case, infrequent electroporation may occur given the stochastic nature of pore formation and, more importantly, the possibility that reduced pore formation energy may occur in association with phospholipid–protein boundaries. In the second case, cells are known to use chemical energy to carry out active transport across cell membranes, and this observation has led to development of theories for electroconformational coupling (*48–51*), a general process involving membrane enzymes that results in transmembrane voltage modulation of transport across cell membranes. The magnetite-mediated pore creation hypothesis now appears the more likely for causing potentially significant uptake into the cytoplasm of cells in the presence of competing influences (*17*).

Significance of Extremely Small Cell Alteration Probabilities and Rates. Two order of magnitude estimates that are now reviewed serve to illustrate the possibility that extremely small cell alteration probabilities and rates are consistent with the stimulated uptake hypothesis. These estimates assume that a subpopulation of N_{min} cells is chemically altered by uptake of an unspecified number of foreign molecules. The hypothesis is based partially on the established fact that chemical alteration of a small number of cells can result in alteration of a much larger biological system such as the human body. In principle, even alteration of one cell ($N_{min} = 1$) because of uptake of foreign molecules could be significant. Following a previous presentation (*41*), the rate of occurrence of cell

alteration is written simply as

$$\nu_a = \nu_t N_{cell} P_{cell} \tag{4}$$

where ν_a is the rate of altered cells; ν_t is the rate of transients, here assumed identical; N_{cell} is the number of cells in the biological system; and P_{cell} is the probability of alteration of a single cell by a transient. If some minimum number of cells, N_{min}, is altered, then an effect on the entire biological system is presumed to follow.

As a first illustration (*41*), we estimate the rate of uptake events due to whole-body magnetic field transients that causes alteration of approximately one million cells ($N_{min} \approx 10^6$) per year (exposure time $t_{exp} \approx 3 \times 10^7$ s) within the human body ($\approx 10^{14}$ cells). For this level of cell alteration to occur, the necessary product of ν_t and P_{cell} is

$$\nu_t P_{cell} = \frac{N_{min}}{N_{cell} t_{exp}} \approx \frac{10^6}{(10^{14})(3 \times 10^7 \text{ s})} \approx 3 \times 10^{-16}$$

whole-body transients per second (5)

For this reason, the rate of effective transients could be very small and yet significant.

In a similar illustration (*41*) we consider the case of a highly localized transient source, such as a dimmer switch, which produces transients at the power-frequency rate (60 Hz in the United States and 50 Hz in Europe). Specifically, we assume that a 1-mL tissue volume is exposed, that is, $N_{cell} \approx 10^9$ cells. We also use a plausible exposure time on the order of 100 s/day, so that the number of transients in 1 year is $N_t = (365)(100 \text{ s})(60 \text{ transients per second}) = 2 \times 10^6$ transients. This result means that if again $N_{min} = 10^6$, a 0.1% subpopulation in this localized volume is altered, and the required probability of cell alteration per transient need be only

$$P_{cell} = \frac{N_{min}}{N_{cell} \nu_t t_{exp}} = \frac{N_{min}}{N_{cell} N_t} \approx \frac{10^6}{(10^9)(2 \times 10^6)} \approx 5 \times 10^{-10} \tag{6}$$

In these order of magnitude estimates, clearly the parameters ν_t, P_{cell}, and N_{min} are important. Most of our ignorance is contained in these parameters, particularly ν_t and P_{cell}. Physical monitoring and surveys of field transients should determine the spectrum of environmental transients (*42, 43, 52*), which here is represented by only a simple mean rate, ν_t. The parameter P_{cell} is expected to be much more complex, as it should depend on the transient magnitude and time dependence, the cell type and biological state, and the type and concentration of foreign molecule. In vitro studies should be capable of elucidating P_{cell} for varied conditions, particularly the transient characteristics.

With this in mind, extremely small cell participation probabilities or rates (fraction of cells affected per time) could arguably be of potential significance.

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Such estimates illustrate the unexplored hypothesis that field-stimulated rare uptake events might be significant for complex biological systems with a large number of cells, for example, the human body. To date, however, essentially no investigation has been carried out for electroporation or other field-stimulated uptake processes that involve multiple pulses, reduced field amplitudes, and very small subpopulations (*41*).

Candidate Interaction Mechanisms

For both pure exposures (field + biological system) and combined exposures (chemical + field + biological system), both electric and magnetic field interactions should be considered. However, with the possible exception of some environmental transients, air electric fields probably result in tissue electric fields that are too small to be taken seriously. In contrast, low-frequency magnetic fields penetrate the body with negligible attenuation, and therefore both direct magnetic field effects (Table II) and induced electric field effects (Table III) are

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Table II. Direct Magnetic Field Interaction Candidates

Type of Interaction	Significant *F*eature	Ref.
Radical reaction modulation	Immune to thermal fluctuations	30–32
Magnetosome rotation	Membrane gating or membrane pore creation	17, 26
Magnetosome lateral displacement	Membrane puncture; requires strong fields	41

Table III. Electric Field Interaction Candidates

Type of Interaction	Significant *F*eature	Ref.
Gated channels	Ubiquitous, transmembrane voltage controlled	57
Membrane enzyme changes	Coupling to membrane macromolecules	58
Lipid-domain membrane fluctuations	Suggested alternative to transient pores	59
Free volume fluctuations	Membrane transport of nonpolar species	60
Transient hydrophilic pores	Stimulated uptake of foreign molecules	41
Membrane macromolecule protrusion changes	Candidate signaling change mechanism	
Extracellular current–charge interactions	Cell density dependent	21
Soluble molecule–electric field interactions	Independent of cells	61

In Electromagnetic Fields; Blank, M.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1995.

candidates. These interaction possibilities are not discussed further here, as they are treated in the original literature. The main point for the present discussion is that, as illustrated in this section and discussed in the literature, models of interaction mechanism can be combined with background and noise to estimate causal thresholds.

Estimates of Thresholds. ***Physical Basis.*** Application of the S/(B+N) ≥ 1 criterion for zero background and only thermal noise has provided simple estimates of minimum electric fields, $E_{e,min}$, to which a cell should respond (*11*). This estimate can be accomplished by using eq 3 written in terms of changes in the transmembrane voltage, $\Delta\psi$, and setting $\Delta W_S \approx \delta W_N$, the fluctuation in energy. For the broad-band case this approach is equivalent to using the equipartition theorem with the capacitance (C) of the membrane (*14*), that is

$$\frac{1}{2}kT = \frac{1}{2}C\overline{(\delta\psi)^2} \tag{7}$$

where k is the Boltzmann constant, T is the absolute temperature, $\delta\psi$ is the transmembrane voltage fluctuation, and the bar denotes a time average. Equation 7 led to a broad-band estimate for the minimum electric field for a response by a spherical cell

$$E_{e,min} \approx \frac{2(\delta\psi)_{kT}}{3r_{cell}} = \frac{2}{3}\left(\frac{kTd}{\pi\epsilon_0 K_m}\right)^{1/2}\frac{1}{r_{cell}^2} \tag{8}$$

where ε_0 is the permittivity of a vacuum and K_m is the membrane dielectric constant. This approach can be applied to other cell shapes (e.g., elongated cells) and can be extended by considering mechanisms that lead to limited frequency bands (Δf) of response and the possibility of signal averaging by the accumulation of transported molecules via the mechanism of electroconformational coupling (*12*) (*see* next section).

Molecular Basis. A careful detailed treatment (*16*) of cellular accumulation of molecules through a rectification process yielded a very surprising result: Contrary to our strong expectations, this signal-averaging process did not yield thresholds below the thermal noise limit (*12*) that had earlier been obtained using purely physical criteria (EMF-induced changes and thermal noise in the transmembrane voltage). We believe that this molecular criterion is correct and more appropriate for a biological system. Not only are fundamental fluctuations involved, but the granularity of molecular processes and the limited rates at which real biochemical processes can proceed conspire to limit the rate at which significant changes in molecular number can accumulate. The effect of a very small field can thus become apparent after an extremely long exposure time, far too long to be relevant to environmental field exposures (*16*).

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The specific but very general prediction of the rectification model has been carried out for the important case of biochemical reactions that depend linearly on reactant concentrations. The primary effect is altered molecular accumulation, $(\Delta n)_{EMF}$. For a spherical cell of radius r_{cell}, this molecular change signal (S) is (*16*)

$$(\Delta n)_{EMF} = \bar{J} t_{exp} \approx \frac{e^{-U0/D}}{D^2} A' J_{cell,max} (zE_{rms} r_{cell})^2 t_{exp} \quad (9)$$

with $J_{cell,max} \approx 10^{12}$ s^{-1} (obtained by assuming a diffusion-limited flux to the cell surface), the largest possible basal activity, and $U_0 \approx 8kT$, an activation barrier for realistic biochemical rates. Here, $A' \cong 0$ to 0.025 is a constant, and E_{rms} is the root-mean-square electric field at the threshold exposure. Random ("white") noise is assumed, for which the spectral density is $D = kT$ for thermal noise and $D > kT$ for more realistic excess noise (*36*). The corresponding individual cell electric field threshold is

$$E_{1\ cell,min} \approx \frac{De^{U0/2D}}{zr_{cell}} (A' J_{cell,max})^{-1/4} (t_{exp})^{-1/4} \quad (10)$$

For thermal noise, a large cell radius $r_{cell} = 100$ μm, a membrane protein displacement charge of $z = 10$, and an exposure time $t_{exp} = 10$ h, the threshold is $E_{1\ cell,min} \approx 10^{-3}$ V/cm. If optimal stochastic resonance is involved (*53*), then $D = U_0/2$, and only about 20 min is needed to achieve the same threshold field (*16*). Achievement of lower individual cell thresholds is greatly constrained by realistic values of (1) basal reaction and transport rates ($J_{cell,max}$), (2) the displacement charge (z), and (3) activation barriers ($U_0 = 8kT$) because the implicit very large barriers that lead to the suggestion of large improvements in S/N by stochastic resonance (*54*) lead to very low rates and correspondingly large shot noise. The possibility of molecular accumulation for a longer time is also unrealistic because of the weak dependence on exposure time, $t_{exp}^{-1/4}$, and this dependence leads to impossibly long times. For example, to achieve a threshold of a few microvolts per centimeter one must wait on the order of 10^{32} s, which is longer than the present lifetime of the universe. A more realistic possibility involves the involvement of many electrically connected cells, particularly those that are organized to form anatomical structures that concentrate electric fields (*14, 55, 56*).

Summary

Causality is considered by reviewing an analogy between classical measurement science and electric and magnetic field interactions with biological systems. This approach leads to the ratio S/(B+N) as a basis for estimating threshold exposure conditions that should separate causal from noncausal cases. An expanded hy-

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch005

pothesis for bioelectromagnetic effects invokes combined chemical and field exposures, with a more specific subhypothesis that considers the possible role of environmental magnetic field transients in causing uptake of foreign molecules into a small but biologically significant subpopulation.

Acknowledgments

We thank T. E. Vaughan, W. C. Thilly, A. Sastre, R. Kavet, I. Guttman, P. Galley, C. Crespi, M. Bier, and R. K. Adair for many stimulating and helpful discussions. This work was supported partly by NIH Grant ES06010 and a computer equipment grant from Stadtwerke Düsseldorf, Düsseldorf, Germany.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch005

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Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch005

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RECEIVED for review June 19, 1994. ACCEPTED revised manuscript March 15, 1995.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch005

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch006

6

Comparison of Endogenous Currents in and Around Cells with Those Induced by Exogenous Extremely Low Frequency Magnetic Fields

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Several epidemiological studies have led to the suggestion that 60-Hz magnetic fields having flux densities of 1 μT or less could have adverse effects on human health. In this chapter I explore the question of whether currents induced by such magnetic fields are likely to be of consequence in comparison to ambient bioelectric fields in the human body. This comparison is accomplished in terms of extremely low frequency current densities at three levels of biological interest: (1) bulk tissue currents, (2) transcellular currents, and (3) extracellular (or pericellular) currents around cell surfaces. At all three levels, current densities likely to be induced by exposure to vertical 60-Hz, 1-μT magnetic fields are found to be much smaller than currents from biological background sources (such as active nerve and muscle). This conclusion still applies when only endogenous current components in the bandwidth near 60 Hz are taken into consideration.

EXPOSURE TO 60-HZ MAGNETIC FIELDS having a flux density (B) on the order of 0.1–1 μT (1–10 mG) could have dire consequences on human health, such as an increase in the occurrence of childhood cancers, according to some epidemiological studies (*1–4*). These studies have relied mainly on "surrogate indicators" of magnetic field strength (such as distribution-line wire configura-

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tions), and thus the statistical associations reported may not be intrinsically linked to magnetic fields. Nevertheless, these epidemiological studies have led to the notion that these commonly found 60-Hz magnetic field exposure levels constitute a type of threshold for producing deleterious bioeffects.

Can exposure to a 10-mG, 60-Hz magnetic field plausibly produce meaningful changes in human physiology (i.e., changes leading to health effects)? One approach to exploring this question is to compare the strength of these fields to those of ambient bioelectric fields, which are a vital part of the human body's natural operation. If the internal fields produced by exogenous sources (e.g., appliances or power lines) are adjudged to exceed the level of ambient bioelectric fields, then the notion that they could have meaningful physiological effects (either good or bad) is plausible. However, if the induced fields were determined to be well below ambient bioelectric levels, this finding would detract from the plausibility of their being bioeffective, or at least it would require a very cogent argument that shows how cells could detect and respond to such weak fields in the presence of much stronger ambient fields.

What is the appropriate anatomic level at which to make such a comparison between exogenously induced fields and ambient (biological background) fields? Mathematically this comparison is most simply made at the "bulk tissue level", that is, by calculating average current densities at various locales in the body and treating tissue as a homogeneous material. This sort of macrocomparison is, however, of very limited validity because the field intensity in and around particular cells determines the bioeffectiveness of an exogenous (or of an ambient) field. Therefore, in this chapter, I have attempted to compare the strength of the two field types at three anatomic levels of organization: (1) bulk tissue current densities; (2) current densities (and transmembrane potentials) across cells (and cell membranes); and (3) current densities in tight extracellular spaces that characterize most human tissue (e.g., muscle, nerve, and bone).

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch006

Bulk Tissue Current Densities

Estimating bulk tissue current densities from exogenous 60-Hz magnetic fields is fairly straightforward and can be accomplished by using a variety of human body compartment models. A somewhat simplified example of this approach is illustrated in Figure 1 for an exogenous magnetic field of 1 μT. Here the body is considered to be roughly cylindrical and an approximate value of 0.5 m is taken for the maximum radius. This rather generous estimate for the maximum torso radius in humans (which corresponds to a circumference of over 3 m) leads to an overestimate of induced current. This overestimate suggests a maximum induced electric field of 10^{-4} V/m at the surface of the cylinder that drops off to zero at the center of the cylinder. Using a typical tissue resistivity of 1 Ω·m yields a maximum current density of 10^{-5} A/m^2, which is actually a high-side estimate compared to those of other authors (*5*) and is probably attributable to two factors: using 1 μT rather than 0.1 μT, and using such a round body model.

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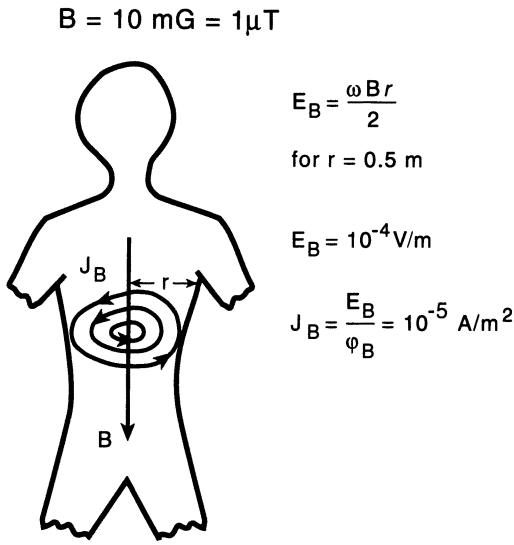

Figure 1. Estimation of bulk tissue electric fields (E_B) and current densities (J_B) produced in a prototypical human standing in a vertical 60-Hz magnetic field having a flux density (B) of 1 μT. r is the approximate torso radius, ω is the sinusoidal frequency (in radians per second), and ψ_B is the average tissue resistivity.

Current Densities Across Cells

Bulk current density is, however, of limited interest from a physiological point of view. The currents that flow in and around particular cells that constitute the tissue are of more relevance biologically. Determining such currents requires assumptions about cell size, shape, membrane impedance, and perhaps most importantly cell-packing tightness. Some modelers have chosen (5) to represent cells as essentially nonconducting spheres surrounded by much larger extracellular spaces. I believe, however, that the picture shown in Figure 2 is more representative of the cellular structure of most human tissue. Figure 2 shows tightly packed cells of various shapes that leave only narrow serpentine passages for extracellular currents to flow through. Taking the cross-sectional area of the extracellular spaces to be 10% of the total area yields roughly a 10-fold enhancement of current density in the extracellular (or "pericellular") spaces relative to bulk current because relatively little current flows across the cells themselves.

How small is this transcellular current? The answer depends on the membrane impedance ascribed to the cells (at 60 Hz). For resting cells (e.g., nerve or muscle) the typical membrane resistance (R_m) is 1000 Ω·cm^2 (or 0.1 Ω·m^2). This value suggests a transmembrane (and thus transcellular) current density (J_m) of only 10^{-7} A/m^2, which in turn suggests a transmembrane potential shift of only 10^{-8} V (compared to roughly 10^{-2} to 10^{-1} V for ambient transmembrane potentials).

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch006

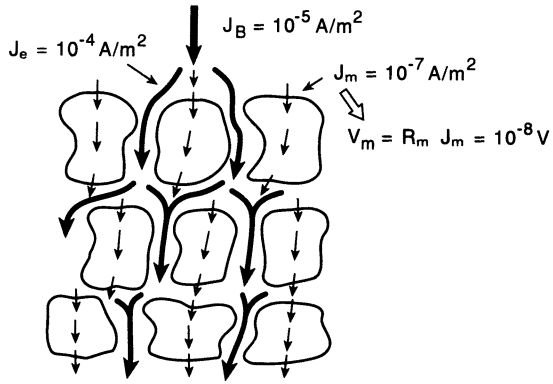

Figure 2. The partitioning of bulk current density (J_B) into extracellular (J_e) and transcellular (J_m) components. V_m is membrane potential; R_m is membrane resistance for a unit area (Ωm^2)

Current Densities in Tight Extracellular Spaces

Thus, exogenously induced currents are apparently much more dense outside cells than within (or across) the cell. However, endogenous (ambient) currents are also presumably stronger (denser) in these tight extracellular spaces. Estimating the strength of such ambient extracellular currents is a more difficult task, but we can approach the issue from the standpoint of some well-established electrophysiological principles, as follows.

When at rest, electrically active cells are known to typically have transmembrane potentials of about 0.1 V (inside negative); during the peak of an action potential this potential goes through a momentary reversal of polarity. The action potential therefore is a pulselike signal that ranges from about 1 ms in duration (typical of human nerve cells) to several hundred milliseconds (typical of human cardiac cells).

As shown in Figure 3, a cell that is completely activated (depolarized) or is completely at rest generates virtually no field outside its own membrane. However, when a cell is in transition from rest to active (or vice versa), currents external to the cell will flow in accordance with extracellular potential gradients. For cells that approximate a long thin cylinder, which is a reasonable representation of nerve axons or of cardiac and skeletal muscle cells, the transition region looks rather like a dipolar current source, and it gives rise to a field that spreads out exponentially (as illustrated in Figure 4). What is the current density in this extracellular region? An answer can be gleaned from the cable model, which neurophysiologists have long used to analyze action-potential propagation (*6, 7*). In this model the longitudinal current flow (from active to resting regions of the cell) is proportional to the spatial gradient of voltage, $\partial V/\partial x$, which can be expressed as the quotient, $(\partial V/\partial t)/(\partial x/\partial t)$. However, $\partial x/\partial t$ is simply the velocity θ

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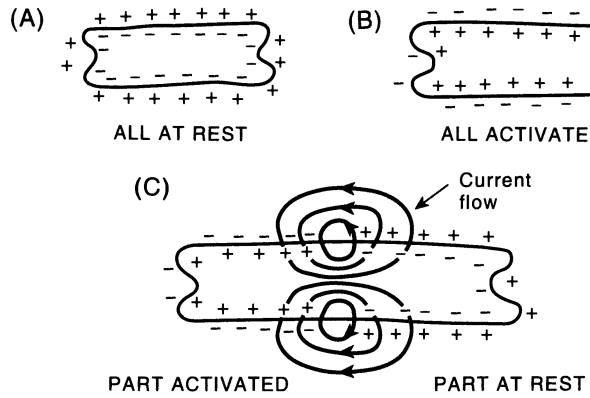

Figure 3. Fields produced by active nerve or muscle cells. A resting cell (A) or a fully activated cell (B) produces virtually no field away from its own membrane. When the cell is in transition from resting to active (i.e., during the action potential propagation), currents flow along the outer surface of the cell (pericellular space) as well as through the membrane and within the cell (C).

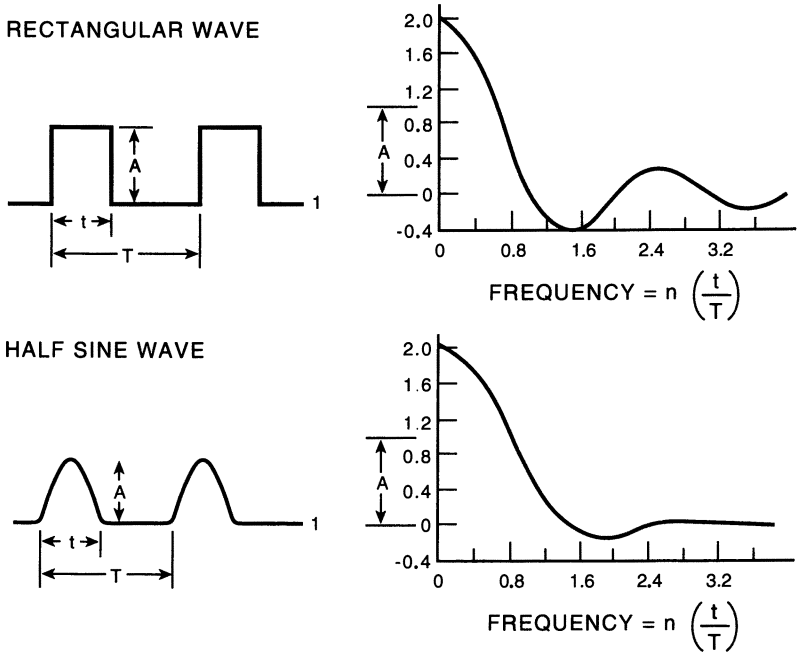

Figure 4. The frequency spectra associated with a rectangular wave and a "half-sine" wave approximation to a nerve or muscle action potential. The curves are shown as continuous functions to encompass all values of duty factor, but for any one of these a set of discrete values for the Fourier series components (harmonics) can be pinpointed. **A** *is the wave amplitude;* **t** *is the wave duration, and* **T** *is the interwave interval.*

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch006

with which the action potential is spreading along the cell. Thus the longitudinal current density J can be written as

$$J = \frac{\partial V}{\partial x}\left(\frac{1}{R_e}\right) = \frac{\partial V}{\partial t}\left(\frac{1}{\theta R_e}\right)$$

where θ is the propagation velocity (meters per second), R_e is the resistivity of the extracellular fluid (ohm meters), J is the longitudinal current density outside the cell (amperes per square meter), and ω is the radial frequency.

For a realistic action potential the rate of change of the transmembrane potential ($\partial V/\partial t$) depends on what point in the action potential is being referred to, and this dependence would lead to an analytically complex expression. Fortunately, however, nerve or muscle action potentials occur repetitively, and they can be treated in terms of their (sinusoidal) frequency components. Thus, if we represent each frequency component of transmembrane potential as $V(t) = V_m \sin \omega t$, the corresponding extracellular current density would become

$$J = \left(\frac{\omega V_m}{\theta R_e}\right)\cos \omega t$$

Some typical values for repetitively active nerve cells (such as those found in human cortex) are $V_m = 0.1$ V, $\theta = 10$ m/s, and $R_e = 5\ \Omega \cdot$m.

Applying these values results in a current density of $J = (2 \times 10^{-3})\omega$ (cos ωt), which denotes a peak current density of $(2 \times 10^{-3})\omega$ A/m^2. Thus, for $\omega = 500$ (which would be a representative firing rate of 80 Hz), this equation predicts a current density of 1 A/m^2.

Comparison with Exogenously Induced Tissue Current Densities

By comparison the tissue current density induced by a 1-μT, 60-Hz magnetic field impinging on the human brain is about 10^{-5} A/m^2. Because most of this extremely low frequency (ELF) current flows around cells, rather than through them, an intensification factor of perhaps 10-fold should be applied to estimate the induced extracellular current density. The resulting value of 10^{-4} A/m^2 is still about 10,000 times lower than the 1 A/m^2 estimate for extracellular current due to ambient neuroelectric activity.

Representing a pulselike action potential by a single sine wave (whose frequency is the repetitive firing rate) may be too crude a model for these purposes. The same concept can, however, be extended to other waveforms by de-

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch006

scribing them as a Fourier series of frequency components (harmonic analysis). This modeling can most readily be done by representing the action potential as a "standard" waveform. Figure 4 shows two waveforms that are reasonable facsimiles of an action potential. Although these two waveforms (rectangular vs. "half-sine" waves) look quite different in the time domain, their spectral contents are really quite similar (for equal durations and repetition rates). The main difference is the small amount of energy falling into the higher harmonic ranges for the rectangular wave. Neither of these waveforms are exact representations of an action potential shape; however, the half-sine wave is more realistic for neural or skeletal muscle action potentials, and the square wave better represents cardiac action potentials.

By using generalized Fourier transforms, these prototypical action potentials can be translated into a specific set of frequency components for any given neural firing pattern simply by choosing an appropriate value for the duty cycle (i.e., the pulse duration as a fraction of the interpulse interval). For example, as shown in Figure 5, using a duty cycle of 10% (which might correspond, for example, to a muscle action potential 10 ms long occurring every 100 ms) gives rise to the spectrum indicated by the dots connected by a dashed line. This spectrum is for the transmembrane signal per se; however, the extracellular current flow is proportional to the time derivative of V_m. Consequently, the frequency components of the extracellular current waveforms are somewhat exaggerated for the higher harmonics: in fact, a "two-humped" frequency spectrum is produced, and it gives peaks at the third and the tenth harmonics. This spectrum suggests that a nerve or a skeletal muscle firing at, for example, 10 pulses per

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch006

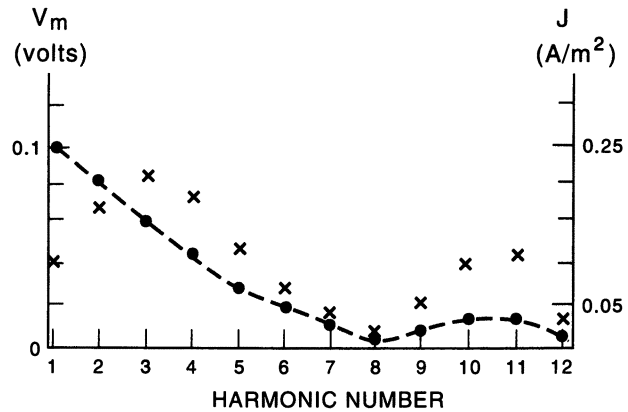

Figure 5. The harmonic content (Fourier series) for a half-sine wave action potential model having a duty factor of 0.1 and a repetition rate of 10 pulses per second. The transmembrane potential (V_m) components are shown as dots connected by a dashed line, whereas the harmonics of current density (J) are denoted by the × marks.

second would give rise to pericellular current densities on the order of several microamperes per square centimeter for frequency components ranging from 10 Hz to several hundred hertz. Several of these harmonics lie close to the 60-Hz frequency of power systems (and to the harmonics thereof). Thus, even if we restrict the comparison to frequencies close to 60 Hz, the intensity of pericellular ambient fields (current densities) is found to be far greater than that inducible by exogenous magnetic fields (e.g., 1 μT at 60 Hz).

Strictly speaking, the foregoing analysis applies to isolated cylindrical cells surrounded by large volumes of extracellular fluid. In reality, of course, brain tissue, and most other tissue as well, is made up of cells in tightly packaged arrays, so that extracellular spaces are actually smaller than the intracellular ones. In these circumstances endogenous bioelectric currents near the cell surface would actually be intensified, just as would currents exogenously induced by electromagnetic fields (EMFs); that is, they would be far more intense than the average (bulk tissue) current density for the overall tissue. This expectation is borne out by the fact that, within the brain, currents underlying the electroencephalogram (EEG) are on the order of a few nanoamperes per square meter. This value is roughly 1% of the current density estimated from the cable equation analysis for the space immediately surrounding a cylindrical cell. In addition to lending support to the calculated extracellular current values, this EEG field current density can itself be compared to the bulk tissue currents induced by a 60-Hz magnetic field in a standing human (in a vertical 1-μT field). This current is on the order of 10^{-5} A/m^2, which is about 0.001 times the EEG current density. These comparisons of extracellular current densities along with the even more disparate values for transmembrane current density are summarized in Table I, which shows that at each cellular level of interest the density of currents flowing in brain tissue due to endogenous neuroelectric activity far exceeds that induced by 60-Hz external magnetic fields (on the order of 1 μT).

A similar analysis can be applied to bioelectric current generated by skeletal muscle, as well as cardiac muscle electric activity, and would suggest that all types of cells (including blood cells) within those tissues are routinely immersed in an endogenous "sea" of electric activity. Furthermore, the fields generated by dynamically loaded bone cells (most likely due to streaming potentials) are also thought to be relatively strong. The bulk current density measured in such active bone is also on the order of a few milliamperes per square meter. These currents would impinge upon bone marrow cells as well as osteogenic cells, and thus they must be reckoned with in any theory that attempts to causally link leukemia with external magnetic fields (60-Hz, 1-μT range).

Table I. Comparison of Bioelectric Current Densities (A/m^2) Produced by Active Nerve or Muscle to Those Induced by a Magnetic Field

Tissue Level	*Produced by Active Nerve or Muscle*	*Induced by 1-μT, 60-Hz Magnetic Field*
Bulk tissue	10^{-2}	10^{-5}
Extracellular space	1	10^{-4}
Transmembrane currents	10	10^{-7}

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch006

Hypothetical Interactions with Weak Magnetic Fields

The foregoing analysis does not, of itself, rule out the possibility that weak alternating current (AC) magnetic fields could alter cellular function, but it does make such a conclusion harder to justify. Some hypothetical arguments could be raised to argue that weak signals generated in living tissue by external magnetic fields could be detected (and responded to) in the presence of much stronger endogenous electric activity. After all, from an engineering standpoint, systems can be designed that can extract signals from backgrounds (noise) thousands of times stronger than the signals. However, the imposed signal must contain some substantially unique property, such as its frequency content, its spatial coherence, or its pattern of repetitiveness over time, that could distinguish it from the bioelectric background. By applying this concept to the possibility of weak magnetic field detection by living cells, several conceivable mechanisms could be imagined:

- very specific frequency selection mechanisms (e.g., a receptor "tuned" to 60 Hz) (*8*)
- signal-averaging processes selective for induced field frequencies
- selective response to the spatial coherence of the induced field
- response to the magnetic field per se, rather than the electric field induced by it (e.g., via magnetite crystals tightly coupled to membrane channels) (*9*)

Endogenous bioelectric activity must be viewed as an important factor in determining what levels of ELF fields are sufficient to perturb normal biological function and thus what levels might be plausibly linked to health effects. Simply demonstrating that an external EMF could induce current flow in or around cells is not a cogent basis for predicting biological effects if those currents are orders of magnitude weaker than the ambient bioelectric background and if no biologically realistic signal-from-noise extractors are demonstrable.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch006

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RECEIVED for review June 19, 1994. ACCEPTED revised manuscript May 31, 1995.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch007

7

Endogenous Electric Fields Measured in Developing Embryos

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Through the use of the noninvasive vibrating-probe technique for detecting extracellular ionic currents as well as standard glass microelectrode techniques, electric fields have been detected in a wide variety of animal tissues. In five of these studies, the electric field was measured directly within the tissue with microelectrodes, and in the remainder it was inferred from the extracellular ionic currents detected. This chapter reviews the measurements of extracellular ionic currents around animal tissues as well as the electric field measurements made by penetrating the tissue with microelectrodes. The range of most endogenous electric fields is from 1 to 100 mV/mm. These data should provide a guide to the range of imposed direct-current electric field strengths that would be expected to influence normal biological functions in living organisms.

HUMANS ARE ELECTRIC BEINGS. Bioelectricity is used by every cell in the human body to perform a wide variety of daily tasks, from membrane transport to cell–cell signaling. Therefore, the first step in determining how environmental electric and magnetic fields might influence cellular function is to identify the endogenous electric fields in living systems. In this chapter I review the evidence for such physiological electric fields in living systems after first providing some basic background information for the nonbiologist so that the description of the endogenous electric field measurements can be more easily

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understood. Several previous reviews (*1–6*) provide a more comprehensive treatment of this literature.

All cells spend a significant amount of energy moving ions across their outer membrane (plasma membrane), which results in an ion concentration gradient across this membrane. This concentration gradient drives K^+ out of the cell through K^+-specific ion channels in the plasma membrane and leaves behind the negative counterions. The net result is a separation of charge across the plasma membrane, which generates a voltage called a membrane potential that is inside-negative and is typically –70 mV in most animal cells and –200 mV in most plant cells. This potential provides all cells with a built-in "battery" that is available to drive ionic currents through the cell. These currents or fluxes are involved in a wide variety of cellular mechanisms because the energy stored in this membrane potential is used for many cellular processes, including the transport of solutes into the cell, signal transduction, and even high-energy chemical bond formation. Not surprisingly, extracellular ion currents are thus commonly found around cells and can be associated with many different activities, as I will review here.

Transcellular ionic currents are by no means restricted to single cells. Multicellular systems are also found to generate ionic currents, mainly because of the nature of their outer layer, which is generally formed by an epithelial sheet of cells that are usually electrically coupled and pump ions across the sheet in a polarized manner. Thus, this sheet of cells is to the organ or organism that it encloses as the plasma membrane is to the cytoplasm that it encloses. One main difference is that most epithelia pump cations in an apical–basal direction, which generates an inside-positive potential, in contrast to the inside-negative potential generated by the plasma membrane in a single cell. Therefore, the natural voltage across an epithelium will drive positive ions *out* in regions of low resistance, whereas that across the plasma membrane will drive positive ions *into* regions of low resistance (typically ion channels in the plasma membrane).

The main experimental evidence for the existence of endogenous ionic currents and electric fields in living cells and tissues comes from two techniques: the extracellular vibrating probe, and glass microelectrodes coupled to high-input impedance amplifiers. These techniques will be briefly explained, and their use in living systems to measure endogenous electric fields will then be discussed.

Methods for Measuring Endogenous Electric Fields

The Vibrating-Probe Technique. The principle of this technique is to minimize the noise of the detection system and to signal-average over long times in order to detect small, steady ionic currents. One can calculate the voltage sensitivity required from Ohm's law in a continuous medium

$$I = E/\rho = -(1/\rho)(\Delta V/\Delta r) \quad (1)$$

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch007

where I is current density, E is the electric field strength, ρ is the medium resistivity, and ΔV is the voltage gradient measured over the small distance, Δr. For $I = 0.1$ $\mu A/cm^2$, and $\rho = 25$ $\Omega \cdot cm$ (seawater), this voltage gradient will be only 6 nV over a 20-μm displacement, 25 μm outside the cell's plasma membrane. Standard 3 M KCl-filled microelectrodes have resistances on the order of 10^6 Ω, and their noise places a limit of 10^{-5} V on their resolution. They are therefore not sufficiently sensitive to detect the required 6-nV potential.

Jaffe and Nuccitelli (*7*) developed a new technique 20 years ago called the vibrating probe that has much greater sensitivity and can detect such small voltages. The main idea is to lower the electrode's noise about 1000-fold both by reducing its resistance and by signal averaging over time using a phase-sensitive lock-in amplifier. The electrode's resistance is reduced by replacing the fluid-filled pipette with a metal sphere with a relatively large capacitance. By vibrating this capacitor between two points at different potentials, the charge on the capacitor fluctuates, and the voltage is detected through these charge variations. Thus, by essentially capacitatively coupling to the external field, the effective resistance is now an impedance that will be reduced as the vibration frequency and capacitance are increased. A lock-in amplifier is used to detect the voltage change; it amplifies only those signals exhibiting the vibration frequency and eliminates interference from other common sources of noise such as the 60-Hz line voltage. Two-dimensional maps of the extracellular current density around living cells and tissues are now routinely generated by computer when the probe is translocated simultaneously in two dimensions (*8, 9*).

This vibrating-probe technique has been used in about 30 laboratories around the world, and a National Vibrating Probe Center is now sponsored by the National Institutes of Health at the Marine Biological Laboratory at Woods Hole, Massachusetts. Literature (*6, 10*) on the use of the vibrating probe to study endogenous ionic currents in both animal and plant systems is available. An updated summary of the ionic currents measured near multicellular animal systems is included in this chapter (Table I). As Table I indicates, endogenous ionic currents on the order of 10–100 $\mu A/cm^2$ have been measured in a variety of tissues from more than 20 different animal species, including both invertebrates and vertebrates. As such currents traverse tissues, they generate endogenous, DC (direct current) electric fields that will be proportional to the resistivity of the extracellular space they traverse. The bulk resistivity of tissues is typically on the order of 1000 $\Omega \cdot cm$ (*69*), and thus current densities of 10–100 $\mu A/cm^2$ are expected to generate endogenous electric fields in the range of 1–10 mV/mm. This range of field strengths must be considered when one evaluates how environmental electric fields might influence living organisms. On the basis of these data, imposed electric field perturbations in the 1–10-mV/mm range could be expected to influence many cellular processes that utilize these fields.

Direct Measurement of In Vivo Electric Fields Using Microelectrodes. Although the likely in vivo electric fields can be estimated by

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch007

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Table I. Ionic Currents Around Multicellular Animal Tissues

Species	*Tissue Type*	*Current*	*Ref.*
Noctiluca miliaris	Dinoflagellate sulcus	11 $\mu A/cm^2$ enters middle of sulcus during localized cytoplasmic movement associated with feeding.	11, 12
Hyalophora cecropia	Oocyte nurse-cell syncytium of silk moth	15 $\mu A/cm^2$ enters anterior, nurse-cell end of growing follicle and leaves most of oocyte.	12
Rhodnius prolixus	Insect telotrophic ovariole	5–10 $\mu A/cm^2$ enters previtellogenic oocytes and leaves oocytes entering vitellogenesis.	13, 14
Drosophila	Insect follicle	5 $\mu A/cm^2$ enters nurse-cell end of follicle and leaves near the oocyte end.	15, 16
Blattella germanica	Cockroach vitellogenic oocyte	10 $\mu A/cm^2$ leaves dorsal region and enters ventral region of oocytes when larger than 0.8 mm.	17, 18
Lymnaea stagnalis; Bithynia tentaculata	Snail egg	0.2–0.6 $\mu A/cm^2$ leaves vegetal pole and enters animal pole. Maximum inward current at animal pole coincides with onset of cleavage; polar lobe formation is also preceded by inward current in that region.	19–22
Pomacea	Eyestalk of mystery snail	5–60 $\mu A/cm^2$ enters eyestalk stump after amputation and declines steadily to 2 $\mu A/cm^2$ in 50 h.	23

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Petromyzon marinus	Lamprey spinal cord	500 $\mu A/cm^2$ enters freshly cut surface spinal cord and falls to 100 $\mu A/cm^2$ within 1 h and 4 $\mu A/cm^2$ in 2 days.	24
Turtle	Urinary bladder epithelium	Outward current was produced by individual cells rich in carbonic anhydrase, and this current quantitatively accounted for total epithelial acidification current.	25
Oryzias latipes	Mid-epiboly fish embryo	No currents in intact embryos, but 5-$\mu A/cm^2$ current pulses enter wounds near pacemaker region with 90-s period.	26
Sarotherodon mossambicus	Fish gill epithelium	400 $\mu A/cm^2$ enters epithelium in highly localized regions above Cl^- cells and is carried by Cl^- efflux.	27–31
Bufo viridis; Rana pipiens	Toad skin epithelium	5–100 $\mu A/cm^2$ leaves mitochondrial-rich cells carried by Cl^- influx.	32–34
Necturus maculosus	Mud puppy gastric mucosa	Up to 500 $\mu A/cm^2$ enters oxyntic cells of gastric crypts carried by Cl^- efflux and 5 $\mu A/cm^2$ enters surface cells by Na^+ influx.	35
Xenopus laevis	Neurula stage embryo	A few microamperes per square centimeter enter most of embryo's surface, and 10 $\mu A/cm^2$ exits the blastopore carried largely by Na^+. Wound healing in these embryos depends on an endogenous Na^+ current.	36–38

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Table I. Continued

Species	*Tissue Type*	*Current*	*Ref.*
Xenopus laevis; Ambystoma mexicanus	Developing hindlimb	7 $\mu A/cm^2$ leaves prospective hindlimb region of stage 43 *Xenopus* embryos, and 0.5–3 $\mu A/cm^2$ exited the same region 1 week prior to placode formation in axolotl embryos.	39, 40
Notophthalmus viridescens	Regenerating newt limb	10–100 $\mu A/cm^2$ leaves stump of amputated limb during 5–10 days after amputation.	4, 41–46
Rana pipiens	Limb stump	20–40 $\mu A/cm^2$ leaves stump of amputated limb 1–10 days after amputation, but currents are depressed in center.	4, 43
Chick	Embryo: primitive streak stage	100 $\mu A/cm^2$ leaves whole streak and enters elsewhere through epiblast.	47, 48
Chick	Embryo: 3-day-old, stage 14–22	100 $\mu A/cm^2$ leaves posterior intestinal portal and enters intact epithelium over remainder of embryo.	49
Chick	Intestinal epithelium	40 $\mu A/cm^2$ enters villi and leaves crypts in open-circuit mode.	50
Rat	Lumbrical muscle	3 $\mu A/cm^2$ enters end-plate region of neonatal muscle up to 5 days after birth when the current reverses and 7 $\mu A/cm^2$ leaves end-plate region.	51–57

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Rat	Lens	Up to 60 $\mu A/cm^2$ enters anterior pole and leaves equator of freshly removed lens.	58–62
Rat	Cultured lung epithelia	Up to 30 $\mu A/cm^2$ exits localized areas of fluid accumulation.	63
Mouse	7-day embryo	20–60 $\mu A/cm^2$ exits along the midline of the embryo with inward current along the extraembryonic ectoderm.	64
Mouse	Metatarsal bone	10 $\mu A/cm^2$ enters articular surface of epiphyses with less dense current entering remaining epiphyseal regions. 100 $\mu A/cm^2$ enters fractured regions.	65
Cow	Wounded cornea	55 $\mu A/cm^2$ exits epithelial wounds, generating 42 mV/mm in the first 0.25 mm from the wound edge.	66, 67
Human	Regenerating fingertip	20 $\mu A/cm^2$ exits stump of fingertip amputation for an average of 8 days following amputation.	68

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applying Ohm's law to the current measured leaving or entering a tissue, measuring the in vivo field directly by placing glass microelectrodes into the tissue is preferable. The main drawback of this technique is the tissue damage resulting from microelectrode impalement and the subsequent injury currents that flow out of the low-resistance pathways usually generated by electrode penetration. These injury currents might significantly alter the local electric fields being measured. Despite this drawback, this direct approach has provided reliable measurements in some embryonic systems.

Among the most recent studies are measurements of embryonic electric fields in chicks (*49, 70*) and amphibians (*71, 72*). Hotary and Robinson (*49, 70*) found that the 2- to 4-day-old chick embryo drives a substantial current of up to 112 $\mu A/cm^2$ through itself, and this current generates a measured voltage gradient of 20 mV/mm along a known migratory route for neural crest cells (Figure 1). One likely target for this endogenous field is the migratory neural crest cell because these cells have been found to actively migrate in vitro toward the negative pole of DC electric fields as small as 7 mV/mm (a process termed "galvanotaxis") (*73–76*). These studies indicate that endogenous fields large enough to influence crest-cell migration do indeed exist in the chick embryo at the proper developmental stage. Moreover, when this endogenous electric field is perturbed by short-circuiting the field with conductive implants, 92% of the embryos exhibited some developmental abnormality, such as tail defects (*70*). These abnormalities were very similar to those observed in rumpless mutant chicks, and the extraembryonic currents measured in these mutants were significantly lower than either wild-type or phenotypically normal embryos (Figure 2). These findings suggests that the endogenous electric field plays a role in tail formation, and perturbations of such fields on the order of 20 mV/mm can have strong developmental effects.

Both axolotl and frog embryos drive about 1–7 $\mu A/cm^2$ out of the developing neural folds during neurulation and into most of the rest of the embryo

Figure 1. The transepithelial potential (TEP) measured in nine rostral–caudal positions in a stage 17 chick embryo. Numbered measurement positions are shown in the drawing at top. Below the drawing is a chart recorder tracing showing the TEP at different measurement positions. At each numbered peak the integument of the embryo was impaled, and a stable positive potential was measured. Times at which the embryo was not impaled are indicated by a solid line below the recording. The spikes shown during these periods indicate electrode resistance measurements. The top bar chart shows the average TEP at each position. Numbers below each bar correspond to the measurement positions indicated in the embryo drawing. Numbers between bars indicate the average distance (in millimeters) between each position. Where this distance is not indicated, the average distance was 0.3 mm. The bottom chart shows the average voltage gradient between each consecutive position. Error bars indicate the SEM; n = 6. *A steep voltage gradient was found between positions 6 and 7. This gradient was significantly different from a zero gradient* ($P < 0.05$, *determined using a Z-score). (Reproduced with permission from reference 49. Copyright 1990 Academic.)*

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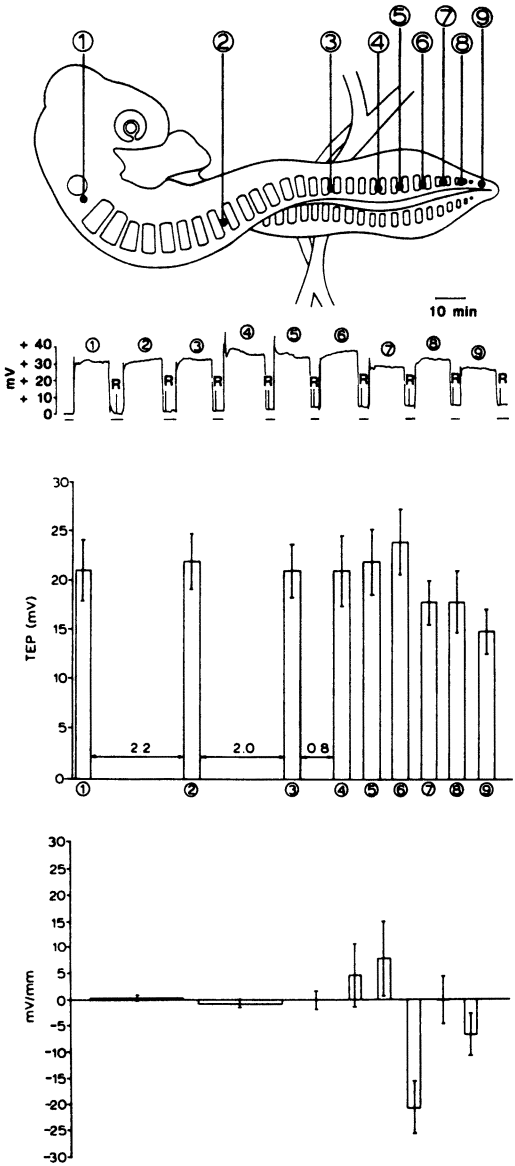

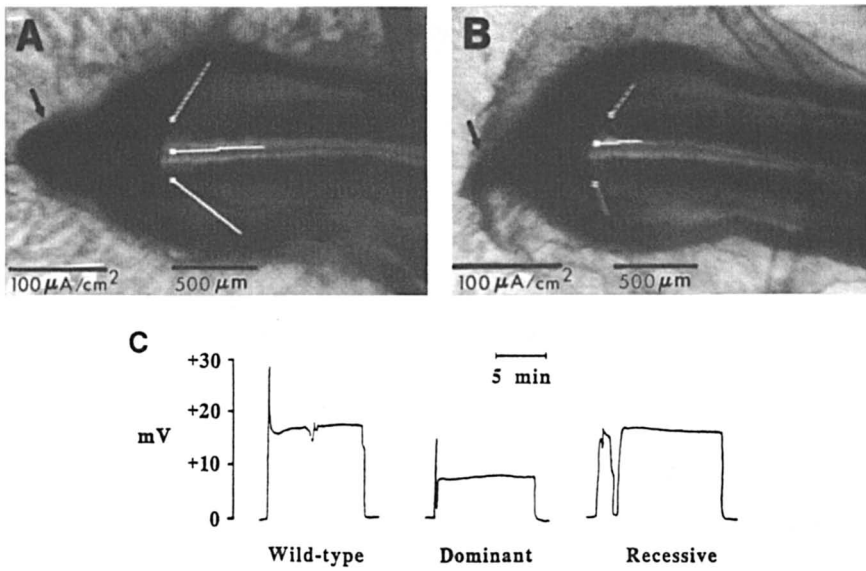

Figure 2. Examples of current and voltage measurements in rumpless mutants. A: currents were measured leaving the posterior intestinal portal (PIP) of a phenotypically normal stage 18 embryo from a batch that contained 25% homozygous-wild, 50% heterozygous-wild, and 25% homozygous-mutant eggs. The genotype of this embryo could not be determined, but it was likely to be either homozygous- or heterozygous-wild type. The currents leaving the PIP of this embryo were comparable in magnitude to those that leave the PIP of wild-type embryos of the same stage. The arrow indicates the normally formed tail. B: currents leaving the PIP of a phenotypically abnormal stage 18 recessive mutant from the same batch as the embryo in A. Arrow indicates abnormally formed tail. The currents in B were much lower than in A and in comparison to wild-type, dominant, and recessive rumpless mutants. Stable, positive potentials were measured in embryos from all three groups. The TEP of the dominant mutant was much lower than both wild-type and recessive mutant, which were of about the same magnitude. (Reproduced with permission from reference 70. Copyright 1992 Company of Biologists.)

(*71*). They also exhibit internally positive transepithelial potentials (TEPs) ranging from 18 to 64 mV. When the TEPs from various regions of the embryo are more closely examined, a caudally negative voltage gradient of 5–23 mV/mm is apparent (Figures 3 and 4). This endogenous electric field is similar in magnitude to that just described in chick embryos. Moreover, when steady DC electric fields are imposed over developing neurula-stage axolotls, developmental defects are most common at the end of the embryo facing the cathode (*72*). The axolotl embryo is more sensitive to imposed fields during neurulation than gastrulation. This sensitivity suggests that the endogenous electric fields detected are important components of the mechanism of neurulation.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch007

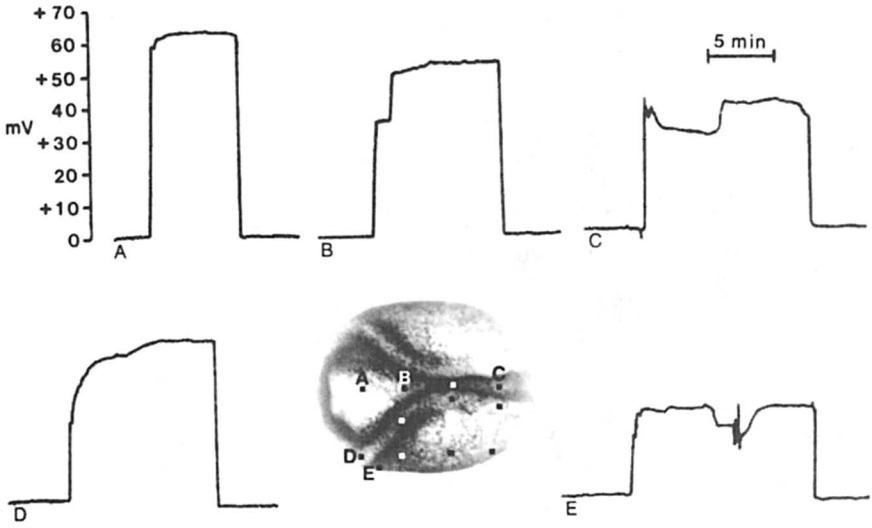

Figure 3. Measurements of TEP in axolotl neurula. The inset shows the 12 standard measurement positions, approximately 400 μm apart in the rostral–caudal axis along the neural plate, in the neural fold, and in flank ectoderm. Records A–E are presented for 5 of these 12 positions. The abrupt shift upward in each record indicates the shift in potential at the moment of microelectrode impalement of the ectoderm. Inwardly positive potentials were relatively stable for 5–15 min of recording, and all records were taken with the same microelectrode. Recorded TEP falls 40–50% in magnitude from that observed at the rostral neural plate as more caudal or more lateral regions of ectoderm are sampled. This fall would be associated with an increasingly more negative gradient of subectodermal potential at caudal or more lateral regions relative to the cranial neural plate. (Reproduced with permission from reference 71. Copyright 1994 Wiley.)

The stage 17 *Xenopus laevis* embryo exhibits a very similar rostral–caudal voltage gradient, caudally negative and averaging 27 ± 4 mV/mm (*77*). In individual embryos this gradient ranged from +8 to –54 mV/mm, and the steepest average voltage gradient was 40 mV/mm, measured between the blastopore and a point 250 μm dorsal to it, with the blastopore being negative.

Four other measurements of endogenous fields also include field strengths in the range of 1–200 mV/mm. Barker et al. (*78*) studied small wounds in guinea pig skin and found that when an incision was made through the glabrous epidermis of the guinea pig, a current of 1 μA flowed through each millimeter of the cut's edge and generated lateral, intraepidermal voltage gradients of about 100–200 mV/mm near the cut. Similarly, Chiang et al. (*79*) measured 66-mV/mm fields near wounds in newt skin. That same group (*80*) found that the electric field directly influences the rate of wound healing using a very elegant experimental design. The rate of wound closure in newts was 15% slower when the endogenous electric field was neutralized by passing an equal and opposite

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch007

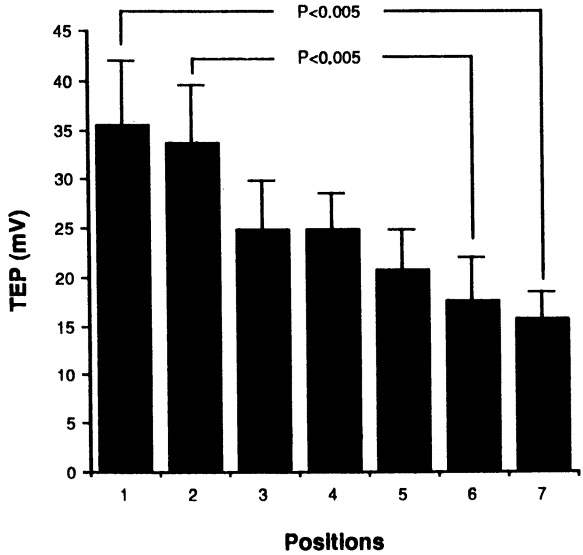

Figure 4. Rostral–caudal voltages beneath the neural plate in stage 16 axolotls. The magnitude of the neural plate TEP in eight embryos was sampled at seven positions along the longitudinal axis. Position 1 was the most rostral margin of the plate and fold; Position 7, the most caudal, was approximately 50 μm from the blastopore. The marked negative slope in potential is in the direction of the caudal margin. (Reproduced with permission from reference 71. Copyright 1994 Wiley.)

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch007

current through the wound. In the third case, McGinnis and Vanable (*81*) measured endogenous voltage gradients of 50 mV/mm in the distal region of the axolotl limb stump shortly after amputation, which fall to 15 mV/mm by 6 h later. The fourth example comes from a study by the same Purdue University group (*66*) that described the wound currents in bovine cornea. The TEP of the cornea drives 55 $\mu A/cm^2$ out of a small wound in the epithelium, and this wound current generates a lateral electric field of 42 mV/mm in the first 0.25 mm from the wound edge.

These studies suggest that the range of endogenous, physiological electric fields naturally occurring in animal tissues is 1–200 mV/mm. Furthermore, these fields are primarily steady, DC fields. Most of the studies completed to date have concentrated on developing or regenerating embryos, or tissues undergoing wound healing. Many of these systems provide evidence that the endogenous currents are important for normal development. Treatments that reduce these currents usually slow the rate of regeneration or wound healing, and treatments enhancing such currents often stimulate regeneration. For example, Borgens et al. (*82*) found that guinea pig spinal cords that had been completely transected exhibited enhanced nerve regeneration following the application of an electric

field across the cut for 50–60 days, and the application of slowly oscillating electric fields significantly improved the rate of recovery from spinal cord injury in dogs (*83*).

These observations suggest that endogenous electric fields play an important role in the guidance of normal developmental processes. Clearly, if environmental electric fields could result in similar field strengths within the body, such fields could disturb these normal processes. However, all of the studies mentioned thus far point to the importance of DC fields in developing systems, and none reports any effect of AC (alternating current) fields, which are found more commonly in the environment. For example, 60-Hz AC fields with peak-to-peak amplitudes of up to 1200 mV/mm have no effect on the galvanotaxis of somitic fibroblasts in vitro (*74*).

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch007

Biological Targets of Imposed Electromagnetic Fields

Several specific biological targets of electromagnetic fields have been identified over the past century. These targets include (1) growing nerves (galvanotropism), (2) migratory cells such as white blood cells and neural crest cells (galvanotaxis), and (3) polarizing or growing tissues. I have previously reviewed this literature (*5*), and only the highlights will be included here.

Galvanotropism. The growth cone of growing neurons in culture actively extends toward the negative pole of a DC electric field with a rather low threshold field strength of 7 mV/mm (*84–89*). When a small, steady electric current is driven through the stump of an amputated adult frog forelimb, the cartilage core of the stimulated regenerates have 40-fold more nerve coursing through them than controls. This observation indicates that imposed fields can stimulate enhanced nerve growth in limbs; galvanotropism has also been demonstrated in guinea pig and dog spinal cords (*82, 83, 90*).

Galvanotaxis. The directed translocation of cells by an electric field has been termed galvanotaxis. Motile cells, including macrophages, leukocytes, epidermal cells, fibroblasts, osteoblasts, osteoclasts, and neural crest cells, commonly respond to imposed fields by migrating toward either the negative or positive pole. Neural crest cells are the best-studied and respond to fields as low as 7 mV/mm by actively extending lamellae toward the negative pole of the field (*see* reference 5 for review).

Polarizing or Growing Tissues. Stem sections from both coelenterates and ascidians grow toward the anode when a field of 1.6 mV per cell is applied. Applied voltages of reversed polarity across isolated sheets of chick epiblast can cause the reversal of some morphological polarity markers (*91*). Finally, the polarity of eight-cell-stage mouse blastomeres is elongated along the field axis by an imposed electric field (*92*).

Determining the Mechanism Through Which Cells Detect and Respond to Imposed Electromagnetic Fields

The galvanotactic response of neural crest cells in vitro involves a very common signal transduction molecule, protein kinase C (PKC) (*76*). In the presence of PKC inhibitors, these cells translocate at the same rate but no longer exhibit any directed response to imposed DC fields. PKC stimulants such as phorbol esters result in a faster directed translocation response.

The other clue to the mechanism of field detection is the dependence on Ca^{2+} influx. The addition of the Ca^{2+} channel blockers, Gd^{3+} and Mg^{2+}, completely eliminates the directed component of translocation. Surprisingly, removal of extracellular Ca^{2+} in the presence of high Mg^{2+} resulted in a reversal of the directed translocation and thus the cells moved toward the positive pole of the field (*75*). This reversal suggests that the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) gradients control directed translocation, and perhaps the imposed electric field modifies the $[Ca^{2+}]_i$ distribution.

Conclusions

This chapter provides a summary of the endogenous electric field measurements that have been made in multicellular tissues during development and wound healing. The physiological range of the DC fields measured to date span a range of 1–200 mV/mm, and thus imposed DC electric fields much smaller than this range are not likely to influence embryonic development or regeneration. However, other chapters in this book address some cellular mechanisms that appear to respond to much lower imposed electric field strengths in the AC domain. Clearly, much more research is needed to identify the mechanisms utilized in the cellular responses to both DC and AC electric and magnetic fields.

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RECEIVED for review April 25, 1994. ACCEPTED revised manuscript September 14, 1994.

8

Streaming and Piezoelectric Potentials in Connective Tissues

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Stress-generated potentials of bone, cartilage, and tendon are reviewed to identify ranges of physiological electric field magnitudes and to relate these fields to tissue properties such as matrix structure and composition, molecular interactions, and interstitial fluid. The range of physiological field magnitudes may indicate the range of physiological or external field magnitudes that can modulate tissue behavior. Because stress-generated potential magnitudes and frequencies depend on tissue properties, potential measurements may be used to assess normal and abnormal tissue properties. Piezoelectric potentials are generated by loading dry tissues, whereas streaming (electrokinetic) potentials (SPs) and currents are generated by loading hydrated tissues. SPs generated by bending cortical bone are 1 order of magnitude smaller, when normalized to strain, and relax 2–4 orders of magnitude faster than SPs generated by compression of cartilage disks. Estimated maximum current densities at cell sites are on the order of 10 $\mu A/cm^2$ for bone and 1 mA/cm^2 for cartilage. These ranges may suggest that external or environmental fields must produce tissue-level current densities of this magnitude to alter connective tissue behavior. Trabecular bone SPs are smaller than cortical bone SPs. Microscopic measurements of bone SPs and models for SP generation in bone and cartilage indicate that field magnitudes and frequencies depend on tissue properties.

0065–2393/95/0250–0125$12.00/0

In Electromagnetic Fields; Blank, M.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1995.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch008

THE STRESS-GENERATED ELECTRIC POTENTIALS that have been observed in a variety of connective tissues including bone, tendon, and cartilage may provide clues for understanding possible field effects on tissues, as well as connective tissue anatomy and composition. These endogenous electric fields may be a biological signal regulating growth, remodeling, and repair of connective tissues. The magnitudes and frequencies of endogenous fields may also indicate the range of externally applied field values that could affect the same tissues. In addition, measurement of the endogenous fields in connective tissues can provide information regarding the tissue structure, such as channel or pore sizes, matrix and fluid composition, and molecular interactions within the connective tissues.

Early investigators attributed the potentials produced by bending dry bone and tendon to piezoelectricity (*1–3*). Piezoelectric potentials are due to polarization produced by mechanical-strain-induced deformation of bonds. Subsequent investigation of stress-generated potentials in hydrated bone, cartilage, and tendon, however, suggested that under physiological conditions, the measured stress-generated potentials are electrokinetic in origin, or "streaming potentials" (*4–6*). This chapter, therefore, will focus primarily on streaming, rather than on piezoelectric, potentials because these appear to dominate macroscopic measurements of stress-generated potentials in connective tissues under physiological conditions. Furthermore, these fields produced by streaming may modulate connective tissue repair and remodeling. Some investigators have suggested (*7–9*) that piezoelectric fields may also be important to bone remodeling, although these fields have not been measured definitively in moist bone.

Streaming potentials (SPs) and currents (SCs) are produced by stress-driven fluid flow past a charged surface. In both bone (*5, 6*) and cartilage (*10*), the matrix is negatively charged at physiological pH. Fluid flow in the charge double layer adjacent to the charged matrix produces a convective current in the same direction (Figure 1). The resulting charge separation creates an electric

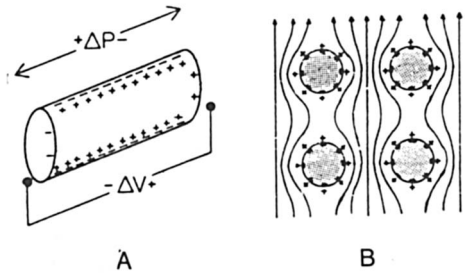

Figure 1. Schematic of models for streaming potential (SP) generation by (a) flow through cylindrical channels (one model for SP generation in bone) and (b) flow past charged cylindrical rod molecules (one model for SP generation in cartilage). ΔP is the pressure difference; ΔV is the voltage difference. (Reproduced with permission from reference 82. Copyright Elsevier 1987.)

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch008

field of the opposite polarity, which drives a conductive current in the direction opposite fluid flow. SPs are measured when the external or measuring circuit impedance is much larger than the tissue impedance. In this case, the total current within the tissue must be nearly zero, so that the conductive current equals the convective current. The SP is the potential at this current equilibrium. SCs are measured when the external circuit impedance is much smaller than the tissue impedance, so that current can flow across the boundary, and therefore the conductive back current is nearly zero. Endogenous fields likely lie between the two limiting cases measured (SP or SC), because tissues surrounding the connective tissue provide an alternative conductive current path to the conductive path through the connective tissue.

Macroscopic, tissue-level SPs have been relatively well characterized for normal cartilage and for cortical bone, the dense bone matrix that makes up the outer shell of bones. Macroscopic, tissue-level SPs have also been measured across healing and remodeling cortical bone, cortical bone with structure and composition modified by in vitro or in vivo treatments, and osteoarthritic and enzymatically altered cartilage, as well as chondrocytes in agarose culture. SPs for trabecular bone, the less-dense, meshlike bone matrix found inside bones, within ends of long bones, for example, and for tendon and ligament have been less well characterized. These macroscopic fields can provide information on the structure, matrix composition, interstitial fluid, and molecular interactions within these connective tissues, using models for SP generation.

Microscopic fields, however, are likely to be more relevant, in terms of identifying signals that may modulate cell behavior. Microscopic SPs have been measured at the surface of polished cortical bone. Models for SP generation in bone and cartilage can make it possible to predict microscopic field levels based on the macroscopic SP measurements.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch008

Stress-Generated Fields of Cortical Bone

The electromechanical behavior of cortical bone has been reviewed elsewhere (*7, 10–15*). The potentials produced by loading dry bone, and by loading wet bone in vitro or in vivo, are thought to be due to piezoelectricity and streaming, respectively. These two topics will therefore be discussed separately.

Macroscopic Stress-Generated Potentials in Dry Bone. Electric potentials generated by deformation of dry bone were first measured by Yasuda (*see* reference 1) and were attributed to piezoelectricity. The piezoelectric potentials of dry bone are typically on the order of a few hundred millivolts (*16*). In classical piezoelectric theory, the polarization tensor is related to the strain tensor by a piezoelectric tensor. Fukada and Yasuda (*1*) demonstrated that the electric field was linearly related to strain (at 2000 Hz), as predicted by classical piezoelectric theory, and measured piezoelectric coefficients for bone. Subsequent investigations of stress-generated potentials across dry bone, by these and other investigators, focused on determining the piezoelectric coefficients (*1–*

3) and on developing a predictive model for these coefficients based on the structure and in particular on the Haversian and collagen fiber orientation of bone (*17–20*). The piezoelectric properties of bone were attributed to collagen, rather than the mineral phase (*1, 16, 17*), because dry collagen displayed electric behavior similar to bone, and the crystal structure of hydroxyapatite mineral is not piezoelectric. Williams and Breger (*21*) developed a piezoelectric model for bone that included a dependence on strain gradients, as well as strain. Williams (*16*) published an extensive review of piezoelectricity in bone and other dry connective tissues, building on previous reviews by Bassett (*22*), Shamos and Lavine (*23*), Williams (*24*), Eriksson (*11*), and Guzelsu and Demiray (*25*).

Macroscopic Stress-Generated Potentials of Physiologically Moist Bone. The much smaller potentials generated by bending moist cortical bone (on the order of 100 μV) are now generally accepted to be electrokinetic (streaming) potentials, rather than electrostatic (piezoelectric) potentials. Cerquiglini et al. (*26*) first suggested that the potentials produced either by bending hydrated bone or by forcing fluid through bone were electrokinetic. Anderson and Eriksson (*27*) also suggested that bone potentials were due to streaming, based on the observed minimum in stress-generated potential for bone soaked in solutions of pH near 5 [the isoelectric point for tendon (*27*)], and the several order of magnitude change in potential as the bone dried.

Subsequent studies of stress-generated potentials tested predictions of the streaming hypothesis. Streaming potential (SP) theory predicts that the SP across a capillary is inversely proportional to conductivity (σ) and viscosity (η) and is proportional to the potential at the fluid slip plane (ζ potential), to permittivity (ε), and to the pressure (P) across the capillary: $SP \propto \varepsilon\zeta P/\eta\sigma$. The ζ potential can be altered by changing the pH or otherwise changing the fluid ionic composition to modulate the matrix charge. The conductivity σ depends on ionic concentration. Furthermore, because SP depends on stress-driven fluid flow, and fluid relaxation in response to stress depends on viscosity and not on ionic concentration, SP relaxation times were similarly expected to depend on viscosity and not on concentration (*5, 6*). Thus, Cignitti et al. (*4*) measured the potentials produced by step loading of bone, and an imposed pressure difference across bone samples kept in fluids with different conductivities, composition, and pH. They measured potentials across both predominantly cortical bone (rabbit tibias) and predominantly trabecular bone (lamb and ox ribs). Although their results were somewhat inconsistent for different samples, they did show that a component of the signal generated by bending varied with conductivity and increased with pH, as did the potentials produced by direct pressure.

Gross and Williams (*5*) showed that the potentials produced by an imposed pressure difference across cortical bone samples and by step cantilever bending of samples from the same specimen decreased with NaCl concentration (and with tabulated fluid conductivity), except at the lowest NaCl concentrations; these potentials also decreased with viscosity and had dependencies on Ca^{2+} ion concentration similar to those measured by Cignitti et al. (*4*). Further-

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch008

more, they found that potentials increased with pH between pH 6.2 and 8.2, consistent with the results of Cignitti et al. (*4*) and Anderson and Eriksson (*27*) and with the streaming hypothesis. The relaxation time of the potential in response to step loading increased with increasing viscosity, as predicted. They also showed that potentials measured across a dry bone sample were much larger than those measured when the sample had been soaked in saline (100 mV vs. 50 μV), consistent with the findings of Anderson and Eriksson (*27*).

Pienkowski and Pollack (*6*) reported results similar to those of Gross and Williams (*5*) for measured potentials as a function of concentration and viscosity using four-point step load and sinusoidal 1-Hz bending of moist bone samples: The measured potential magnitude normalized to strain decreased with NaCl concentration (and measured conductivity) for near-physiological conductivities and with viscosity. The potential reached a plateau at low NaCl concentrations (<0.009 mol/L), as expected theoretically for concentrations low enough that the double-layer thickness approaches the pore radius. The dependence of relaxation time on NaCl concentration and viscosity was also similar: The relaxation time increased with viscosity and did not depend on NaCl concentration.

The results differed, however, in that the ζ potential calculated by Gross and Williams (*5*) from the measured SP was independent of concentration, but that calculated by Pienkowski and Pollack (*6*) from their measurements did depend on concentration. Furthermore, Pienkowski and Pollack observed a sign change in ζ potential for high NaCl concentrations and viscosities, which was not observed by Gross and Williams. However, taken as a whole, these studies indicated that the dependence of stress-generated potentials on ionic concentration, on viscosity, and on pH and hydration was consistent with a streaming rather than a piezoelectric hypothesis. Piezoelectric fields may, however, be generated locally, resulting in local displacement currents with relaxation times on the order of microseconds, which are not measured macroscopically (*6, 7, 9, 16*).

Macroscopic Streaming Potentials and Currents of Normal Cortical Bone. SPs generated by bending bone have been measured across bovine (*5, 6, 28–31*), equine (*28*), human (*6, 29, 32*), canine (*33, 34*), feline (*35*), rabbit (*36*), rat (*37*), and sheep (*38*) bone in vitro, and across canine (*32, 34, 35, 39*), feline (*36*), rabbit (*37, 40*), and sheep (*41*) bone in vivo. Although most SP measurements have been conducted in vitro, SP measurements in vivo (*35, 36, 38, 39*) indicate that differences between in vitro and in vivo measurements are small.

Otter (*36*) quantitated the difference between SPs measured in vivo and in vitro. SP magnitude for normal intact canine cortical bone was similar in vivo and in vitro when normalized to strain difference across the cortex, whereas SP normalized to periosteal strain was 4 to 7 times larger in vitro than in vivo (Figure 2). A slightly slower increase with bending frequency, and faster decay time in vivo compared with in vitro, may be related to (1) modification of organic matter in bone pores, (2) different electric or fluid boundary conditions on

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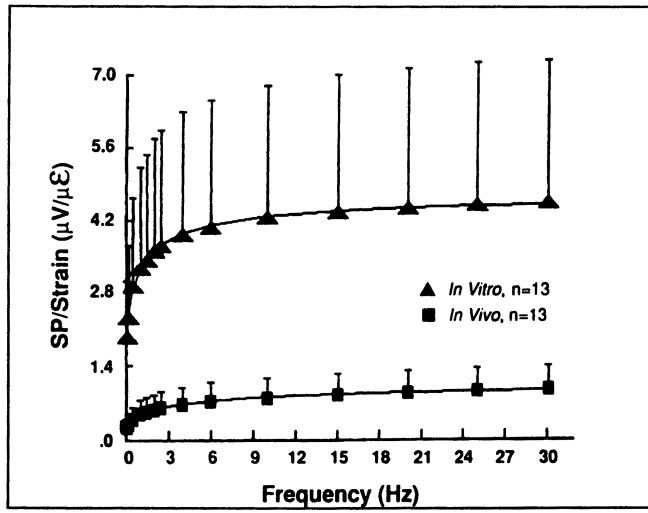

Figure 2. SP normalized to periosteal strain as a function of frequency for SPs measured in vivo across intact bone cortex and SPs measured at the same sites in vitro across strips of bone cortex. (Reproduced with permission from reference 41. Copyright 1992.)

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the bone surfaces (e.g., with soft tissue vs. with no soft tissue), or (3) vascular pressures in vivo.

SPs in response to a step load typically look like a decaying exponential in time, whereas SP magnitudes in response to sinusoidal loading increase with frequency monotonically toward an asymptote (on a linear scale) near 10 Hz (Figure 2). SP phase relative to bending, for sinusoidal loading, decreases monotonically from $\pi/2$ radians at low frequencies toward zero near 10 Hz (*28–30*). Typical SPs measured across normal intact cortical bone at or above 10 Hz are on the order of a few $\mu V/\mu\varepsilon$ (where strain is assessed on the sample surface) in vitro (*28, 29, 33, 41*) and slightly less in vivo (*33, 41*). Thus, for maximum physiological loads, on the order of 2000–3500 $\mu\varepsilon$ (*41*), fields across bone cortex would be on the order of 10 mV/cm. Field magnitudes in vivo might be much smaller because of current shunting through the soft tissues.

Streaming currents have been measured across normal intact and healing cortical canine bone (*42*). Current densities normalized to periosteal strain are on the order of 1–10 $nA/(\mu\varepsilon \cdot cm^2)$. Thus, for maximum physiological loads, current densities would be on the order of 1–10 $\mu A/cm^2$.

Pulse-related SPs, apparently generated by physiological variation in blood pressure, have also been measured across canine tibial cortex in vivo. The magnitude of these potentials is on the order of 7 μV for cortical canine bone or 23–35 $\mu V/cm$ for a typical cortical thickness of 2–3 mm (*43*).

Macroscopic Streaming Potential and Current Dependence on Bone Structure and Composition. Macroscopic electrokinetic fields depend on bone matrix and fluid composition and on matrix structure. Herein the focus will be on changes that occur under physiological conditions, such as during bone growth and maturation, healing, and remodeling and in osteoporosis.

SP magnitude varies as bone matures (*37*) and may also vary with bone age after maturation (*44*). Pollack et al. (*37*) measured SPs, generated by spike loading using four-point bending, across opposite sides of femurs from rats of different ages. SP normalized to periosteal strain increased with rat age by approximately a factor of 4 between 7 and 13 weeks and then did not significantly increase further between 13 and 21 weeks. Bone geometry also changed during this same time period, such that SP normalized to periosteal strain and cortical thickness increased by less than a factor of 2 between 8 and 13 weeks. Subsequently, Spirt and Pollack (*44*) found that ζ potential of rat femur, as assessed by particle electrophoresis, decreased from 1.5 to 6 months and then increased between 12 and 24 months. Thus SP may vary with age because of changes in bone geometry, ζ potential, and bone microscopic structure.

Several studies indicate that SPs, although measurable across healing bone, are smaller than those of normal bone and tend to increase with healing stage, when measured in vitro (*33, 45, 46*) or in vivo (*33, 46*). Steinberg et al. (*45*) measured SPs in vitro across rat femurs, following osteotomy and healing and subjected to variable loading rates. SPs increased from 0.08 mV at 3 weeks to 1.13 mV at 16 weeks and were smaller than the 6.53 mV measured across normal intact bone. Strain differences between femurs, however, were not recorded.

MacGinitie et al. (*33*) measured SPs at the site of healing drill holes in the cortex of canine tibia after 2, 4, or 12 weeks of healing both in vivo and in vitro. SPs were measured in vivo, using a modification of the method of Otter et al. (*41*), and in vitro across cortical bone strips subjected to four-point bending, at frequencies between 0.1 and 30 Hz. SPs were smallest for drill holes 2 or 4 weeks after surgery, were somewhat larger for drill hole sites 12 weeks after surgery and for the adjacent remodeling bone (ipsilateral controls), and were largest for normal intact bone (contralateral controls) both in vivo and in vitro (Figure 3). Bone impedance of drill holes relative to control sites varied in a manner similar to SPs, such that SP normalized to bone impedance was similar for all sites. SP magnitude tended to increase less rapidly with bending frequency for healing and remodeling sites (drill holes and adjacent bone) compared to control sites as assessed by several measures (fit of frequency parameters to models for SP generation, increase in SP magnitude over the last decade of frequency, and phase shift over the first decade of frequency).

Cochran et al. (*46*), in a follow-up study, measured SPs generated by sinusoidal bending at the callus of healing gap osteotomies in canine tibia in vivo, 6 or 12 weeks following surgical creation of the osteotomy. SP magnitude was greater on the callus relative to an adjacent remodeling cortical bone site when the callus was immature and flexible. For more mature rigid callus, SP at the

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch008

In Electromagnetic Fields; Blank, M.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1995.

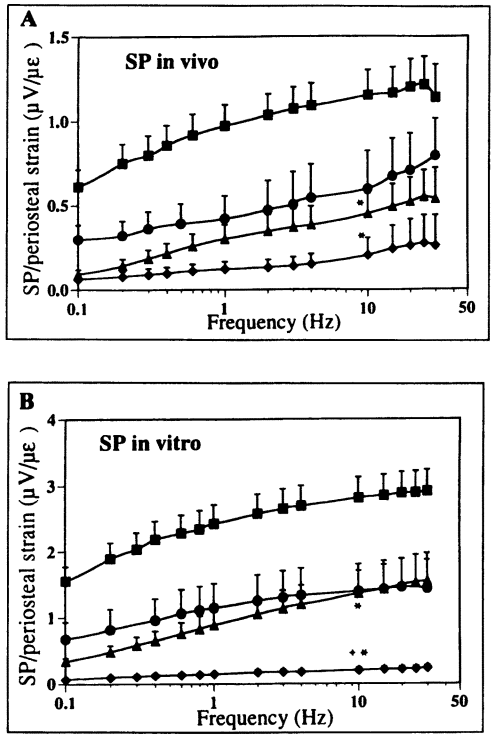

Figure 3. SP normalized to periosteal strain as a function of frequency of bending for SPs measured in vivo (A) and in vitro (B) across normal intact cortical bone (■), remodeling cortical bone adjacent to healing drill holes (●), healing drill holes 12 weeks after surgery (▲), and healing drill holes 2–4 weeks after surgery (♦). (Reproduced with permission from reference 33. Copyright Liebert 1993.)

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch008

callus was smaller and more like the SP measured at the adjacent cortical bone site. SP normalized to surface strain was somewhat, but not statistically, smaller for the callus (0.11 ± 0.11 μV/με) than for the porous adjacent cortical bone (0.16 ± 0.11 μV/με). Linear regression showed that SP normalized to strain for both callus and adjacent cortical bone sites tended to increase with decreasing porosity. SP vs. frequency of bending was variable for both callus and adjacent electrode sites.

The difference in bone structure and composition between osteoporotic and normal cortical bone results in smaller SPs with shorter relaxation times. Otter et al. (*46*) studied the effects of disuse osteoporosis on SPs using a canine tibia model. Disuse osteoporosis was produced by surgically removing a segment of the Achilles tendon, dividing the patella tendon, and then maintaining the foot in dorsiflexion by bandaging for 12 weeks following surgery. SPs for

the more porotic disuse bone were slightly but significantly smaller than for normal intact bone. The relaxation time for SPs generated by bending osteoporotic bone was also significantly shorter than for normal control bone (a result implying that SP magnitude increases more slowly with bending frequency).

Corcoran et al. (*47a*) compared the ζ potential of osteoporotic human bone (as assessed by clinical examination, cortical thickness, and Singh index) to normal bone by using particle electrophoresis. The ζ potentials were larger, although not significantly larger, for osteoporotic bone. This measured increase in ζ potential suggests that measured SP magnitudes should be larger for osteoporotic bone because SP is proportional to ζ potential. However, because SP also tends to decrease with bone porosity, as observed in healing bone (*33, 46*) and osteoporotic bone (*47b*), the effect of a change in bone porosity may prevent measurement of an increase in SP magnitude due to an increase in ζ potential. The increase in ζ potential may be a response to compensate for the increase in porosity.

In addition to examining how physiological changes in bone structure and composition and fluid composition alter SPs, a number of investigators have used various treatments of bone in vitro, and administration of various agents in vivo, to explore the relation between bone structure and composition and SP generation. Treatments in vitro to alter bone structure and composition have included boiling (*37, 48*), formaldehyde (Formalin) fixation (*37*), and demineralization or digestion (*48–51*). Treatments in vivo to alter bone structure have included administration of β-aminopropionitril (BAPN) during bone growth (*37*). Other studies suggest that SP magnitudes are affected by changes in the double-layer interface due to fluid composition both in vitro (*5, 6, 30, 48, 51–54*) and in vivo (*34*).

Microscopic Potentials and Models of SP Generation in Bone. Although tissue-level fields have been measured under a variety of conditions, the microscopic, cellular-level fields are less well known. Two approaches for determining fields at the cellular level are microscopic measurements and models that relate the macroscopic fields to bone anatomic structure.

Microscopic measurements are the most direct approach for determining fields near cells. Iannacone et al. (*55*) and Starkebaum et al. (*56*) measured microscopic SPs on the polished bone surface of fully hydrated samples tested in four-point bending and in compression. These SPs were observed to have a nearly symmetric cusplike dependence on distance from the Haversian canal, with local fields 1–2 orders of magnitude greater than the macro fields. The sign and magnitude of the cusps depended only on the macro stress for four-point bending (*56*). An abrupt change in magnitude was observed at cement lines. Pienkowski and Pollack (*6*) found that microscopic fields measured on the bone surface depended on fluid concentration in the same manner as macroscopic potentials. One drawback of these measurements is that potentials measured at the surface may not be the same as potentials within the tissue.

Microscopic measurements have not yet been made within the bone ma-

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trix. Thus models relating macroscopic fields to bone structure and composition are essential to determine microscopic field magnitudes. Furthermore, such models may enable prediction of fields for different bone structure and composition.

A number of models that have been developed relate SP generation to bone fluid spaces. These spaces include Haversian canals (radius $R \approx 10$–100 μm), canaliculi ($R \approx 0.05$–0.1 μm), and interstitial matrix spaces ($R \approx 100$–600 Å) (*15, 28, 29*). Some models have assumed (*5, 57, 58*) that macroscopically measurable SPs arise from fluid flow within the canaliculi. Others have proposed (*59, 60*) that SPs arise from flow to the bone surface through a homogeneous interstitial matrix porosity. Microporous space in fully mineralized bone has not been positively identified histologically, although electron microscopy has suggested that fluid space exists in the matrix (*61*). Canaliculi have been clearly identified and have been shown to be partially occluded by cell processes in fresh bone (*62*). The largest "pores", Haversian and Volksmann's canals, either have been neglected in these models or assumed to play a role in fluid relaxation from the smaller pores and thus modulate measured SP magnitude and frequencies (*13, 63*). To date, no single model can explain all experimental SP data.

Gross and Williams (*5*) and Pollack et al. (*57*) developed microscopic models for SP generation by flow in canaliculi to the Haversian system. In both cases, the flow was assumed to be driven by compression of lacunar spaces. The model of Pollack et al. (*57*) incorporated the idea of flow to the Haversian system and as a result explains the cusplike dependence of microscopic potentials. Neither of these models, however, can be used as a predictive model for macroscopic potentials, except in terms of proportionalities (e.g., to fluid properties such as viscosity and concentration).

Salzstein et al. (*59*) developed a macroscopic biphasic continuum model for flow in small pores for rectangular bone samples in four-point bending. Spirt and Pollack (*60*) extended the Salzstein model to a cylindrical geometry to model SPs produced by loading the diaphysis of whole long bones. The model of Salzstein et al. (*59*) provided the first reasonable fits to macroscopic SP data as a function of frequency. Fits to SP data as a function of frequency were used to identify the radius [on the order of 105–350 Å (*28*) and 200–600 Å (*29*)] of the pores involved in SP generation. The size range suggested that these pores were interstitial pores. This estimate of the effective pore radius, R, is close to estimates ($R < 200$ Å) obtained by another method (*30*) and is consistent with pore radius estimates from the 0.1–3-s relaxation times for macroscopic SPs measured across cortical bone [R near 45 Å (*37*) and $R << 0.1$ μm (*64*)]. However, the homogeneous model predicts that SP frequency response should depend on sample thickness, contrary to experimental results (*31*). Frequency response was independent of sample thickness for SPs measured across bovine bone strips in sinusoidal cantilever bending for thicknesses between 1 and 4 mm (*31*), and this finding supports an alternative hypothesis (*64*).

Johnson (*64*) predicted that SP should be independent of sample thickness for thicknesses greater than the inter-Haversian distance, if the fluid from small

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pores relaxes to the Haversian system and then relaxes to the surface very rapidly via the Haversian system. This hypothesis, that fluid relaxes via Haversian canals, also could explain the faster relaxation time for healing, remodeling, and osteoporotic bone. The greater porosity ($R < 5$ μm) visualized in these cases suggests, if Johnson's hypothesis is correct, that shorter relaxation times are due to the shorter distance fluid must travel between resorption lacunae, Haversian canals, or the large fluid channels between the woven bone trabeculae.

The homogeneous models of Salzstein et al. (*59*) and Spirt and Pollack (*60*) require, in addition, a no-flow boundary condition at the bone surface to obtain nonzero SPs, as postulated in another model (*64*) for measurements in a humid environment. For a no-flow boundary condition, there is a net pressure difference across a bone sample in uniform bending, and therefore nonzero SP. This no-flow condition may not be valid, especially in vivo, where soft tissues surround the bone. The alternative, a free-flow boundary condition, has been assumed to be valid (*59*) for fluid-covered bone. This alternative boundary condition results in no net pressure difference across a sample in uniform bending, so that for a homogeneous model there should be no macroscopic SP. The validity of the no-flow boundary condition has been argued for SP measurements in a humidity chamber on the basis that the surface is not wetted, and capillary pressure will keep the fluid within the bone (*59, 64*). This hypothesis has some experimental support, in that Iannacone et al. (*55*) observed menisci in lacunae and Haversian canals at the bone sample surface during microscopic SP measurements. However, SP magnitude and frequency response are the same whether measured with an agar bridge contacting the bone, with a pool of fluid contacting the bone, or with an Ag–AgCl electrode directly contacting a dry bone surface (*65*). Thus, either the no-flow condition pertains equally to a fluid-covered surface, or models requiring this boundary condition are incorrect.

Petrov et al. (*63*) developed a microscopic model for a homogeneous matrix surrounding a Haversian canal. SP was calculated as a function of distance from the canal, based on the assumptions of zero pressure (instantaneous fluid relaxation) and zero potential in the Haversian canal, and no flow at the cement line. This model predicts an SP magnitude as a function of distance from the canal that is similar to microscopic measurements.

Weinbaum et al. (*58*) developed a microscopic model in which flow in canaliculi around the cellular processes gives rise to SPs. This model is the first to explicitly take into account the cellular processes in the canaliculi.

Kowalchuk et al. (*51*) developed a model for the bone interface, based on ζ potential measurements of bone and hydroxyapatite particles, in various fluids and following various treatments. The proposed bone–fluid interface consists of a bone substrate (including mineral and proteins), a stationary layer (including proteins and ions), and the bone fluid. The ζ potential is predicted to depend on all three components.

To date, therefore, no macroscopic model has been developed that incorporates both SP generation due to flow through small pores, and relaxation via the Haversian system. The microscopic models to date cannot be used to predict

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macroscopic data and thus cannot be used to find microscopic field magnitudes from macroscopic measurements.

Stress-Generated Fields of Trabecular Bone

Wu et al. (*66*) measured SPs across moist cubes of trabecular bone in sinusoidal nonuniform axial loading and compared these with SPs of cortical bone under the same conditions and from the same specimens. Trabecular bone SPs were more than 1 order of magnitude smaller than the cortical bone SPs (approximately 0.001 μV/με vs. approximately 0.3 μV/με at 20 Hz). Trabecular bone SPs also increased less rapidly with bending frequency than cortical bone SPs.

Trabecular bone anatomy suggests that the trabecular bone SP frequency response should be similar to that of cortical bone SPs but that the SP magnitude should be smaller, because of the more porous structure of trabecular bone. Distances to the intertrabecular fluid space or Haversian canals within trabeculae are similar to the inter-Haversian distances in cortical bone (*67, 68*). Trabecular bone resistivity, however, is much smaller than cortical bone resistivity [approximately 0.2–1.0 kΩ·cm (*69*) vs. approximately 15–55 kΩ·cm (*70*)]. If the intertrabecular spaces in trabecular bone contribute to fluid relaxation as the Haversian canals do in cortical bone, then SPs of trabecular bone (neglecting any contribution by the marrow) would have a similar frequency response but smaller magnitude compared with SPs of cortical bone. Thus, measured SP magnitude in trabecular bone is approximately as expected on the basis of trabecular structure, but the frequency response is not. Measured differences in the frequency response between trabecular and cortical bone SPs therefore cannot be explained in terms of fluid relaxation to the Haversian canals or intertrabecular spaces alone.

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Stress-Generated Fields of Cartilage

SPs of cartilage due to hydrostatic pressure gradients were first observed by Maroudas et al. (*71*). Subsequently, electric potentials were also measured in response to tissue stress (*72, 73*). These stress-generated potentials were identified as SPs by Grodzinsky et al. (*74*), on the basis of their dependence on fluid concentration, pH, and the similar relaxation behavior of the electric potential and stress. Furthermore, following digestion with hyaluronidase to reduce the net negative matrix charge, the potential decreased. The SPs across disks of cartilage were then more fully characterized by Lee et al. (*75*) and Frank and Grodzinsky (*76*) for sinusoidal loading in confined compression, and by Kim et al. (*77*) for loading in unconfined compression.

Macroscopic Streaming Potentials and Currents of Normal Cartilage. SPs generated by compressive loading of articular cartilage are

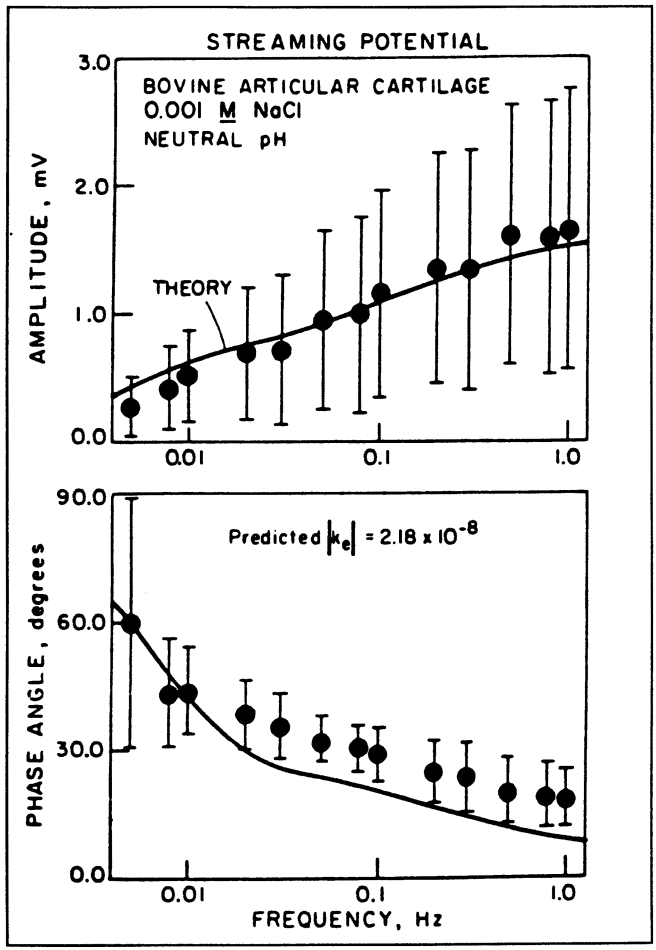

Figure 4. SP magnitude and phase as a function of frequency of compression across a cartilage disk in 0.001 M NaCl. The line indicating theory is the fit obtained using the model of Frank and Grodzinsky; k_e is the ratio of the electrokinetic coupling coefficient to the electrical conductivity. (Reproduced with permission from reference 82. Copyright Elsevier 1982.)

larger than those generated across bone, whereas the frequency response (Figure 4) is similar in shape to that of SPs generated by bending bone, although the characteristic relaxation time is longer. SPs of cartilage in physiological saline increase monotonically with frequency in the physiological loading range between 0.001 and 20 Hz. At the higher frequencies (e.g., near 10 Hz), SP magnitude is on the order of 20 μV/με for confined compressive sinusoidal loading of plugs of articular bovine cartilage 1 mm thick (*75*). SP phase relative to dis-

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placement decreases monotonically with frequency from a value less than $\pi/2$ below 0.01 Hz toward an asymptote near zero at higher frequencies. The characteristic relaxation time, $\tau = l^2/H_A k$, where l is a characteristic length, H_A is the bulk modulus, and k is the permeability, is on the order of 10 min (*78*) for 1-mm-thick cartilage disks, compared with 0.1–3 s for cortical bone. Streaming current (SC) densities on the order of 1 $\mu A/cm^2$ were measured for compressive sinusoidal loading of bovine articular cartilage plugs approximately 800 μm thick in 0.005 M NaCl [for 10 mε, near 1 Hz (*76*)]. Because theory predicts that SCs should not depend on NaCl concentration (assuming that ζ potential is independent of NaCl concentration), similar currents should be expected at physiological saline concentrations. Frank and Grodzinsky (*76*) predicted that in vivo currents would be 50–100 times higher because of lower impedance at the articular surface in vivo compared with measurement conditions.

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Macroscopic SPs of Osteoarthritic and Enzymatically Digested Cartilage. SPs have been measured for cartilage taken from a rabbit model for osteoarthritis, and following digestion by trypsin or chondroitinase ABC to model the proteoglycan (PG) loss that accompanies osteoarthritis. Hoch et al. (*79*) measured SPs of articular cartilage disks cored from rabbit knee joint following meniscectomy and subjected to a 0.1-Hz sinusoidal compression. The SP of osteoarthritic-like knee cartilage compared with control cartilage followed the same trend with time after surgery as the mechanical stiffness and glycosaminoglycan (GAG) content, as assessed by uronic acid assay. Lee et al. (*75*) measured SPs of articular bovine cartilage in confined compression before and after a 6-h digestion with trypsin to remove GAG and noncollagenous protein. SPs decreased to approximately one-third the initial value following digestion by trypsin for 6 h. Frank et al. (*80, 81*) similarly observed that SPs generated by sinusoidal compression of cartilage disks decreased with time as these disks were digested using trypsin, chondroitinase ABC, and hyaluronidase. SPs were <20% of the initial value after 12 h in trypsin. SPs were approximately 60% of the initial value after 20 h in chondroitinase ABC, which can remove approximately 85% of the chondroitin sulfate. SPs were approximately 80% of the initial value after treatment with hyaluronidase, which disaggregates PG aggregates. These experiments demonstrated that the matrix charge responsible for SP generation in cartilage is primarily due to PGs.

Microscopic Potentials and Models of SP and SC Generation in Cartilage. SPs have not been measured on a microscopic level, as have SPs on the surface of bone. However, the models developed for SP generation in cartilage have been relatively more successful at predicting cartilage electrokinetics than have those for bone. Macroscopic models (*75, 82*), based on whole-tissue properties, and a model based on the microscopic structure of cartilage (*83*) have been developed.

Frank and Grodzinsky (*82*) modeled SP generation in cartilage in confined compression using an assumed poroelastic homogeneous matrix, similar to

the model for SP generation in bone by Salzstein et al. (*59*). Because cartilage is relatively homogeneous and isotropic compared with bone and is avascular and therefore lacks the canalicular and Haversian structure of bone, this assumption more closely approximates cartilage structure than it does bone structure. For this cartilage SP model, tissue properties were obtained from independent measurements of the mechanical properties, conductivity, and fixed charge density of cartilage. Subsequently, models for tissue property dependence on pH and ionic strength were developed to predict SP dependence on these parameters (*84*). This electrokinetic model provides a good fit to experimental data. Thus, from this model, the local currents (fields) can be estimated. For SP measurements in confined compression, a porous platen was used for the top surface, whereas an impermeable surface was used for the bottom surface, to mimic the articular and cartilage–bone surfaces in vivo, respectively. Thus, fluid flow is greatest at the articular surface, and therefore fields are expected to be greatest in this top layer. On the basis of the model of Frank and Grodzinsky (*82*), current densities as high as 1 mA/cm^2 (*77*) may exist near the surface of cartilage under physiological loading conditions.

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Stress-Generated Fields of Tendon

Stress-generated potentials of dry tendon were first measured by Fukada and Yasuda (*85*) and were attributed to piezoelectricity. Subsequently, Anderson and Eriksson (*27*) measured potentials for hydrated tendon and suggested that the potentials in the hydrated case were SPs because these potentials decreased to zero near pH 4.7. This pH value was identified as the isoelectric point, the pH at which the ζ potential changes sign, by measurement of electrophoretic velocities of tendon particles. Gross and Williams (*5*) confirmed the observations of Anderson and Eriksson.

Summary

SPs in response to stress are common to many biological tissues. They have been observed in response to imposed stress or pressure gradients across bone, cartilage, and tendon, as well as other tissues. Piezoelectric fields have been positively identified only for dry tendon and bone, although these fields may still contribute under physiological conditions. Typically, the magnitude of tissue-level SPs in bone is on the order of 1 μV/$\mu\varepsilon$ in vivo and in vitro. Local magnitudes may be higher because microscopic measurements indicate that SPs depend on distance from the Haversian canals. Current densities for maximum physiological loading are on the order of 1–10 μA/cm^2. The magnitude of tissue-level SPs in cartilage is somewhat larger, on the order of 10 μV/$\mu\varepsilon$. Local current densities may be as large as 1 mA/cm^2 in the upper layer of articular cartilage. Thus, if external fields must induce currents near the physiological range to modulate connective tissue behavior, external fields must produce fields on the order of 1 μA/cm^2 in bone to 1 mA/cm^2 in cartilage. The structure and composi-

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tion of the matrix, and interstitial fluid modify SP magnitude and frequency of both bone and cartilage. In cartilage, PGs contribute the negative matrix charge responsible for SPs at physiological pH, and thus molecular interactions can also modify SPs. SP measurements, combined with models, can therefore elucidate the structure, composition, and molecular interactions of connective tissues.

Acknowledgments

This research was supported in part by National Science Foundation Grant BCS9209046, the New York State Department of Health, and Pacific Lutheran University.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch008

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Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch008

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RECEIVED for review March 8, 1994. ACCEPTED revised manuscript March 24, 1995.

9

Electric Stimulation of Protein Synthesis in Muscle

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Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch009

Research on biological effects of electromagnetic (EM) fields has focused on changes in biosynthesis as a key to relevant cellular mechanisms. Cells exposed to EM stimuli show changes in biosynthetic patterns that are similar to the changes caused by known stresses, such as heat shock. This similarity suggests that weak EM fields stimulate the stress response, which comprises the normal protective mechanisms found in all cells. This chapter considers EM-stimulated changes in biosynthesis in the context of research on chronic electric stimulation of biosynthesis in mammalian muscle. The studies on muscle, especially the frequency dependence of specific protein synthesis, suggest possible molecular mechanisms in EM stimulation.

Electromagnetic Stimulation of Biosynthesis

Living systems are composed of many charged species, such as ions and polyelectrolytes, so it is not surprising that externally applied electric fields can affect reactions within cells. Because environmental electromagnetic (EM) fields induce currents inside cells, at appropriate intensities they too should affect biological processes in cells.

In recent years, concern about environmental EM fields has grown as

0065–2393/95/0250–0143$12.00/0

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many biological effects have been reported. The published proceedings of the First World Congress on the subject (*1*) contain an up-to-date cross section of these studies. One effect, a change in normal biosynthetic processes in cells, has been demonstrated (*2, 3*) in several laboratories and with different types of cells. Evidence from clinical studies, which reports improved growth and healing with EM fields (*4*), indicates that the fields cause an acceleration of biosynthetic processes. Data from epidemiological studies, which show that the probability of developing leukemia in children increases with exposure to EM fields from power lines, may also be related to changes in biosynthetic processes.

Because much of the evidence in these studies was obtained using transformed cells in culture, damaged tissue, or cancer cells, the mechanisms by which EM stimulation leads to changes in biosynthesis might appear to involve unusual or unnatural pathways. This chapter, however, examines studies on the biosynthetic effects that result from chronically stimulating normal living tissue, or more specifically, changes in mammalian muscle composition that are due to repeated electric stimulation by nerves or electrodes. These data raise the possibility that EM fields interact with parts of cells that normally respond to electric stimuli, and in some cases the response system may even be optimized for this purpose. Related effects are presented elsewhere in this volume by Bassett on bone repair and by Sisken on soft tissue healing. The demonstrated effects of repeated stimulation on learning in the central nervous system will not be discussed in this chapter because the relation between electric stimuli and biosynthetic responses is not as well documented at the molecular level (*5*) as in muscle.

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EM Stimulation as a Cell Stress

Changes in protein synthesis, stimulated by low-frequency EM fields, have been analyzed in *Sciara coprophila* salivary gland cells using two-dimensional gel electrophoresis. The many proteins synthesized in a cell have been separated according to molecular weights (MW) and isoelectric points (pI), and the amount of each protein has been determined. Analysis of protein distribution curves for mass (percent mass vs. MW) and charge (percent mass vs. pI) in different ranges of MW and pI, and comparison of the proteins under control conditions to those of cells exposed to EM fields or to sudden high temperatures (i.e., heat shock) indicate that both magnetic and thermal stimuli lead to similar cellular responses (*6, 7*). Both stimuli change the percent mass distribution around 30 and 70 kDa in similar ways. In the pI distribution, both shift pI peaks from 5.8 to 6.3 and show a new peak at 7.1. Increased levels of transcripts for the heat-shock protein hsp70, which are found in the EM-stimulated samples at normal growth temperatures (*8*), indicate that a characteristic stress protein is turned on by both stimuli. According to these results, the magnetic field is apparently interpreted by the cell as a stress, and the system responds via the stress–response mechanism.

The stress–response mechanism reacts similarly to increasing stimulus en-

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ergy of both 60-Hz EM fields (from 0.8 to 800 μT) and temperatures (from 20 to 45 °C). Many similarities in the sequence of changes in the distribution of proteins and in individual proteins are evident (*7*). At the highest intensity of each stimulus, the values return to the level of the control, which indicates that a similar response range was covered by both stimuli. Differences between responses to the two stimuli can be explained by acceleration of reactions at elevated temperatures. EM fields appear to stimulate a natural pathway that is similar to the cellular response to sudden rises in temperature and other physical stresses.

The observed similarities in the response system are surprising in view of the great differences in stimulus energies. Cells respond to EM fields at very low energy densities (*7*), about 10^{-7} J/m^3 for a 0.8-μT magnetic field, which is orders of magnitude lower than the 10^{+7} J/m^3 energy density for a 5.5 °C rise in temperature at the thermal response threshold. Although responses to the two stimuli do not depend upon the energy input, the responses are graded with increasing energy for each individual stimulus. This grading would occur if parallel sensors for the two stimuli fed into a common response pathway.

The aforementioned studies on biosynthetic changes with EM stimulation were done on normal salivary gland cells of *Sciara coprophila* larvae. Similar studies on mammalian muscle would have distinct advantages, because a relatively limited number of proteins are synthesized in muscle, and a relation has been found between the frequency of electric stimulation (using electrodes or nerves) and changes in muscle protein composition (*9–12*). Unfortunately, muscle has not been studied in this context, and the literatures of the two research areas have not even been considered together. Because the related information on muscle can contribute to understanding molecular mechanisms in EM-stimulated biosynthesis, I now briefly review the muscle studies and relate them to the *Sciara* experiments.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch009

Structure and Function of Skeletal Muscle

Some information about the structure and composition of skeletal muscle is included here to provide a framework for understanding the experimental results to be discussed.

Muscle is an electrically excitable tissue that generates tension to produce or resist movement. It also generates heat as a result of the chemical reactions that occur during its function. This section considers skeletal (or voluntary) muscle, which is one of three different types: skeletal, cardiac, and smooth muscle. The structure of skeletal muscle is hierarchical. Each muscle is made up of muscle fibers (large, multinucleated cells), which are made up of bundles of smaller myofibrils, which in turn are made up of repeating units called sarcomeres.

The contractile properties of skeletal muscle can be understood in terms of the molecular structure. The sarcomere, the basic contractile unit of skeletal muscle, is composed of interdigitating thick and thin protein filaments. The re-

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actions between the proteins on the thick and thin filaments convert ATP energy into tension or shortening, and the filaments slide together to increase the degree of overlap during shortening. The sliding-filament hypothesis has been used to explain tension generation and shortening by the interaction of myosin cross bridges (thick filament) with actin (thin filament) that results when the filaments slide past each other without changing their lengths.

The thick filaments are made up of the protein myosin that consists of two heavy chains that form a twisted helix, and four light chains that interact with the two globular heads at the end of the helix. The globular heads have both an actin binding site and the adenosinetriphosphatase (ATPase) activity that is critical for converting chemical into mechanical energy. Myosin is made up of two subunits, heavy and light meromyosin, connected at a relatively flexible point that allows the heavy meromyosin to form a cross bridge to the thin filament.

The thin filament contains the proteins actin, tropomyosin, and troponin. The two chains of F-actin (that activate the myosin ATPase) are arranged in a helix, and tropomyosin, a long rodlike molecule, lies in the grooves between the two F-actin chains. Tropomyosin inhibits the ability of actin to interact with myosin. Troponin regulates the actin–myosin interaction, primarily through inhibition. Troponin has three subunits, troponin T (TnT; binds to tropomyosin), troponin I (TnI; binds to actin), and troponin C (TnC; binds Ca^{2+} ions). When Ca^{2+} binds to TnC, it causes a conformational change that allows actin to interact with myosin. At rest, the Ca^{2+} concentration is approximately 10^{-7} M, and contractile activity is inhibited. Contraction is initiated by the release of Ca^{2+} that is sequestered and stored in the sarcoplasmic reticulum, a specialized structure found throughout muscle.

When skeletal muscles are fully differentiated, they differ in function and composition. The basic design framework of skeletal muscle includes a number of muscle fiber types, which differ depending upon the specific protein isoforms (e.g., the different molecular variants of myosin) present:

- fast-twitch, glycolytic fibers that are specialized for short-duration bursts of intense exercise mostly under anaerobic conditions (e.g., leg and arm muscles) and that contract rapidly in times as short as 7.5 ms. Examples of fast muscles that have been used in many studies are extensor digitorum longus (EDL) and tibialis anterior muscles in the leg.
- slow-twitch, oxidative fibers that are specialized for sustained periods of isometric contractions of moderate intensity under aerobic conditions (e.g., leg and back muscles involved in posture) and that can take up to 100 ms for one contraction. An example of a frequently studied slow muscle is the soleus in the leg.

These categories of muscle are overlapping; thus, for example, fast-twitch, oxidative–glycolytic fibers have properties intermediate between fast- and slow-fiber types, and many muscles contain mixtures of different fibers. Also, muscle function and composition can change with changes in innervation or stimulation rates, as the next section indicates.

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Interactions between the thick and thin filaments are stimulated by electrically induced signals from neurons. There are 100–3000 spinal motor neurons per muscle, and each innervates from 10 to 2000 muscle fibers. A given motor neuron innervates only one type of fiber, and the nerve properties are correlated with muscle fiber type. The maximum firing rate, cell body size, and axonal conduction velocity are greater in motor neurons that innervate fast fibers than in those that connect to slow fibers. Although the range of values is wide, fast fibers are normally stimulated at rates in the range of 100 Hz and slow fibers at about 10 Hz. (Slow fibers, however, usually fire for extended periods of time, whereas fast fibers usually are active in bursts that occur at frequencies below 10 Hz.) When a motor neuron fires an action potential, all of the innervated muscle fibers twitch in synchrony.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch009

Chronic Electric Stimulation of Protein Synthesis in Muscle

Muscle mass is very dependent on electric stimulation for its development and integrity. In the absence of normal stimulation by nerves, or when nerves are cut and limbs are immobilized, disuse muscular atrophy results. For normal functioning muscle, exercises at different speeds and levels of force can be used to develop specific muscles. Electric stimulation can lead to specific molecular changes. It can suppress certain muscle proteins while it enhances the synthesis of others. Also, the frequency of the stimulation has a great bearing on the specific proteins that develop and consequently on the contractile properties of muscle.

Electric stimulation is apparently as critical in muscle development as in early development of all tissues. This relationship has been demonstrated in studies (*9*) on myogenin and myoD, two proteins that regulate genes for making muscle proteins during differentiation. In early development, the levels of these proteins are high, but they decrease when the nerves attached to the muscles start to produce action potentials. Eftimie et al. (*9*) showed that levels of coding by mRNA for the two proteins remain high in denervated preparations but that electric stimulation with electrodes (100-Hz trains of 1-s duration applied every 100 s for 6 days) can depress the concentrations, as do normal nerve action potentials. When electric stimulation is stopped, the transcripts coding for these proteins reappear. The changes that occur with myogenin and myoD appear to be specific because they do not occur with actin, which is unaffected by the level of electric stimulation.

Mature muscle is also subject to structural and functional changes in response to electric stimulation. A review by Pette and Vrbova (*10*) summarized much research on changes in skeletal muscle fibers due to chronic electric stimulation. Much of their work focused on the fast-to-slow transition in muscle. When the fast muscle EDL, normally stimulated at about 100 Hz, is stimulated at 10 Hz, each contraction is slower and resembles that of a slow muscle after several weeks. At the same time, compositions of some proteins change to resemble those of a slow muscle. A particularly graphic demonstration of this ef-

fect can be seen in the changes of TnT (*see* Figure 1), in which the isoforms that are characteristic of the fast EDL change to those that are characteristic of the slow soleus after 82 days of stimulation at the slow rate (10 Hz, 12 h daily). Frequency-dependent changes in other proteins have been described, but the responses are not usually so simple. Responses are also significantly different between species.

Lomo (*11*) worked on both fast-to-slow and slow-to-fast transitions in muscle and demonstrated changes in contractile properties and protein composition of muscle due to chronic electric stimulation. The fast EDL and slow soleus muscles in rat are characterized by differences in their nerve stimulation rates and muscle contraction rates and in the ATPases on the heavy meromyosin (which determine the contraction rate). Experiments have shown (*11, 12*) that the nerve stimulation rate appears to control muscle composition and contraction rate.

When the nerves to the two muscles are cut and interchanged so that the fast firing nerve stimulates the slow muscle and the slow nerve stimulates the fast muscle, the function and composition of both muscles change after several weeks. The fast muscle contracts more slowly, and the slow muscle contracts more rapidly. The changes in contraction time are shown in Table I. The slow muscle does not change with slow-frequency stimulation but speeds up with fast-frequency stimulation using nerve or electrodes. The fast muscle does not change with fast-frequency stimulation but slows down with slow-frequency stimulation. The slowdown of the fast muscle is not as marked as the speeding

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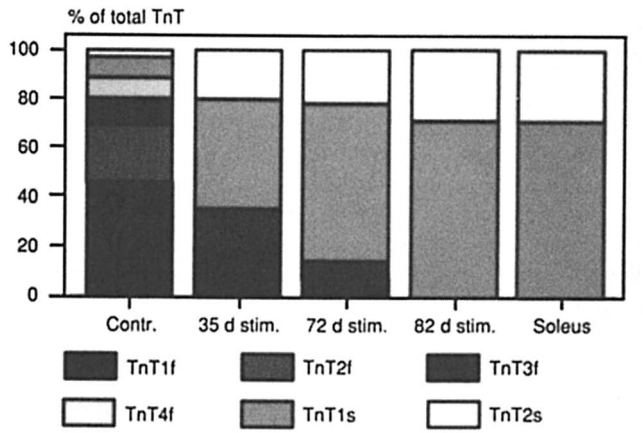

Figure 1. Changes in the distribution of troponin T (TnT) isoforms in a fast muscle [extensor digitorum longus (EDL)] as a result of stimulating it over a period of weeks at a slow frequency. Contr is normal EDL muscle; TnT_{1f}–TnT_{4f} are fast-type TnT isoforms; TnT_{1s} and TnT_{2s} are slow-type TnT isoforms. Data are from Härtner et al. (19). (Reproduced with permission from reference 10. Copyright 1992 Springer-Verlag.)

Table I. Average Muscle Contraction Times (ms)

Type of Stimulation	*Soleus (Slow)*	*EDL (Fast)*
Innervation		
EDL nerve (fast)	14.3	12.7
Electrodes after denervation		
Fast pattern (150 Hz)	12.2	11.1
Fast pattern (100 Hz)	15.0	11.0
Innervation		
Soleus nerve (slow)	37.4	22.5
Electrodes after denervation		
Slow pattern (20 Hz)	37.7	21.1

NOTE: EDL, extensor digitorum longus.
SOURCE: Based on a table in reference 11. (Reproduced with changes with permission from reference 11. Copyright 1989.)

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch009

up of the slow muscle. The changes in function are reflected in changes in protein composition.

Studies of expression of different isoforms of myosin heavy chain (MHC), which contain the ATPase activity, show changes in composition that parallel the changes in contraction (*12*). The molecular changes, shown in Table II, indicate changes after denervation and after different rates of stimulation. These data clearly show that the speeding up of the slow muscle occurs without the type 2B isoform that is characteristic of fast muscle.

Both the EDL and soleus muscles can change their twitch-contraction times over a two- to threefold range in response to changes in stimulation frequencies, but the changes in the MHC isoforms are not as marked. Neither EDL

Table II. Relative Amounts of MHC Isoforms in EDL and Soleus Muscles

Muscle Type and Stimulation	*%Type 1*	*%Type 2A–2X*	*%Type 2B*
EDL (fast muscle)			
Control	0.2	26.8	73.1
Denervated	4.8	79.2	16.0
Electrodes			
20 Hz	8.8	91.3	0.0
150 Hz (every 15 s)	2.2	89.0	8.8
150 Hz (every 15 min)	2.2	52.6	45.2
Soleus (slow muscle)			
Control	96.9	3.1	0.0
Denervated	78.5	21.5	0.0
Electrodes			
20 Hz	93.7	6.3	0.0
150 Hz (every 15 s)	25.0	75.1	0.0
150 Hz (every 15 min)	15.7	84.3	0.0

NOTE: MHC, myosin heavy chain.
SOURCE: Reproduced with permission from reference 11. Copyright 1989.

nor soleus can express certain proteins characteristic of the other muscle, so the range of molecular adaptability has limitations. Most notably, the gene for the type 2B isoform, which constitutes 75% of the MHC expressed in the fast EDL muscle, appears to be downregulated in the slow soleus muscle.

A number of other studies (*13–15*) have documented changes in the levels of different enzymes that also correlate with the rate of electric stimulation. For example, changes in lactate dehydrogenase appear to be correlated with aerobic-oxidative capacity of the muscles (*13*). Changes in parvalbumin, a calcium-binding protein that may be involved in muscle relaxation, also correlate with electric stimulation (*14*). In that system, changes for short-term stimulation (less than 5 days) are different from those for longer-term stimulation (up to 28 days). In addition, the response to electric stimulation differs among species (*15*). Many aspects of this subject, including molecular changes due to EM stimulation, remain to be explored.

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Certain aspects of the electric stimulation process in muscle appear to be fairly clear. The changes in proteins are due to the frequency of action potentials and not to trophic secretions from nerve endings. This conclusion was reached by eliminating the nerves totally, stimulating with electrodes, and then showing exactly the same effect. Fast stimulation results in synthesis of proteins that are found in fast muscles, and slow stimulation leads to proteins found in slow muscles (*11*).

Chemical changes associated with ionic fluxes during action potentials in muscle are apparently not as important as the frequency of the signals. When experiments are carried out with electrodes, stimulation can be easily controlled so that the number of action potentials during the slow (200 pulses at 20 Hz every 15 s) and fast (25 pulses at 150 Hz every 15 s) stimulation patterns are almost the same. In these experiments, the frequency (in hertz) appears to be the important factor in determining the pattern of proteins synthesized (*11, 12*).

However, frequency is not the only factor. When stimulation is carried out at the rate of 25 pulses at 150 Hz every 15 min, or 60 times fewer impulses than the earlier fast pattern but at the same frequency, the changes are different (*see* Table II). The reason for this difference is not clear, but the changes may reflect a threshold in the total energy input per unit time or a difference between acute and chronic stimulation, as was noted in the changes in parvalbumin that correlate with the duration of electric stimulation. The parvalbumin system displayed slight increases during the first 5 days and larger decreases for longer terms.

Electric and EM Stimulation

In the studies on electric and EM stimulation of protein synthesis, I found no reports of a stress response in muscle following electric stimulation. A normal heat-shock response does occur in muscle, with the characteristic HSP70 and other proteins (*16*), but this response occurs after an acute thermal stress. The research literature on muscle has been concerned with the biosynthetic response to chronic electric stimulation.

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A possible reason for the absence of a stress response is that muscle may respond differently to electric than to magnetic fields. This explanation does not appear to be likely. Preliminary unpublished studies (*17*) on rat gastrocnemius muscle suggest that acute EM stimulation (2-Hz square waves with 0.3-mT peak, repeated for 4 h) causes changes in protein similar to the stress-related changes measured in *Sciara* cells stimulated with 0.8–800-μT, 60-Hz sine waves for 45 min. Because the magnitudes of magnetic field used were comparable for the two systems, the similar changes in the molecular weight distribution and shifts in the isoelectric point distribution could be due to similar mechanisms.

Acute and chronic stimulation in muscle may produce two different types of response. Apparently, the distribution pattern of proteins synthesized by muscle after acute stimulation is similar to the stress response in *Sciara* but very different from the biosynthetic response to chronic electric stimulation. A better understanding of the properties of the two responses and of the transition between them may be obtained from studies of the refractoriness of the stress–response system, as well as the changes due to repeated stimulation.

Studies with HL-60 cells and *Sciara* salivary gland cells show similar biosynthetic responses to physical stimuli of different modalities and widely differing input energies (*6, 7*). On this basis, the response mechanisms of the muscle cells are thought to be the same for both electric and EM stimulation. HL-60 cells and *Sciara* salivary gland cells respond to 1000 times weaker magnetic stimuli than electric stimuli, and the electric currents in the muscle cytoplasm due to action potentials are in the range in which they should be as effective as the weak magnetic fields.

The ability of muscles to synthesize stress proteins appears to be directly related to the types of fibers they contain (*16*). Slow fibers make HSP70 constitutively, whereas fast fibers do not. This distinction may be an evolutionary development to protect cells from overreacting to the high temperatures that can be reached in fast muscles when they contract rapidly. (The aforementioned changes in lactate dehydrogenase also correlate with the aerobic-oxidative capacity of muscles.) The response of the gastrocnemius muscle to acute magnetic stimulation suggests the presence of a significant concentration of slow fibers. In any case, both electric and magnetic fields appear to stimulate changes in muscle proteins.

The chronic changes in muscle composition do not appear to be a stress response but rather are part of a functional adaptation to the pattern of repeated stimulation. Nevertheless, chronic control of muscle composition may occur via the same molecular mechanism: electric currents that flow through tissues when muscle membranes carry electric impulses. During action potentials, the eddy currents in the muscle cytoplasm pass around nuclei near the membrane, and repeated currents (depending upon the frequency) may possibly activate particular segments of DNA that result in specific proteins. It is difficult to imagine how weak currents or magnetic fields directly affect DNA chains, which are tightly wound and stabilized by bound histones into the compact chromatin structure. Clearly, the external fields do something to initiate or accelerate the

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level of transcription and translation. Murphy et al. (*18*) showed that large electron shifts over a distance of 30 Å within a DNA molecule are possible. Electric and magnetic fields could affect such charge movements and possibly stimulate the transcription process in exposed segments of DNA.

Frequency of Stimulation and Protein Synthesis

The data for electric stimulation of biosynthesis in muscle tissue suggest the presence of a code that relates the frequency of stimulation to the synthesis of a particular protein. This code may reflect the activation of a particular promoter segment on the DNA in nuclei that is stimulated directly by magnetic fields or by induced currents.

Studies of increased Na^+/K^+-ATPase activity in magnetic fields, described in another part of this book, suggest that magnetic fields affect charge transfer during the ATPase reaction. At normal operating temperature, the optimal enhancement of the reaction in a magnetic field at 60 Hz is very close to the measured rate of the enzyme. A similar correlation between optimum frequency and reaction rate has been seen in studies of HL-60 cells, in which an optimal enhancement of transcription occurs at a frequency of 45 Hz in stimulation by electric and magnetic fields (*2, 8*). This frequency value is in the range of RNA synthesis rates (bases per second) and suggests that magnetic fields may also have a direct effect upon the transfer of charge during the RNA polymerase reaction.

If the frequency determines the charge-transfer process stimulated, then selectively stimulating an individual response by choosing a frequency that applies only to that reaction should be possible. The spectral content of EM fields in the background and of the stimulating signal would have to be known. The correlation between magnetic field frequency and the rate of charge-transfer reactions offers great promise for clinical applications and for establishment of safety standards.

Researchers in the field of chronic electric stimulation of biosynthesis in mammalian muscle have pointed out potential beneficial health effects. The same is true of research with EM fields. In the bone-healing studies mentioned, the EM signals were apparently stimulating muscle cells as well. Such chronic stimulation would undoubtedly have been important in overcoming the disuse atrophy (muscle tissue loss) that normally occurs in immobilized limbs. This stimulation probably played an important role in the healing process and contributed to the observed efficacy of the medical intervention.

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RECEIVED for review March 8, 1994. ACCEPTED revised manuscript December 23, 1994.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch010

10

Epidemiologic Studies and Their Role in Identifying Potential Risks from Exposure to Electromagnetic Fields

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This chapter discusses primarily studies of populations with possible exposure to nonionizing radiation and diseases that appear to be associated with such exposure. The epidemiologic studies of residential exposure are reviewed for childhood and adult cancers, and confounding factors are discussed. The epidemiologic studies of occupational exposure are reviewed for brain cancers, leukemias, and breast cancer. Finally studies of possible reproductive effects from electromagnetic fields are summarized.

ELECTRICITY IS AN ESSENTIAL PART of the human environment. Since the discovery of electricity, humans have used this resource to heat their homes, to provide light for their daily living, to run the tools that simplify their work, and to power the equipment for their recreational activities. For more than a century, the use of power has grown with increasing population size and industrial development. Scientists studied environmental effects from power use, but no one seriously questioned whether the exposure of humans to electricity was safe.

In 1966 two Russian scientists (*1*) suggested that exposure to electricity associated with work in a high-voltage power switchyard could cause neurologi-

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cal effects in workers. Other scientists felt that any effects from nonionizing radiation would result from heating, which would not occur with exposures at low frequencies. This position was supported by the fact that no risks had been seen in biological experiments except under conditions that generally caused heating of tissue. However, transient changes in physiological function such as heart rate had been noted (*2*).

By the early 1970s, scientists in the United States began to question the possible damaging effects from extremely low frequency electromagnetic fields (EMFs). Initially, the emphasis focused on specific exposure scenarios related to government security activities. One involved the U.S. Embassy building in Moscow where high levels of microwave flux densities had been detected. The speculation was that the Russians were trying to adversely affect the health and performance of Americans housed in the building. A review of the health status of these workers did not identify any serious illnesses (*3*). The microwaves were probably used to gather intelligence information. Concern arose as well because of a Navy communication system used to contact submarines (*4*). The antennae used by the system transmitted at low frequency and high power but at much lower electric fields than are found near high-tension distribution lines. The scientists reviewing the preliminary studies suggested that the nonionizing EMF exposure appeared to increase stress and lower immune resistance (*4*).

Research into the nonthermal effects of nonionizing radiation has expanded rapidly in the past several years. The studies on humans (cited throughout) have investigated both deleterious health outcomes as well as beneficial physiological changes in subjects exposed to electric and magnetic fields at different frequencies and strengths. Many patients have had deliberate exposure to EMFs for therapeutic purposes in bone and wound healing. However, this chapter will discuss primarily studies of populations with possible exposure to nonionizing radiation and diseases that appear to be associated with such exposure.

The uncontrolled studies of EMF exposures in humans suffer from the same criticisms common to most epidemiologic studies of chronic diseases: Exposure assessment is imprecise because data must be collected retrospectively. With some exposures, this problem has not been a serious drawback because the exposures are unique and well-remembered, such as exposure to the atomic bomb detonation or a special drug therapy. However, humans are virtually surrounded by nonionizing radiation in their daily living. In addition, scientists are uncertain at present whether all, some, or none of the components and configurations of these fields can cause health effects.

Studies of Cancer and Residential Exposures

Childhood Cancers. Wertheimer and Leeper (*5*) conducted the first epidemiologic study of the possible association between EMF exposure and cancer in children in Colorado using cancer cases identified from death certificates

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch010

and controls selected from birth records. They coded residences according to electric wiring configuration and called the different configurations "wire codes". High-tension wires carry current at 50 kV, which is then stepped down at the substation and subsequently to primary distribution lines. At poles locally the current is further reduced to 110–220 V at a transformer, and secondary distribution lines carry currents to individual homes. The Wertheimer and Leeper study grouped wires by size assuming that wires larger in circumference carried more current. Wertheimer and Leeper reported an increased risk of childhood cancer associated with an exposure measure derived from the proximity of the house to "high wiring configurations". These configurations were defined as (1) homes less than 40 m from large-gauge primary distribution lines (primaries) or six or more "thin" primaries (small-gauge primary wires thought to carry lower current than the large-gauge wires); (2) homes within 20 m of three to five thin primaries or high-tension wires; and (3) homes within 15 m of "first-span" secondary wires that arose directly from a transformer without prior service drops. All other wiring configurations were considered to result in low-current exposures.

Measurements taken near the various wiring configurations and over plumbing sites where wires were grounded showed changes in magnetic fields that correlated weakly with the hypothesized gradient in exposure. The risks from high EMF exposure based on the wire code measure were about twofold or more for all cancers, leukemia, and brain cancer. The odds ratio (OR, an estimate of the relative risk in exposed versus unexposed) was as high as 3.1 when only subjects with stable residences were included.

The study was criticized for several reasons. The investigators classifying the wiring configuration knew the disease statuses of residents. However, subsequent blind reviews of the wire codes reportedly resulted in the same classification as in the original study. The controls were selected from children born at the same time as the case and living as long as the case. These controls had to be contacted to locate residence and establish vital status, so the family had to remain in Denver, CO. The investigators did not collect in-house measurements to confirm whether measured EMF exposures would reflect presumed variations based on wire code. They provided little information on any confounding factors that might have influenced the risk of childhood cancer. Subsequent studies tried to improve on the exposure measures especially.

Numerous studies (*6–15*) followed the original report of increased childhood cancers associated with residential exposures to EMFs. These studies focused on either risks of a single cancer or all cancers. The studies also used different methods of assessing exposure, including distance from power lines and substations as well as use of the wire code of Wertheimer and Leeper. In some cases, both a proxy exposure and a measured exposure were used to characterize the subject's electromagnetic environment. Studies on cancer in children have separately reported risks for the two common cancers in that age group, leukemia and brain cancer. Other cancers in that age group are infrequent and are of-

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch010

ten reported as a group. The studies also can be divided by the methods used to identify exposure.

The results of the studies of leukemia in children associated with EMF exposure using proxy measures (distance from the power lines or a wire code scheme similar to that of Wertheimer and Leeper) are shown in Table I. All of the studies compared the exposures of cases to those of controls that were selected in different ways (*5–14*). Wertheimer and Leeper (*5*) used controls who had been born in Denver about the same time as the cases with leukemia. The Savitz et al. study (*7*) and the London et al. study (*8*) used controls selected by random digit dialing. In this design neither the cases nor the controls had to be

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch010

Table I. Childhood Leukemia Related to Residential Exposure to EMFs Determined by Wire Code or Distance from Source

Study	*No. of Cases*	*OR*	*95% CI*	*Comments*
Wire Code				
Wertheimer and Leeper, 1979 (*5*)	155	3.0	1.8–5.0	Residence at death
Fulton et al., 1980 (*6*)	119	1.1	NR	All addresses of cases, birth address for controls
Savitz et al.,[a] 1988 (*7*)	97	1.5	0.9–2.6	Residence at diagnosis, positive trend with code
London et al., 1991 (*8*)	211	1.7	1.1–2.5	Longest residence
NRPB[b] meta-analysis, 1992 (*9*)		1.4	1.1–1.8	Studies above
Distance from Source (<50 m compared to 50 m or more)				
Tomenius, 1986 (*10*)	243	1.1	0.3–4.6	Source: 200-kV wires, electrical construction
Coleman et al., 1989 (*11*)	84	1.5	0.7–3.4	Source: substations, few exposed to high-tension wires
Myers et al., 1990 (*12*)	180[c]	1.1	0.5–2.5	Source: substations, transformers, high-tension wires
NRPB meta-analysis, 1992 (*9*)		1.4	0.7–2.5	Studies above
Lin and Lu, 1989 (*13*)	NR[d]	1.3	0.8–2.2	Source: power lines, substations, transformers
Feychting and Ahlbom, 1993 (*14*)	38	2.9	1.0–7.3	Source: power lines

[a]This study used revised wire code measure as reported in text.
[b]NRPB is National Radiation Protection Board.
[c]All nonsolid tumors are included.
[d]NR indicates data not reported.

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born in the city. Some controls in the London et al. study were friends of the cases with leukemia. Other studies used other cancers as controls (*11*). The Swedish study (*14*) used a nested case–control design that selected controls from the same cohort of individuals from which the cases were identified. The entire cohort consisted of people whose property was located within 300 m of any 220- or 400-kV power lines. This type of design would increase the probability of exposure in the entire population. In fact, the latter three types of controls (friends, other cancers, and persons living near power lines) might be considered to be groups that would be closely similar to cases in regard to exposure. The use of any of these three types of controls might minimize risk.

Some critics also questioned the use of controls selected by random digit dialing as in the Savitz study and some controls in the London study because these controls may differ from the cases in ways that could influence the apparent risk as, for example, variation in socioeconomic factors. Some critics suggested that some study designs, especially those using random digit dialing, differentially increase the stability of controls compared to cases because cases can move after diagnosis of disease and controls are selected from the areas where cases were originally diagnosed. Other authors (*16–18*) suggested that differences in stability of cases and controls may explain the findings, and not EMF exposure.

Familial stability is related to several characteristics such as poverty, family size, ages of family members, type of dwelling, and, a factor very important in this situation, disease in the family. However, the data do not suggest that stability explains the findings, because when the association between leukemia and EMF exposure is based only on stable households, the risk increases in some studies (*5*), and when Savitz and Kaune (*15*) corrected for stability in multivariate analysis, the risk persists. The fact that different methods were used to select controls in several studies that yielded similar results suggests that bias in control selection is not the best alternative explanation for the findings. However, there is no information about how the selection of controls may have influenced the results of these studies.

The four studies in the upper half of Table I used the proxy measure of wire code similar to that used in the Wertheimer and Leeper study (*5*). The studies in the lower half used a proxy measure of distance from a power source. The advantage to both of these measures is that they do not require access to the dwelling but can be calculated on the basis of outside review of the home. Consequently, more cases and controls had estimated exposures for at least one residence. Furthermore, cooperation of the families could not bias the results. The National Radiation Protection Board (NRPB) pooled the data from the two sets of studies shown in Table I and found the same odds ratio of 1.4 using either proxy measures, a result indicating an estimated 40% higher risk of leukemia in exposed residences, but only the wire code measure was significant.

The odds ratio of 2.9 reported for the Swedish study (*14*) falls outside of the upper limit of the pooled odds ratios of the previous studies. That result may

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be due to the design of that study in which the residences of cases and controls were selected to be within a certain distance from power lines. This type of design may have controlled for other confounding variables related to a residence, much as occurs when matched controls are selected. Such a design could result in overmatching, which could dilute the risk. The high odds ratio reported in this study suggests this was not a problem. The range of odds ratios for all studies is from 1.1 to 3.0, with the best estimate being about 1.4.

Table II presents the data from studies in which the risk of leukemia was estimated on the basis of household measurements of exposure (*7–10, 14*). These values usually represented spot measurements or 24-h measures, but none represent exposures measured over long periods. The results of the individual studies showed risks in some and not in others. The meta-analysis of these studies by the NRPB (*9*) indicated that the odds ratio was only slightly elevated at 1.2 and the confidence intervals included 1.0. The odds ratio is low and not significant, but the value is similar to that reported for proxy measures. Investigators have tended to agree that the measured values are more likely to reflect exposures in households than are proxy measures such as distance from power lines. However, all studies had problems because EMF measurements could be made only on a limited number of residences of cases and controls in the study. In addition,

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Table II. Childhood Leukemia Related to Residential Exposure to EMFs Determined by Measured or Estimated Values

Study	*No. of Cases*	*OR*	*95% CI*	*Comments*
Tomenius, 1986 (*10*)	243	0.3	0.1–1.1	Measured at door of residences at birth and diagnosis ≥3 mG compared to <3 mG
Savitz et al., 1988 (*7*)	36	1.9	0.7–5.6	Average spot measures in several rooms, low power, residence at diagnosis ≥2.0 mG compared to <2 mG
London et al., 1991 (*8*)	164	1.7	0.8–3.6	24-h average in child's bedroom, low power, ≥2.68 mG compared to <2.68 mG
NRPB meta-analysis, 1992 (*9*)		1.2	0.7–2.1	Studies above
Feychting and Ahlbom, 1992 (*14*)	38	3.1	1.1–8.6	Calculated exposure based on model and average annual line currents over 28 years, level of ≥2 mG compared to <1 mG, adjusted for multiple variables
	24	0.6	0.2–1.8	Spot measures, same levels as comparison

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some have questioned whether a lifetime exposure even for a young child can be represented by a single household measurement.

Feychting and Ahlbom (*14*) helped to clarify some of the problems arising from different exposure estimation methods. The results reported for spot measurements in the Feychting–Ahlbom study (Table II) showed no association between leukemia and these measures, even though a significant association existed between the distance from power lines and cancer in the studies using proxy measures as shown in Table I [OR 2.9, 95% confidence interval (CI) 1.0–7.3]. The investigators estimated lifetime exposures using the location of the house in relation to power lines and the load that was carried on those lines during each previous year of residence. These estimates represent the surrogate exposures of subjects over their lifetimes. The investigators established the validity of their exposure model by testing it against spot measures and found a high correlation with present line loads and house location but not with previous levels. The conclusion from these observations is that recent spot measures do not represent lifetime exposure.

The calculated measure of exposure based on line loads over the child's lifetime showed a 3.1-fold excess risk of leukemia when the highest exposure level was compared to the lowest level. This recent method of assessing exposure appears to be a helpful method of representing true lifetime exposures. However, similar data may not be available for other locations. The report does demonstrate that EMF exposure from residential sources may vary over time. Thus, the best exposure measure may be a proxy measure that integrates differential exposures throughout a lifetime.

Savitz and Kaune (*15*) attempted to refine the wire code by using measurements to improve the estimation. The new code eliminates some subclassifications of wire thickness and types of wire span that were present in the original Wertheimer–Leeper scheme. The new simplified code classifies residences within a 20-m distance from transmission lines or those with three-phase primary distribution lines as having high wire codes. The results of the analysis using the new code indicate odds ratios of 3.8 (95% CI 1.6–9.0) for leukemia and 2.4 (95% CI 0.8–7.6) for brain cancer after correcting for age, race, sex, mother's smoking, per capita income, year of diagnosis, and residential stability. Thus, refining the exposure increased the calculated risks of both leukemia and brain cancers, but the greatest effect was on leukemia cases where the risk now is significant.

As in the previous studies, the re-evaluation of the data shows some evidence of a dose response, but the odds ratio for the group with intermediate exposure levels was not significantly higher than in the low wire code. Most of the risk appeared to be in cases diagnosed after 1980, the odds ratios being 7.1 (95% CI 2.3–22.1) for cases diagnosed in or after 1980 and 1.8 (95% CI 0.5–6.3) for cases before 1980. There was some difference in the level of association of leukemia and high wire code by age, but the risk of brain cancer was highest in children aged 10–14 years. Females had a risk of leukemia associated with EMF

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exposure that was about 3 times that of males (for females, OR 7.0, 95% CI 1.9–26.3; and for males, OR 2.4, 95% CI 1.0–6.0), but the ratios do not differ significantly from each other. The risk of brain cancer with EMF exposure did not differ by sex. Because of differences in the method of ascertaining cases in the early versus the later time periods in the Savitz study and the small number of cases that were available in subcategories used to evaluate effect modifiers, the most reliable information from this study probably relates to the overall risks for leukemia and brain cancer corrected for confounding variables, as presented earlier. However, these other variables should be examined further in other studies.

The Swedish study (*14*) noted that the risk of leukemia occurred only in single-family houses. Control for potential confounding variables did not change this relationship. There was no obvious reason for the difference. However, the calculated contemporary measurements were more similar to the present spot measures for single-family than for apartment houses. This finding suggests that wire codes or distances from transmission lines may not be as representative a measure of household exposure for an apartment dweller as for a person in a single-family home. This observation needs further study. The Swedish study also suggested that the risk is higher in recent periods rather than in the period 20 years earlier. As noted, Savitz and Kaune (*15*) also found the apparent risk to be higher in recent years. Although the investigators had taken line currents into effect in the various time periods, these measures may not have taken into account all possible variations in the exposures in past time periods. Small numbers of cases or some unrecognized confounding factor also may account for the differences.

Brain cancer is the second common cancer in childhood. The relationship of this cancer and exposure to EMFs has been studied in case–control studies similar to those described for leukemia. In fact, many of the same investigators have examined all cancers using the same study design (*5, 7, 10, 14*). Table III examines the data for several studies in which exposure is based on a proxy measure of distance from power source and wire code (*5, 7, 10, 13, 14*). The number of studies are fewer, but the strength of the association is higher, in the three studies in which associations were found. However, no association was found in two studies. On the basis of all studies, the odds ratio appears to be about twofold.

Exact values of EMFs based on spot measurements or the calculated fields over all times of exposure in the Swedish study are shown in Table IV (*7, 9, 10, 14*). The results suggest that only the Tomenius study (*10*), among the three providing data, continues to show an effect. This finding indicates that the determination of the risk of childhood brain cancers in association with EMF exposure has the same problem as that seen for leukemia, namely, that the association between the cancer and EMF seems to be higher when a proxy measure of exposure is used instead of a real measure of household magnetic fields. As discussed previously, Savitz and Kaune (*15*), in a recalculation of the risk based on a re-

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch010

Table III. Childhood Brain Cancer Related to Residential Exposure to EMFs Determined by Wire Code or Distance from Source

Study	*No. of Cases*	*OR*	*95% CI*	*Comments*
Wire Code				
Wertheimer and Leeper, 1979 (*5*)	66	2.4	1.1–5.1	Residence at death
Savitz et al.,[a] 1988 (*7*)	59	2.0	1.1–3.8	Residence at diagnosis
Distance from Source (<50 m compared to 50 m or more)				
Tomenius, 1986 (*10*)	294	4.0	0.9–27.0	Source: power lines, electrical construction
Lin and Lu, 1989 (*13*)	NR	1.1	0.5–2.4	Source: power lines, substations, transformers
Feychting and Ahlbom, 1992 (*14*)	33	0.5	0–2.8	Source: power lines

[a] This study used revised wire code measure as reported in text.

Table IV. Childhood Brain Cancer Related to Residential Exposure to EMFs Determined by Measured or Estimated Values

Study	*No. of Cases*	*OR*	*95% CI*	*Comments*
Tomenius, 1986 (*10*)	294	3.9	1.2–17.3	Measured at door of residence, ≥3 mG compared to <3 mG
Savitz et al., 1989 (*7*)	25	1.0	0.2–4.8	Average spot measures in several rooms, low power, residence at diagnosis, ≥2 mG compared to <2 mG
NRPB meta-analysis, 1992 (*9*)		1.9	0.9–3.77*	Studies above
Feychting and Ahlbom, 1993 (*14*)	33	0.7	0.1–3.2	Calculated exposure based on model and average annual line currents over 28 years, levels ≥2 mG compared to <1 mG, matched analysis
	23	1.5	0.4–4.9	Spot measures, same levels for comparison

*Correction of original value in report

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch010

vised Wertheimer–Leeper wire code, found an odds ratio of 2.4 when comparing the high- to the low-configuration wire code, a value similar to what had been reported originally but with wider confidence intervals that include 1.0. The number of cases with complete information for the new classification are fewer than in the previous report.

A study of the possible risks of childhood cancers from residential EMF exposure in Denmark was reported by the Danish Cancer Registry and was summarized briefly in a bulletin from the NRPB (*18*). Following the review of the original data and the Swedish and Danish studies, the NRPB concluded that the new studies were well-controlled and better than those previously reported, but the numbers of cases were small. Therefore, they concluded that, although "they do not establish that exposure to EM fields is a cause of cancer, they provide weak evidence to suggest the possibility exists".

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch010

Adult Cancers. Wertheimer and Leeper (*19, 20*) duplicated their childhood study for adult subjects and reported an excess risk of all cancers of about 1.3 (95% CI 1.1–1.5) based on 1179 cases. However, following this observation, very few additional studies of the risks from residential exposure in adults have been done (*11, 21–23*). Those reported used different methods of assessing exposure, most including only proxy measures such as distance from a field source. The results focused on many different cancers and were reviewed in two recent documents (*9, 24*). The data do not indicate an association between all cancers or leukemia and EMF exposure in individual studies or in the report of the pooled studies (*9*). The recent study from Sweden (*25*) also examined the risk in adults and found no association between leukemia and EMF exposure. In summary, most studies of adult cancers have shown no risks from EMFs based on estimated exposures from place of residence.

Confounding Factors. Not only have questions been raised regarding whether inappropriate selection of controls may be causing the apparent association between EMF exposure and childhood cancers, but also the incomplete investigation of possible confounding factors may lead to incorrect conclusions. Just because a person lives in a house near a specific electric field source does not necessarily mean that if the person gets ill that source is the reason for the problem. Other factors that are common to houses near power lines may increase the risk of leukemia or brain cancer. Some recent studies investigated these other possible factors. Increased traffic density near residences, which would imply exposure to gasoline combustion fumes, appears to increase the risk of childhood cancers in the same Denver population studied by Savitz and Feingold (*26*). Smoking of the parents and occupations of the father also might have increased the probability that the children were exposed to benzene or other hydrocarbon compounds that are thought to increase the risk of leukemia and brain cancer in adults. Savitz and Feingold reported a higher proportion of children with cancer having possible exposure to these chemicals than children without cancer.

In Electromagnetic Fields; Blank, M.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1995.

Savitz and Kaune (*15*) reported that exposure to EMFs based on the revised wire code showed an association of the high wire code with leukemia and brain cancer in children independent of other confounding factors. Other studies (*8, 14*) investigated possible confounding factors, including socioeconomic variables, type of housing, and stability of population, and found that these factors do not account for the association that has been observed between residential proximity to power sources and the risk of childhood cancers.

The problem is that little is known about the etiologies of leukemia and brain cancer, and so it is very difficult to be sure that there are not other confounding variables that should be taken into account. To be a confounding factor, the variable would have to be associated with power-line configurations and also associated with the risk of these cancers. Now that studies have been done in many different sites and populations, it becomes harder to imagine what factors might be common to both the EMF exposure surrogate measure and the outcome under all the various housing configurations in multiple locations. Still, such an explanation for the findings is possible, especially considering that the investigators have not been able to tie the risks to direct measured exposures.

The study (*8*) in Los Angeles, CA, showed some association between leukemia and the use of specific electric appliances. However, the leukemia risk appeared to be associated with high wire code independent of other exposures after appropriate corrections. There is no apparent explanation as to why children living in proximity to field sources would have different personal activities within their residences than would children at other sites, especially after socioeconomic factors are taken into account.

In summary, it is hard to conceive of a single confounding factor that could explain the apparent association between the EMF wire codes and childhood cancer that has been reported from so many different populations in diverse locations. However, confounding factors still could explain the finding if they were virtually inseparable from the proxy measures of EMF exposure that are being used today. Investigators must continue to consider other explanations, especially as they investigate other locations, because the lack of association with measured levels still is a concern.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch010

Studies of Cancer and Occupational Exposures

Brain Cancers. Early studies that attempted to associate exposures to electricity in the workplace and cancer classified occupations as exposed without verifying the fact of exposure. The investigators grouped occupations with possibly different types of electrical exposures into the same category. Most individuals in the studies were classified according to a single job used to represent the person's lifetime exposure. Thus, any relationship of a job title with electrical exposure was based on conjecture.

Milham (*27*), in one of the first studies that addressed the risks of exposure to electricity in the workplace, focused on "usual" occupations as reported

on death certificates and used a proportional mortality ratio to determine whether an association existed between cancers and these jobs, which had been combined into electrical and nonelectrical occupations on the basis of presumed exposures. Multiple groups with different types of occupations were combined without reported information on the specific characteristics of the electrical exposures.

Subsequent research into exposures has suggested that, although many of the groups were exposed, the types of exposure differed in intermittency, intensity, and frequency. Several groups called "nonexposed" actually have been shown, by using direct measurements, to have been exposed. Little is known about exposures in the past. Misclassification of exposure is usually thought to increase the probability that a real risk will be missed. However, although that is the usual situation, some unsuspected bias may have influenced the decision regarding the grouping of jobs and tasks used to assess exposure status, and thus created an artificially inflated or deflated risk. The more precisely investigators can identify the exposures, the more they can account for other potentially confounding exposures in the jobs, the more often the results of different studies show consistent results, then the more likely that the finding of an association between EMF exposure in the workplace and cancers represents a true association.

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As seen in Table V, the earliest studies of possible relationship between EMF exposure and brain cancers included both Milham's study of proportional mortality using deaths from the United States or individual states or case–control studies using dead cases and dead controls (*27–31, 34*). Exposure was based on the job as recorded on the death certificate. The classification of exposed jobs differed from study to study. Only one of the early studies (*29*) confirmed the occupations of the deceased as reported on death certificates through interviews with the next-of-kin. Most of these studies suggested an association between brain cancer and jobs that might have had exposure to EMFs. The studies identified variation in risks by subgroups of presumed exposed jobs, but the high-risk occupational groups were not the same from study to study. Electronics workers and those who had exposure to radiowaves and microwaves were identified as high-risk occupations in some studies. Astrocytomas were associated with the highest odds ratios. The ratios ranged from 1.2 to 3.9.

By 1989, investigators had begun to report studies in which information had been obtained from living cases and controls that increased the accuracy of the job information even though there still were no data collected on exposures for the jobs (*32, 33, 35, 36*). The study of Floderus et al. (*37*) in 1992 was the first case–control study that collected measurements of EMFs in a systematic way at those jobs identified by workers' histories. The studies of Tynes et al. (*38*) and Theriault et al. (*39*) also developed careful schemes to estimate the exposures of the workers to EMFs, but these studies were done within an industry.

In the studies that included data on all jobs, the total group of electrical workers usually did not show a significant increase in the odds ratio associating

Table V. Occupational Exposure to EMFs and Brain Cancer Related by Study Design

Study	*Exposure*	*No. of Cases*	*OR*	*95% CI*	*Comments*
Proportional Mortality Analysis					
Milham 1985 (*27*)	Electrical occupation on D.C.[a]	101	1.2	1.0–1.4	
Case–Control Studies					
Lin et al., 1985 (*28*)	Electrical occupations with "definite" exposure; jobs on D.C.	951	2.2	1.1–4.1	Dead controls Gliomas and astrocytomas
Thomas et al., 1987 (*29*)	Radio or microwave exposure	435			Dead controls, jobs identified by kin, jobs classified by industrial hygienist
			1.6	1.0–2.4	RF and MW[b]
			2.3	0.7–2.5	Electronic and electric repairers, etc.
			4.6	1.9–12.2	Astrocytomas
Magnani et al., 1987 (*30*)	Electrical worker, occupation on D.C.	432	1.3	0.7–2.5	Dead control
Speers et al., 1988 (*31*)	Electrical, electronic and utility workers, occupation on D.C.	202	3.9	1.5–10.2	Gliomas only
			13.1	1.3–129.0	Utilities
Reif et al., 1989 (*32*)	Electrical worker, occupation at cancer registration	452	0.8	0.4–1.6	Other cancer cases
			4.7	1.7–13.6	Electrical engineers
			1.9	0.8–4.3	Electricians
Preston-Martin et al., 1989 (*33*)	Jobs by interview				Neighborhood controls, live cases
	Jobs 10+ years	202	1.7	0.7–4.4	Gliomas
		—	10.3	1.3–80.8	Subgroup astrocytomas
		70	0.7	0.1–5.8	Meningiomas
Loomis and Savitz, 1990 (*34*)	Jobs on D.C., 16 states	2098	1.4	1.1–1.7	Dead controls
			2.7	2.1–3.4	Electrical or electronic engineers and technicians
			1.6	1.1–2.4	Telephone repairers
Lewis and Buffler, 1991 (*35*)	Jobs by interview	375	0.7	0.4–1.1	All central nervous system tumors

Continued on next page

Table V. Continued

Study	*Exposure*	*No. of Cases*	*OR*	*95% CI*	*Comments*
Ryan et al., 1992 (*36*)	Jobs by interview	110	0.8	0.3–1.9	Glioma
		60	0.9	0.2–4.1	Meningioma
		50	5.0	1.4–17.4	Females with cathode ray tube exposure
Floderus et al., 1992 (*37*)	Jobs classified by measured exposures	258	1.4	0.9–2.1	Upper quartile to lower quartile based on job mean exposure
		52	1.1	0.5–2.2	Astrocytoma I–II
	Jobs by interview	191	1.5	1.0–2.4	Astrocytoma III–IV
Case–Control Nested Within Cohort					
Tynes et al., 1994 (*38*)	Railway workers with estimated exposures over time	39	0.82	0.38–1.78	
Theriault et al., 1994 (*39*)	Electric power workers exposures measured and estimated in past	108	1.54	0.85–2.81	Astrocytoma using highest 90th percentile compared to below median OR 12.29 (1.05–143.5)

[a]D.C. stands for death certificate.
[b]RF stands for radio frequency; MW stands for microwave.

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brain cancer and occupational exposures. The ratios for those studies (*32, 33, 35–39*), as shown in Table V, vary from 0.7 to 1.7, and none are significant. However, these studies consistently indicate that, if there is a risk of brain cancer, it is for the specific cancer, astrocytomas, especially in its most malignant form (*35*). A high risk occurred in the Los Angeles study (*33*) when the analysis was restricted to exposure to electrical jobs held for 10 or more years and occurrence of astrocytomas (OR 10.3, 95% CI 1.3–80.8). The study of Theriault et al. (*39*) showed a significant 12-fold excess risk of astrocytomas when persons exposed at the 90th percentile were compared to individuals below the median (OR 12.29, 95% CI 1.05–143.5). This high risk also contributed to the observed significant trend of increasing risk for increasing dose in this group.

In the studies that examined meningiomas, there is no association between exposure to electrical jobs and the risk of this brain tumor. One study (*36*) reported an odds ratio of 5.0 (95% CI 1.4–17.4) for women who had a cathode ray tube exposure, but this observation may have been the result of examining several exposure subgroups, and further confirmation is needed from other studies. The NRPB, in examining the three new studies of occupational groups from Sweden, Denmark, and Norway, concluded that the addition of these studies suggested that, although industries with EMF exposure might have an increased

risk of leukemia, the evidence did not suggest an increased risk of brain cancers (*18*). No studies indicated whether any observed excesses in the populations were "due to exposure to EM fields or to some chemical associated with the work" (*18*).

In summary, the results of these studies suggest that, although studies using jobs on death certificates to classify exposure suggested some association between brain cancers and exposure in electrical occupations, this finding was not as strong when individuals reported on their personal work history in case–control studies. However, when investigators examined the risks among subgroups of the exposed and specific histopathologic types of brain cancer, especially astrocytoma, the studies suggested an association between electrical occupations and astrocytomas but not other brain tumors. If EMFs act as a promoter as has been proposed, an association of this exposure with the most malignant brain cancer, namely, astrocytoma III–IV lesions, might be expected. The argument that reported associations may be due to exposure to a confounding factor becomes less tenable as an explanation for the findings when multiple occupations with different exposures are combined as an exposure category to examine risk. The same confounding exposure factor probably is not present in all electrical occupations. Selected subgroups of workers may have apparently elevated risks because of the specific characteristics of EMF exposure associated with that subgroup or an interaction between EMFs and another job exposure common to the group. These possibilities as well as the presence of an unrecognized confounding factor must be examined in future studies.

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Leukemia. The studies of leukemia in occupational settings (Table VI) include several proportional mortality studies as well as both population-based case–control studies and selections of cases and controls from within a cohort. Many of the early studies used occupation as identified from death certificates as in the studies of brain cancer (*27, 34, 41*). Later studies used either self-reported job histories in population-based case–control studies or jobs identified from work histories in studying cases and controls from within an industry cohort of workers (*37–39, 42–49*). Recent studies (*37–39, 47, 49*) attempted to measure exposures for the jobs listed in workers' histories. The Floderus–Persson study (*37*) used all jobs in the subjects' working lifetimes across all industries. The nested case–control studies (*38, 39, 49*) measured only jobs within an industry that had presumably exposed workers. Exposures to other jobs outside the industry were not measured, and often few residential measurements were made.

The results of both types of case–control studies and the proportional mortality studies of the association of all types of leukemia with EMF exposure are somewhat consistent, especially if job titles are used to indicate exposure. Most studies, except for those of Loomis and Savitz (*34*), and Tynes et al. (*38*), showed elevated odds ratios associating the risk of leukemia with EMF exposure; ratios ranged from 1.2 to 3.2. In all but three of these studies (*39, 44, 49*) the elevated odds ratio indicates a significant association between the risk of all

Table VI. Occupational Exposure to EMFs and Leukemia Related by Study Design

Study	*Exposure*	*No. of Cases*	*OR*	*95% CI*	*Comments*[a]
Proportional Mortality Analyses					
Milham 1985 (*27*)	Electrical occupation on D.C.[b]	146	1.4	1.1–1.6	AL = 1.6 (1.3–2.1)
Milham 1985 (*40*)	Amateur radio operators from license	24	1.9	1.2–2.8	AML = 2.9 (1.4–5.2) CML = 2.7 (0.7–6.8)
Robinson et al., 1991 (*41*)	Electrical occupations on D.C.	183	1.2	1.0–1.4	AML = 1.1 (0.9–1.5)
Case–Control Studies (Population-Based)					
Flodin et al. 1986 (*42*)	Self-identified electrical worker	59	3.8	1.5–9.5	AML only included
Pearce et al. 1985 (*43*)	Electrical workers	456	1.7	1.0–3.0	Highest occupation groups
			8.2	1.5–44.7	Electronic assembler
			4.6	1.6–14.2	Radio repair
Pearce 1988 (*44*)	Electrical workers	534	1.4	0.7–2.7	AML = 1.2 (0.4–3.9) CML = 0.9 (0.1–6.4) CLL= 3.4 (1.3–8.9)
Loomis and Savitz, 1990 (*34*)	15 electrical occupations from D.C.	3400	1.0	0.8–1.2	Dead controls (16 states) AML = 1.1 (0.7–1.7) CNLL = 1.1 (0.8–1.7) ALL= 1.5 (0.7–3.4) CLL= 0.6 (0.3–1.1)
			1.3	1.0–1.7	Electrical and electronic engineers and electricians
Cartwright et al., 1988 (*45*)	Electrical workers self–report	161	2.4	1.0–6.0	AML only included
Bastuji–Garin et al., 1990 (*46*)	Electrical workers	185	3.2	1.2–5.3	AL only
	self–report		1.3	0.6–2.9	Welders Adjusted for all exposures
Floderus et al., 1992 (*37*)	Jobs classified by measured exposures	250	1.6	1.1–2.4	Comparison upper to lowest quartile based on job mean exposure. Positive trend.
	Interview				AML = 1.0 (0.6–1.9) CLL= 3.0 (1.6–5.8)
London et al., 1994 (*47*)	Measured values of electrical and nonelectrical jobs				Cancer controls except brain. Ages 20–64
	Occupation at diagnosis	2355	1.3	1.1–1.6	All leukemia; electrical occupations; ANLL, CLL, CML similar rates; dose–response % time >25 mG; OR per 10-unit increase
			2.2	1.3–3.7	CML

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Table VI. Continued

Study	*Exposure*	*No. of Cases*	*OR*	*95% CI*	*Comments*[a]
Case–Control Nested Within Cohort					
Stern et al., 1986 (*48*)	Shipyard workers, jobs on work record	53	3.0	1.3–7.0	Electricians: ML OR = 2.3 (0.8–7.1), LL OR = 6.0 (1.5–24.5)
			2.3	0.9–5.3	Welders: ML OR = 3.8 (1.1–11.5), LL OR = 0
Matanoski et al., 1993 (*49*)	Telephone Co., jobs on work record classified by measured exposure	35	2.5	0.7–9.6	Positive significant trend for peak cumulated exposure
Tynes et al., 1994 (*38*)	Railway workers with estimated exposures over time	52	0.72	0.37–1.40	
Theriault et al., 1994 (*39*)	Electric power workers, exposures measured and estimated in past	140	1.54	0.90–2.63	ANLL OR = 2.41 (1.07–5.44); AML OR = 3.15 (1.20–8.27)
Summary of 10 Studies					
Savitz and Calle, 1987 (*50*)			1.2	1.1–1.3	AL OR = 1.4 (1.2–1.6) AML OR = 1.5 (1.2–1.8)

[a]ABBREVIATIONS: ALL, acute lymphocytic leukemia; ANLL, acute nonlymphocytic leukemia; CLL chronic lymphocytic leukemia; CNLL, chronic nonlymphocytic leukemia; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; AL, acute leukemia; ML, myeloid leukemia; LL, lymphocytic leukemia.
[b]D.C. stands for death certificate.

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leukemia and possible EMF exposure in the workplace. However, the studies have not been consistent in identifying which type of leukemia is involved in the excess risk. Several studies showed an increased ratio for myeloid leukemia, but the studies of Pearce (*44*) and Floderus and Persson (*37*) suggested that chronic lymphocytic leukemia was associated with the exposure to EMFs. The nested case–control study of Stern et al. (*48*) suggested that the risk by type of leukemia might differ by occupation: Welders appear to have a risk of myelocytic leukemia, and electricians appear to have a risk of lymphocytic leukemia. Tynes' study was conducted in railway workers exposed to 16.67-Hz fields, and this is one of the few populations among the case–control studies with no risk for either leukemia or brain cancer (*38*).

The recent nested case–control study of Theriault et al. (*39*) from Canada includes cases of cancer in a cohort of about 200,000 workers in the electric power industry in Canada and France. Despite the large size of the cohort, the study has limited ability to show any effects because most of the cohort came from the electric power industry in France where workers could not be followed

after leaving the industry. Thus, retirees could not be traced, and workers who may have left their jobs because of disease and then died will also be lost. Despite these limitations, the combined populations showed an odds ratio of 2.41 (95% CI 1.07–5.44) for acute nonlymphocytic leukemia and an odds ratio of 3.15 (95% CI 1.20–8.27) for acute myeloid leukemia within this group. This study measured current exposures for jobs, estimated changes in exposure over time, and classified individuals by the summed measures of their jobs. The odds ratios represent the estimated risks associated with exposure above a median of 3.1 μT-years compared to below that level. However, the data have conflicting information because the risk is not consistent in all three utilities located in three different geographic areas. There was no risk of all brain cancers with occupational exposure in this population, although a comparison of the risk in persons in the upper 90th percentile of exposure levels to those below the median revealed an increased risk for astrocytoma. No other cancers showed an association with the exposure.

London et al. (*47*) compared the occupational EMF exposures of leukemia cases and of other cancer controls excluding brain cancer identified in the Los Angeles County Cancer Registry for men aged 20–64 years. Occupations judged to have electrical exposure as noted in previous studies and a sample of nonelectrical occupations were measured using personal monitors on a sample of current workers employed in those occupations. Other exposures in these occupations were estimated. The risk of all leukemia showed a weakly positive increased risk with increase in average magnetic field exposure (OR per 10-unit increase, 1.2; 95% CI 1.0–1.5). The risk was slightly higher for chronic myeloid leukemia, especially when exposure was estimated on the basis of percent time >25 mG.

In 1988 Coleman and Beral (*51*) reported on the studies of leukemia published at that time, many of which are included here and in the cohort section that follows. The combined results of 11 studies of leukemia that had used different methods of study design, some of which are considered to offer more reliable results than others, suggested that the overall risk ratio was 1.18 (95% CI 1.09–1.29). This meta-analysis indicated a small increased risk of leukemia in electrical workers as defined in these studies, and that risk was significant. The excess appeared to be higher for acute myeloid leukemia, the risk ratio being 1.46 (95% CI 1.27–1.65). The data from England and Wales suggested that the risks of leukemia in these occupations might be higher in the recent time periods compared to the past.

The case–control studies that measured exposures and applied these values to the jobs identified in workers' histories to determine a cumulated exposure also assessed whether there is a trend of increasing risk with increasing estimated dose. In epidemiologic studies, the observation of a positive dose response is considered strong evidence that an apparent association may be real. Floderus and Persson (*37*) reported an increase in risk with increasing level of exposure as measured by mean values and time above 0.20 μT for all leukemia

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch010

and chronic lymphocytic leukemia. London et al. (*47*) also reported a positive trend that was significant for all leukemia and for chronic myeloid leukemia when using an average measure of daily exposure or percent time spent above 25 mG.

The nested case–control study of utility workers by Theriault et al. (*39*) used the time-weighted mean exposures by job to calculate cumulated exposure and found positive dose–response trends for all leukemias, but the increase was not significant. Matanoski et al. (*49*) examined the risks of all leukemia except chronic lymphocytic leukemia in relation to several cumulated exposure measures and, using peak exposures cumulated over jobs and time, showed a significant dose response.

Thus, when measurements have been used to estimate risk, the data show some increase in risk with increase in dose, but the response is often not significant, and the observed response differs by type of leukemia in different studies. Exposures characterized by intermittent high peaks show a stronger dose–response relationship. More data are needed on measured exposures and differences in characteristics of the EMF exposures, such as peak exposures, to determine whether an association between EMF exposure and leukemia exists.

In summary, the studies of the association of leukemia with electrical jobs have shown some consistency in results. Many have shown an association, usually weak, between leukemia and presumed exposure to EMFs in the workplace. When measurements have been obtained on exposures to EMFs, an increasing risk with increasing level of exposures was reported by some (*37, 47, 49*), but others have shown no trend (*39*). Questions still remain as to whether specific characteristics of EMF exposure that vary by occupation may also be associated with differences in risk. This topic, in turn, will raise further questions as to whether any observed risks specific to certain occupations are due to the EMF exposures associated with these jobs or some other factor common to that workplace. New studies must be large enough to examine the risks for specific types of leukemia.

All studies of residential exposure to EMFs and leukemia in children represent predominantly one type of leukemia, acute lymphocytic leukemia, a type of cancer that is very rare in adults. Chronic forms of leukemia and acute myeloid leukemia are common in adults. Only one specific type of leukemia in adults might be expected to occur in relation to EMF exposure as, for example, ionizing radiation is specifically associated with myeloid leukemias. However, in studies of nonionizing radiation, the leukemia types reportedly associated with EMF exposures have varied. Another question that remains is why no risks are reported for specific occupational groups with different types of exposure to EMFs, for example, welders or workers exposed to the high-intensity electromagnetic pulsed fields used by the military (*52*).

Finally, investigators have suggested that exposures to other substances on the job might be the explanation for apparent associations between leukemia and EMF exposure, but in most cases, the studies have not been able to adequately

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch010

control for confounding exposures. The presence of other agents in the workplace may create confusion in results because these agents interact with EMFs to cause cancer. A possible hypothesis is that different types of leukemia or cancer might occur depending on exposures to another agent as a chemical that initiates the cancer. EMFs would promote only the specific type of cancer that has been initiated, and the result would be different cancers in different work settings. It will be difficult to identify this sequence of events in epidemiologic studies.

Cohort Studies of Cancers. Several cohort studies (*53–63*) of workers in occupations that had potential exposure to EMFs have been completed. The investigations provide an opportunity to examine cancers at several sites, but many have reported only the results for selected cancers of interest, leukemia and brain cancer. Some studies included only specialized groups of workers who are likely to have electric field exposure, such as telecommunications workers, amateur radio operators, telephone workers, utility workers, and electrical engineers. Others combined workers in multiple jobs with potential exposure to EMFs into a single cohort, such as Törnqvist et al. (*59*), Tynes et al. (*60*), and Guénel et al. (*62*). Diseases were identified by matching the cohort of workers to sources of death records or cancer registries. Some studies (*56, 59, 60*) compared the electrical workers to external populations. In these studies, the investigators examined the possibility that these workers' risks are different from those of the general population. In such cases, the workers usually are healthier than the general group and excess risks of disease in workers would be unusual. Other investigators (*57, 61, 63*), compared workers with exposures to others in the same study population. In this situation, exposed workers must be identified and compared to nonexposed workers. Errors in exposure classification may reduce an apparent risk. The latter type of analysis should produce similar answers to those of nested case–control studies. The results of these studies are shown in Table VII.

The early studies reported only on the risk of brain cancer or gliomas in electrical workers or engineers (*53–56*). With the exception of the study of amateur radio operators (*56*), the other studies indicated no risks. The results of subsequent cohort studies varied, but in each the reported number of brain cancers was small and for most studies no excess risk was found. The cohort studies that examined the risks from leukemia generally showed an excess risk.

The study of New York telephone workers (*57*) selected working groups who by measurement had higher exposure to EMFs. Only two groups of line workers were exposed, the cable splicers and the central office technicians, but the characteristics of exposures in these two groups were very different. Only the cable splicers were exposed to power-line currents. This group showed an excess of leukemia based on a very small number of cases. The workers included were all under age 65 years. With the exception of the Sahl et al. (*61*) and Savitz and Loomis (*63*) studies of utility workers, most other studies did not group workers

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In Electromagnetic Fields; Blank, M.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1995.

Table VII. Occupational Exposure to EMFs and Cancers Related in Cohort Studies

Study	*Exposure*[a]	*Type of Cancer*[b]	*OR*	*95% CI*	*Comments*
Olin et al., 1985 (*53*)	Electrical engineers $N = 1254$	Brain	1.0	0.1–3.7	
Vagero et al., 1985 (*54*)	Telecommunications $N = 2051$	Brain	1.0	0.3–2.3	
McLaughlin et al., 1987 (*55*)	Electricians and electronics workers	Gliomas	0.9	0.7–1.1	
Milham, 1988 (*56*)	Amateur radio operators	Brain	1.4	0.9–2.0	
Matanoski et al., 1989 (*57*)	NY telephone workers	Leukemia	7.0	–	Cable splicers
	Exposed line jobs	Lymphomas	3.6	–	compared to
	by measurements	Brain	1.8	–	non-line workers
	$N = 4547$	All cancers	1.8		(internal)
Garland et al., 1990 (*58*)	U.S. Navy; electrical jobs by record N = approx. 400,000	Leukemia	1.5	0.8–2.4	
Törnqvist et al.,	Electrical occupations	Leukemia	1.3	1.0–1.3	
1991 (*59*)	$N = 19,170$	AML	1.2	1.0–1.6	
		CLL	1.1	0.9–1.4	
		CML	1.0	0.8–1.4	
Tynes et al.,	Electrical workers	Leukemia	1.4	1.1–1.8	
1992 (*60*)	$N = 37,945$	AML	1.6	1.1–2.3	
		CML	1.5	1.0–2.1	
		CLL	1.3	0.8–1.9	Leukemia by exposure in jobs
		Brain	1.1	0.9–1.4	Radio frequency
		Bladder	1.2	1.1–1.4	OR 2.9 (1.3–5.4)
		Pancreas	1.2	1.0–1.4	Heavy magnetic
		Larynx	1.4	1.1–1.8	1.8 (1.1–2.8)
		All cancers	1.1	1.0–1.1	
Sahl et al.,	Electric utility workers	Leukemia	1.1	0.5–2.3	Internal cohort
1993 (*61*)	Cohort and nested case–control analysis	Brain	1.1	0.4–2.7	analysis
	$N = 36,221$	Lymphomas	1.3	0.7–2.3	
		All cancers	1.1	0.9–1.3	
Guénel et al., 1993 (*62*)	Electric workers	Breast	1.4	0.2–4.9	No risk intermittent
	Intermittent N = 154,000	Brain	0.7	0.4–1.0	No risks women
	Continuous N = 18,000	Leukemia	1.6	1.2–2.2	
	Danish occupational survey	AL	1.6	0.9–2.6	
		Other leukemia	1.7	1.1–2.5	

Continued on next page

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Table VII. Continued

Study	*Exposure*[a]	*Type of Cancer*[b]	*OR*	*95% CI*	*Comments*
Savitz and Loomis, 1995 (*63*)	Electric power company workers, *N* = 138, 905 men	Relative risk in highest exposure ≥4.3 μT-years			Internal analysis adjusted for age, calendar year, race, social class, work status (active or inactive), PCBs[c] and solvents
		Total mortality	1.23	1.16–1.30	
	Measured TWA exposures for jobs	Total cancer	1.221	1.09–1.37	
		Leukemia	.11	0.57–2.14	
		Brain cancer	2.29	1.15–4.56	
		Dose–response increase relative risk per μT-year			
		Total mortality	1.02	1.01–1.03	
		Total cancer	1.22	1.09–1.37	
		Leukemia	1.11	0.57–2.14	
		Brain cancer	2.29	1.15–4.56	

[a] *N* is the number of cases.
[b] Abbreviations are the same as in Table VI.
[c] PCBs stands for poly(chlorinated benzene)s.

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into exposure categories based on measured values for the jobs. However, the studies except for these two showed small excesses of leukemia for workers in electrical occupations. In the Tynes et al. study (*60*), the excess was highest for the myeloid leukemias. These authors also indicated that the excess is highest for jobs associated with exposure to radio-frequency radiation and those jobs with exposure to high magnetic fields.

The recent cohort study (*62*) of the risk of cancer based on data from the Danish Cancer Register used industry–occupation data collected 17 years previously and a classification for EMF exposure based on published reports. Guénel et al. (*62*) reported a significant increased risk of leukemia for men continuously exposed to magnetic fields [standardized incidence ratio (SIR), 1.64, 95% CI 1.20–2.24] but no increased risk of brain cancer or melanoma. Women with electrical occupations were most frequently employed in the data processing field and had no increased risk of these cancers. Breast cancer was increased in men in occupations that allowed continuous exposure, but not significantly, and the observation was based on only two cases. There was no increase in women.

The two studies that showed no increase in leukemia with EMF exposure occurred in utility workers and used an internal analysis. The study of Sahl et al. (*61*) used measurements to classify jobs. However, for about 20% of workers who had left employment, no occupational history was available. The data using both a cohort and a nested case–control approach showed no association of electrical jobs and leukemia, brain cancer, lymphomas, or cancers.

In a large cohort study (*63*), 138,905 workers employed in the electric power industry between 1950 and 1986 were followed for all causes of death, and no excess of any cause was noted in comparison to U.S. mortality. Exposure to EMFs was characterized using an integrated daily-shift measure. Savitz and Loomis (*63*) used exposures and duration by job to estimate dose and calculated relative risk per microtesla-year. Brain cancer mortality increased with exposures; the highest increase was in the group with exposures estimated for the period 2–10 years before disease, in which the relative risk increased 1.94 per microtesla-year (95% CI 1.34–2.81).

Leukemia showed no increase with dose, but the relative risk was 2.50 (95% CI 1.06–5.76) for electricians (the occupational group with highest mean magnetic field measure) with 20 years of employment. This cohort had a small but significant elevation of risk with dose for all mortality and for all cancers. This finding has not been reported in any other previous study. Brain cancers are an infrequent cause of death, and therefore the excess of this cancer cannot explain the all-cancer and all-cause excesses. Either one or more common causes of death may be increased, or the method of analysis of these investigators, which differs from that of other studies, may explain these observed excesses. The excess is small, but the result of even a small increase on total mortality or total cancers would carry a serious implication over a lifetime exposure, if true.

Tynes et al. (*60*) suggested that the workers may have excess cancers at other sites, such as the bladder, pancreas, and larynx. However, the lack of consistent reporting of excesses of cancer at the same sites in other studies may mean that these are chance observations that have resulted from the examination of multiple cancer sites.

In summary, the data from the cohort studies suggest risks of leukemia, except when electric power company employees are studied. In the largest recent study of power company employees only brain cancers, all-cause, and all-cancer mortality were increased. The observed differences in risk of electric power workers compared with other workers exposed to electric fields suggest that further information on specific characteristics of EMF exposures, such as peaks, intermittency of exposure, and frequencies, may be important. Other agents associated with work in these occupations may be important, as already suggested.

Breast Cancer. An unusual observation has been the reported excess of breast cancer in men working in electrical occupations (Table VIII). Because of the rarity of this cancer and good survival among men with the cancer, some

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Table VIII. Occupational Exposure to EMFs Related to Breast Cancer

Study	*Exposure*	*No. of Cases*	*OR*	*95% CI*	*Comments*
Males Only					
Matanoski et al., 1991 (*57, 64*)	Cohort: NY Telephone Co.	2	6.5	0.7–23.4	Compared NY State rates. Electromagnetic switching exposure jobs from personnel records
Demers et al., 1991 (*65*)	Case–control	227	1.8	1.0–3.7	Jobs by interview
Tynes and Andersen, 1990 (*66*)	Cohort	12	2.1	1.1–3.6	Highest ratio in electrical transport SIR 4.0 (1.1–10.1)
Floderus et al., 1994 (*67*)	Cohort	2	8.3	2.0–34.3	Engine drivers
		4	4.3	1.6–11.8	Railway workers
Females Only					
Loomis et al., 1994 (*68*)	Case control	27,882	1.4	1.0–1.8	68 cases exposed
	Jobs on D.C.[a]		2.2	1.2–4.0	Ages 45–54
	Electric workers				

[a]D.C. is death certificate.

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of the early studies of death certificates may not have examined this risk. Matanoski et al. (*64*), on the basis of a few cases, reported a risk of male breast cancer in the subgroup of telephone workers who were exposed to electromagnetic switching operations. Tynes et al. (*66*) reported an excess in electrical occupations, but the highest risk appeared to occur in those who were working in the railroad industry.

The recent Floderus et al. report (*67*) of the Swedish workers suggested that the risk of breast cancer was eightfold among engine drivers in the first 10 years of the follow-up after classification into job categories in 1960. The risk was high for the whole railway industry. The risk disappeared in later time periods, possibly because employment in the industry declined by 50%, so the worker classification in 1960 may not have been correct in later time periods. The investigators also suggested that younger workers may have higher risks. Floderus et al., in the same paper, reported a threefold excess risk of pituitary tumors in railway workers, a result that also may suggest hormonal effects from some exposure in this occupation.

The Danish study (*62*) of a cohort of men and women potentially exposed to magnetic fields above a threshold of 0.3 μT reported a possible excess of breast cancer in men (SIR 1.22, 95% CI 0.77, 1.83) and no excess in women (SIR 0.96, 95% CI 0.91, 1.01). A population-based case–control study (*65*) in the United States reported excesses of breast cancers in males working in electri-

cal occupations, but these investigators did not describe a specific type of exposure. One study (*68*) of women in electrical occupations reported on the risk of breast cancers and suggested a 1.4-fold excess risk, which is higher in the younger ages. This study, unlike the others, identified cancers through the use of death certificates. This approach introduces another factor into the risk, that of survival, because breast cancer is not a rapidly lethal disease.

Railway trains in Norway, Sweden, and Switzerland may expose some workers to high levels of magnetic fields. These fields may be at different frequencies than those experienced from power lines. In addition, the telephone workers who showed the risks of breast cancer had very unusual exposures to rapidly changing magnetic fields. This observation again emphasizes the need to search for the differences in exposure that may provide clues as to where the important etiologic factors may lie, including other exposures to confounding variables that may differ by specific occupation.

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The observation that men may develop breast cancer from exposures to EMFs is important because it supports a prior hypothesis regarding the mechanism of EMF effects. Laboratory scientists have reported changes in melatonin in animals in the presence of EMFs (*69a, 69b*). These observations prompted Stevens (*69c*) to hypothesize that breast cancer might occur in excess among women exposed to high-power lines. Melatonin, which is secreted by the pineal gland, may influence the risk of breast cancer because it is a neurohormone that controls the day–night variation in estrogen as well as many other bodily functions. The estrogen level is high in the day, and when melatonin secretion increases in the evening, the level is reduced. Many cancer biologists believe that the risk of breast cancer is increased in the presence of unopposed estrogen. Hence, the theory would be that continued exposure to EMF might decrease the cyclic levels of melatonin and thereby cause continuous high levels of estrogen day and night and a consequent increased risk of breast cancer. Melatonin levels could exert an even more direct effect on the risk of breast cancer because laboratory experiments have indicated (*70*) that this agent can suppress the growth of estrogen receptor positive breast cancer cells.

Using cell systems, Liburdy (*71*) recently attempted to answer some of these questions directly. Liburdy's results indicated that EMF exposure will not increase the proliferation of breast cancer cells directly, but it will block the oncostatic effect of melatonin on cells. The mechanism is unknown, but Liburdy suggested that it may act through a modulation of the signal transduction associated with melatonin's regulation of cell growth. The effect may differ in breast cancer cells with and without estrogen receptors, but most of melatonin's effects have been observed in estrogen receptor positive cells. Male breast cancers are frequently estrogen receptor positive, but the cancers in females have different proportions of receptor-positive cancers depending on age. Therefore, the effect in females might be diluted because of these differences.

The recent study of Floderus et al. (*67*) reporting an excess of pituitary tumors could be related to the melatonin theory as well because changes in hor-

monal feedback mechanisms are thought to be related to these tumors. Epidemiologic studies may not have focused on these neoplasms because they are benign and often not reported in cancer registries or on death certificates. Thus, they are rare tumors and difficult to follow in populations.

Tynes et al. (*60*) reported an increase in soft tissue sarcomas in electrical workers. This increase may have been a chance observation, but, as the investigator noted, it might also be related to changes in the melatonin regulation in the body. This neurohormone is known to influence the immune response. These cancers have also been related to immune function. These cancers are rare and difficult to identify in death records, and therefore investigators should begin to watch for these and other cancers that might be related to changing melatonin control of physiological function.

Melanomas were reported in two studies of telecommunication workers and in other populations of workers who may have had EMF exposures as welders (*72a, 72b*). The infrequency of the observation and the importance of sunlight exposure as a risk factor for this cancer make it difficult to place much weight on the reports. However, the observation needs further attention in future studies because laboratory evidence (*72c*) suggests that oral administration of melatonin can inhibit growth of melanomas in BALB/c athymic mice.

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Other Exposures. Reports of cancers have occurred with other exposures to nonionizing radiation under specific circumstances. Muhm (*52*) reported an increased risk of leukemia in workers who were exposed to rapid pulsed electromagnetic exposures and continuous-wave fields of 10^4 and 10^8 Hz. The exposure, which mimics the nonionizing radiation exposures from nuclear blasts, is very unusual, and the observation is based on only two cases. Barregard et al. (*73*) examined the risk of all cancers in chloralkali workers exposed to static electric fields and found no excess, but the population consisted of only 157 men. Robinette et al. (*74*) examined the risks associated with exposures to radar and microwaves (*74*). The study had short follow-up, and the analysis could not identify any risks.

Hill (*75*) reported on a study of the laboratory workers and professionals who originally developed radar equipment and found significant excesses of Hodgkin's disease and gall bladder and bile duct cancers. Because these sites have not been mentioned in other studies, the importance of the observations is unknown. The study of amateur radio operators (*40*) was discussed earlier. Differences in health outcomes from exposures that vary from those associated with power lines may help determine whether specific characteristics or frequencies of nonionizing radiation exposures are associated with risks.

Discussion of Findings

The studies that examine the risks of cancer related to residential and occupational exposures to EMFs generally have identified increased occurrences of

leukemia, brain cancers, and possibly male breast cancer. The residential risks have identified a possible association of brain cancers and leukemias in children with type of housing, but few studies of adults have indicated any associations with cancers. This result is not entirely surprising because children are likely to have had fewer residences before diagnosis of cancer than are adults. Therefore, there is less possibility of misclassification of exposure to the residence of interest.

The exposure variable most likely to be associated with cancer is distance from a power-line source or a measure that takes the distance into account, as in the Swedish retrospective exposure assessment (*14*). A measure of the current magnetic fields in the home in all studies has been less likely to be associated with cancer. But recently measured fields may bear only a weak relationship to past exposures (*14*). The question that is raised is whether any other factor can be characteristic of a house situated near a power source that is a confounding variable associated with this type of residence. In Colorado, the two studies showed that the houses near sources with high-exposure wire codes were more likely to be near intersections (*5, 7*). It is not clear whether correcting for that variable would still leave a significant risk from EMFs. It is also not clear whether that characteristic would be common for sites other than Denver. The fact that the studies show consistent results regardless of the city makes it less likely that there is a common confounding variable for all residential studies. Meta-analysis suggests a small increased risk from all studies. The excess is significant only when the proxy exposure measure of wire code is used.

The fact that the risk is small is not surprising. If all types of low-frequency EMF exposures are associated with a risk, then both cases and controls have the exposure, and any effect would be diluted. If only a certain type of exposure to EMF is related to a risk and it is not being measured using the current exposure classification, then again the risk would be diluted. Finally, low risks also can indicate a measure that is not actually directly related to the risk of disease but is related to the true etiological agent, in which case EMF is just a confounding variable. At present, the findings in multiple studies of cancer in children and EMF exposure are not explained.

The leukemias and brain cancers of children are usually acute lymphocytic leukemia and neuroblastomas, respectively. These are totally different cell types from leukemias and brain cancers that occur in adults. Yet the adult occupational studies suggest an increased occurrence of leukemia and brain cancers. The leukemia cell type associated with electrical occupations originally appeared to be acute myeloid leukemia, but recent studies suggested an association with chronic nonlymphocytic and chronic lymphocytic leukemia. This lack of specificity in the type of cancer is disturbing because it suggests that the results may not represent a true association.

Recent studies (*18*) have been more likely to identify an association with leukemia than brain cancer. When brain cancer is associated with exposures to EMFs in an occupation, the risk appears to be more likely to be associated with

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astrocytomas. However, gliomas, of which astrocytomas are a subset, are the most common cell type in adults. The differences in cell type may simply be a reflection of the role of a promoting agent increasing the growth and malignancy of the cancer. Whatever cell type of cancer is initiated because of mutations caused by other agents, the presence of EMFs in the environment as well could be just increasing the risk.

The major problem with the occupational studies may be the imprecise measure of exposure. Often the investigators in early studies combined jobs they presumed may have an exposure. However, measured exposures often classified jobs differently or showed marked variation in exposure within a job. Misclassification usually will dilute any apparent effect. The investigation of other potential exposures associated with the jobs has not been very complete. Consequently, the observed effect could be due to some other exposure in the electrical workers' jobs. However, most investigators combined several occupations, so it is hard to imagine an exposure that is common to all of them. Of course, the risk may be common to only one job in the group, not all, and that job has the exposure that causes the risk. Those investigators who based their risk estimates on jobs classified by measured exposures and those who did nested case–control studies within a potentially exposed cohort usually showed higher risk estimates. The odds ratios would be likely to increase in these situations if the exposure classification were more accurate and other confounding factors were accounted for in the case–control design. The only exceptions have been the studies of electric power company workers whose exposures were measured, but one study reported no excess risks and the other reported excesses of total mortality, all cancers, and brain cancer.

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Reproductive Effects

Examining reproductive risks from possible toxic exposures leads to a wide range of potential outcomes, from infertility to early or late fetal losses to congenital malformations. Even cancer at early ages may represent an intrauterine exposure to a toxic agent. In general the studies of possible reproductive effects from EMFs have not reported a full review of all possible adverse reproductive outcomes. The studies focused on exposures primarily to appliances such as electric blankets, electric heating units, and video-display terminals (VDTs). Some examined the effects from occupational exposures of the fathers. Many of the exposures, such as those of VDTs, are very different from the fields related to proximity to distribution lines. An outcome of cancer in a child is certainly related to a different sequence of possible biologic mechanisms than an outcome of infertility in the parents. Despite these limitations of the studies, the public's concern over EMF exposure has focused on adverse reproductive events as well as cancer.

Wertheimer and Leeper (*76*) investigated the risks to the fetus associated

with use of electrically heated beds and blankets. They reported an increase in length of gestation for infants of parents who had those devices in the home, but only when the infants were conceived during months when temperatures were low. They also reported that, in households using electrically heated beds, fetuses conceived during colder months were more likely to end in spontaneous abortion compared to those in households with unheated beds. They did not confirm that the heating unit was actually used.

The same investigators conducted a second study (*77*) in Oregon examining the ratio of spontaneous abortions to live births in households with ceiling heating according to month of the year. The overall proportion of spontaneous abortions was not higher for houses with electric heating in the ceiling, but the proportions were higher in the colder months, a result that the authors equated with greater use of heat. These studies were not well-controlled for other potential confounding variables that may be related to spontaneous abortion, and the methods of identifying populations for study was somewhat unusual (use of birth announcements and spontaneous abortions following live births). The findings do raise interesting questions.

A recent study from Finland (*78*) evaluated the household measures of magnetic fields in cases of early pregnancy loss versus controls. The population was part of a study in which women who were attempting to get pregnant were enrolled prior to pregnancy and their levels of serum human chorionic gonadotropin were measured monthly to determine when a woman became pregnant and when the pregnancy ended. The pregnancies were classified as early losses if the loss occurred before the pregnancy was clinically apparent. The women with cases of early pregnancy loss (EPL) were compared to controls with normal pregnancies within the same cohort. Magnetic field measurements were made of the residences at the time of participation in the study. The highest level of exposure was associated with a 5.1-fold odds ratio, which was of borderline significance, but there was no dose response. However, the numbers in the "high exposure" category were very small. EPL is considered to be a sensitive indicator of embryonic damage from possible environmental hazards.

In 1983, Nordstrom et al. (*79*) queried wives of current and former workers in a Swedish electric plant about the outcomes of their pregnancies. Among the various pregnancy outcomes examined, the only difference observed was an excess of congenital malformations among offspring of workers exposed to high-voltage switchyard work. Several other studies (*80–82*) examined the sex ratio of children born to workers in the electric power industry and found conflicting results. Nordstrom (*79*) also suggested that men in the electric power industry may have relative infertility. The finding could be explained primarily by differences in fertility of switchyard workers. Examinations of radioelectrical workers showed (*83*) an increase, which was not significant, in azospermia and oligospermia. .

The studies on the reproductive effects from both household exposures in women and occupational exposures of men do not lead to any conclusion about

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the reproductive risks associated with EMF exposures to 50–60-Hz fields. The data are very limited at present, so no firm conclusion can be drawn.

Women in the workplace have increasingly been exposed to VDTs. The amount of exposure from low-frequency EMFs may differ by instrument, and the type of exposure is not the same as found from some other electrical workplace exposures. Several studies (*84–87*) examined the risk of spontaneous abortion associated with the use of these machines and found no risk. Two additional studies (*88, 89*) did show a risk of spontaneous abortion and one (*90*) a risk of first-trimester abortion. Except for the study of Schnorr et al. (*87*), most of these studies used only time at a terminal as the criterion for exposure. Machines vary in level of worker exposure to EMFs; therefore, measurement of the machines' EMF output is an essential part of studies of these relatively short-term outcomes. Exposure measurements may be difficult to ascertain for a chronic disease that occurs years after first exposure, but the data can be feasibly collected for pregnancy outcomes.

A recent study (*91*) reported from Finland examined the risk of spontaneous abortion from exposure to VDTs in women employed in three companies. The particular models of terminals used in each company in each time period were tested to determine the level of exposure of the subjects. The use of VDTs in general was not associated with spontaneous abortions in a comparison of cases and controls. However, the odds ratio for spontaneous abortions in women exposed to the machines having high magnetic fields compared to those using the machines with the lowest exposure was significantly different. There was some suggestion of a dose response in the study. The investigators have corrected their findings for other known risk factors for spontaneous abortion that might be confounding factors.

The final group of studies regarding possible adverse reproductive effects from exposures to EMFs relate to the studies of the risk of brain tumor in children in association with their fathers' occupations. Four studies (*92–95*) looked at this possible association. The number of cases is large, ranging from 101 to 499. In the studies of Spitz and Johnson (*92, 93*) and Wilkins and Hundley (*94*), the occupations of fathers were identified from the birth certificates. The study of Nasca et al. (*95*) looked at both the occupation at birth and at death of the child as ascertained from an interview with the mother. In general, the studies all showed odds ratios that were about 1.6–2.0 but were only significantly elevated in some of the studies. The subgroup of electronics workers in two of the studies had the highest risk. Because the possible biological mechanism for risks to the fetus from a prenatal exposure of the father to EMFs are not clear, further studies are needed before accepting this apparent association. Control for other exposures related to these occupations is needed.

The data on reproductive hazards do not reflect any consistent pattern of results. The studies tended to focus on varying types of exposures, including occupational exposure to VDTs in women and electrical work in fathers. The adverse outcomes reported vary from cancer in children from father's exposure

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to early pregnancy loss from household exposure of mothers. The mechanisms are not clear. The only outcomes for which there are several studies are those relating VDT exposure in women and spontaneous abortion. These seem to show no effect, although the recent study suggests that the investigations should have been looking at early pregnancy loss rather than clinically recognized abortion. There is a need for further investigation in this area.

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RECEIVED for review June 6, 1994. ACCEPTED revised manuscript May 10, 1995.

11

Magnetic Field Exposure Assessment

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Epidemiology studies seeking to determine whether magnetic field (MF) exposure is linked to health outcomes, particularly cancers of specific types, have relied on a range of exposure assessment techniques. For residential studies, a wire coding technique for categorically classifying presumed exposure on the basis of the type and proximity of power line adjacent to study subjects' homes has been used in several key studies in the United States. Since its introduction, wire coding has been joined by other techniques in epidemiology studies, involving direct measurements of fields in homes and personal exposure monitoring. Engineering research has identified outdoor power lines and residential grounding systems as important sources of residential MFs. Occupational studies initially relied on an intuitive relationship between job title and MF exposure. Here too, measurements of MFs on job sites and personal monitoring studies have enhanced our understanding of occupational exposure, particularly within the electric utility industry. Outstanding issues concern (1) the use of contemporaneous measurements for retrospective estimation of MF exposure, particularly when a substantial time has elapsed since the exposure of interest, and (2) the absence of a biologically based metric for characterizing the aspect(s) of MF exposure potentially relevant to health outcomes.

VIRTUALLY EVERYONE IS EXPOSED TO MAGNETIC FIELDS (MFs). The question of whether exposure to magnetic fields in the extremely low frequency

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In Electromagnetic Fields; Blank, M.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1995.

(ELF) range, 3–3000 Hz, is a risk factor for cancer or other health outcomes has received significant attention worldwide (*1–3*). In pursuit of answers to this question, a number of important issues regarding MF exposure assessment have developed, and these constitute the subject of this chapter. Although ELF electric fields are usually produced by the same sources responsible for MFs, the emphasis of contemporary health-related research, both epidemiologic and experimental, has focused on MFs. This chapter, accordingly, restricts its attention to MFs.

In a generic sense, exposure assessment is vital at several points in the health-risk assessment process, which is shown in Figure 1. Certainly, one needs to know the population distribution of exposures to chemicals or physical agents that are considered hazardous. This knowledge, combined with dose– or exposure–response characteristics, enables the estimation of the public health impact that results from exposure.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch011

Further upstream at the head of the risk assessment process is hazard identification, which "qualitatively answers the question of how likely an agent is to be a human carcinogen" (*5*). Quite often, the outcome of a hazard identification for a chemical substance or physical agent depends, in large measure, on the results of epidemiologic studies. The quality of the exposure assessment component of epidemiologic studies can limit the interpretation of a body of epidemiologic evidence. This condition certainly applies to our ability to draw valid inferences about potential health effects from MF exposure.

Several informative reviews of MF exposure assessment have been published (*6–8*). This chapter emphasizes exposure assessment techniques and results and refers, in more general terms, to epidemiologic results. The chapter deals with three broad issues:

1. How do the surrogate exposure measures used in epidemiology relate to the actual characteristics of MFs measured where exposure occurs (residence or workplace)? Surrogate measures have included, for example, physical characteristics of outdoor electric power lines formalized into a "wire code" or job titles for occupational studies.

2. How do measurements taken today relate to exposures that may have occurred during proposed etiologic periods that are years or decades in the past?

3. What aspect of field exposure, often called the "exposure metric", is related to biological effects that could lead to health consequences? For example, what is the importance of time-weighted-average (TWA) exposure over a period of years, or the frequency with which intermittent fields (changes in the steady state) are encountered over a similar time span?

In Electromagnetic Fields; Blank, M.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1995.

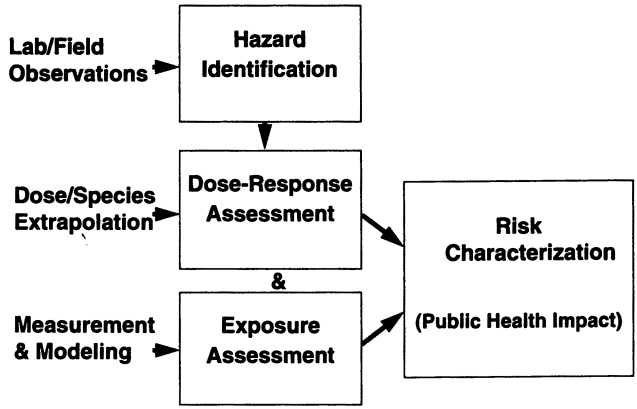

Figure 1. Elements of risk assessment. (Adapted from reference 4.)

Residential MF Epidemiology, Wire Codes, and MF Measurements

In their landmark case-control study of childhood cancer in the Denver, CO, area, Wertheimer and Leeper (*9*) introduced electric wiring configurations as a surrogate index for residential exposure to MFs. Because each residence was coded according to specific rules, the term "wire code" found general use as the term for a home's electric wiring configuration. Each subject's exposure was categorized on the basis of the type of electric utility wiring adjacent to the residence and the distance from that wiring to the residence.

Several factors led Wertheimer and Leeper to pursue wire coding of electric utility lines to assess residential MFs in preference to direct MF measurements. Perhaps the most practical consideration was that wire coding did not require resident participation. Unless the electric lines near the homes were modified over time or the homes were demolished, exposure assessment without intrusion was possible for cases and controls with verified addresses, and study size was maximized. Subsequent studies bore out this advantage when comparing the size of wire-coded populations to the size that participated in residential measurements (*10–12*). Wertheimer and Leeper also surmised that, across the entire living space of a house, the outdoor electric lines and the associated grounding system were the dominant sources of long-term MF exposure.

Wertheimer and Leeper used a dichotomous exposure classification, wherein subjects were classified as living in either high- or low-current configuration (HCC or LCC) homes. The HCC classification was based primarily on proximity to overhead transmission lines or three-phase primary distribution lines, although sufficient proximity to secondary lines coming directly from a transformer (i.e., prior to a service drop) also qualified as HCC. To support their

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch011

classification method, Wertheimer and Leeper presented field measurements they had taken under various line types, but no measurements were taken inside the residences of study subjects. On the basis of cases between 1950 and 1973, they reported that childhood leukemia mortality, as well as childhood deaths from several other cancers, were associated with residence in HCC homes. However, the lack of residential MF data rendered this study difficult to interpret.

In the 1980s, Savitz et al. (*10*) conducted a study of childhood cancer in the Denver area, based on cases incident from 1976 to 1983 [i.e., same area as, but different cases than, Wertheimer and Leeper (*9*)]. Savitz et al. (*10*) used a five-tiered wire code, summarized in Figure 2, based on a refinement introduced by Wertheimer and Leeper (*13*) in a Denver-based study of adult cancer. In addition, to address a key methodological deficit of the Wertheimer–Leeper study, Savitz et al. took point-in-time (or "spot") measurements in as many study

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch011

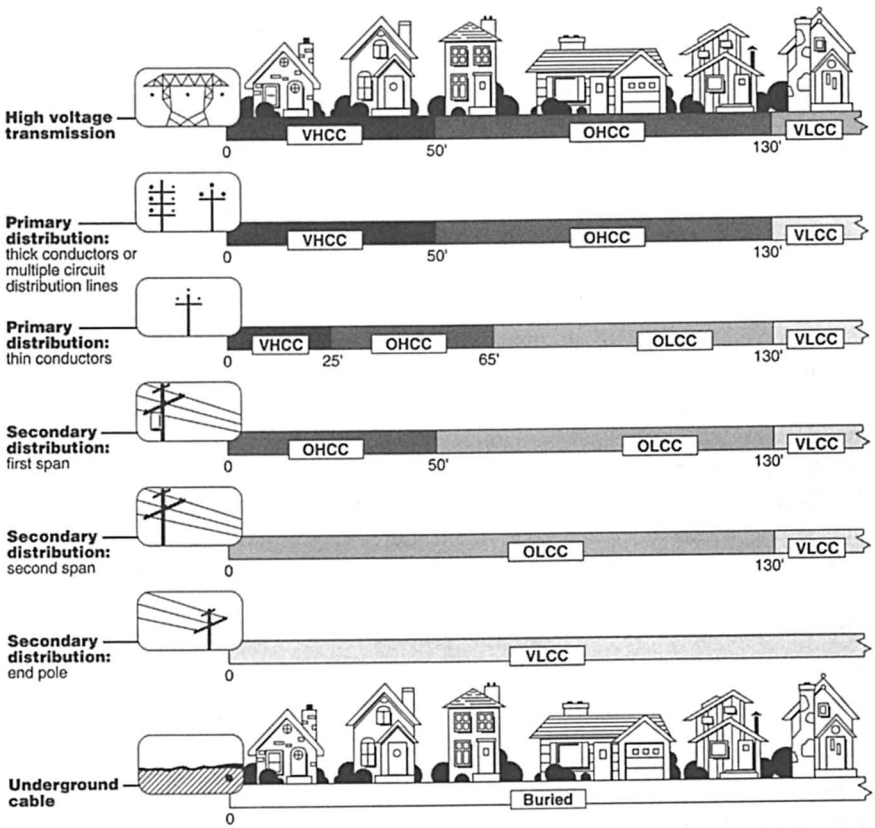

Figure 2. Summary of criteria for five-tiered Wertheimer–Leeper code. HCC and LCC are as defined in text. The prefix V means very, and O means ordinary.

homes as possible. The underlying idea was that direct measurement of the agent of primary interest should produce a stronger association with disease than would a wire code surrogate. The Savitz study produced several important results that stimulated markedly increased scientific interest in the MF–health issue:

- A positive relationship was observed between wire code and average spot-measured fields (Figure 3). (The average spot-measured field is the sum of the measurements recorded for each room within a residence divided by the number of rooms.) Using the arithmetic mean field data provided by Savitz (*14*), one can estimate that the wire code explained about 19% of the variability of the measurements across residences (arithmetic data are not optimal because the measurements are right-skewed with more of a log-normal appearance). Furthermore, about 26% of HCC residences in the study's data set registered spot measurements greater than 2 mG [including 7 of 12 very HCC (VHCC) residences], whereas only 4% of LCC residences exceeded that value (*15*).

- For the dichotomous HCC–LCC classification, Savitz et al. reported a significant association of all cancers [odds ratio (OR) 1.54, 95% confidence interval (CI) 1.0–2.3] with wire code, and a statistically significant trend was reported for all cancers across the five wire codes. Among the individual cancers, brain cancer was significantly associated with HCC (OR 2.0, 95% CI 1.1–3.8), and leukemia was suggestive (OR 1.5, 95% CI 0.9–2.6).

- For measured fields, the associations were generally (although not always) weaker and less robust, the latter owing to the smaller sample of study subjects with MF measurements.

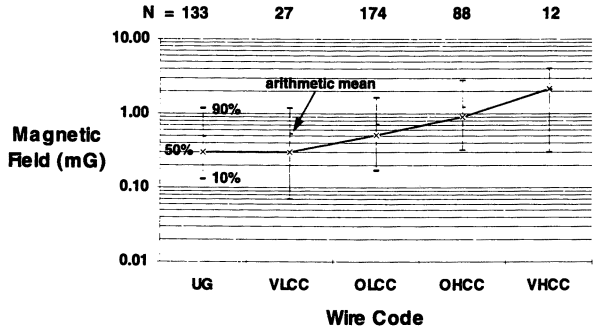

*Figure 3. Distribution of "low power" spot-measured fields by Wertheimer–Leeper wire code in the study of childhood cancer by Savitz et al. (*10*). Low power refers to the power consumption within the residence minimized prior to the measurement.* N *is the number of residences. (Adapted from reference 14.)*

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch011

On two occasions the Savitz data were reanalyzed on the basis of modifications to the wire coding scheme. First, Leeper et al. (*16*) reclassified HCC homes associated with secondary wiring. According to older practice, secondaries were strung in an "open" (or clothesline) configuration with conductors a short distance apart; for more recent service connections, triplex wiring is used with the conductors "spun" together. Because of the relative electrical properties of open versus spun wiring, Leeper et al. anticipated and measured higher MFs for the open type. (Open secondaries offer greater electrical impedance than spun secondaries to ground currents "seeking" the utility neutral. Consequently, relatively more current in open secondary systems is diverted to alternate ground pathways. This causes increased current imbalance on the distribution line, which results in higher residential MFs.) Leeper et al. also presented case-control data for all cancers that suggested that risks within the open secondary category were consistent with risks for HCC, and that risks for spun were consistent with LCC.

More recently, Savitz and Kaune (*17*) published a reanalysis of the original Savitz data based on a modified, and in fact simplified, wire code. The modified system (Table I) consists of low, medium, and high wire code (LWC, MWC, and HWC, respectively) categories and is based on proximity to transmission, three-phase primary lines, and open secondary lines. The leukemia and brain cancer HWC–LWC risk estimates were slightly higher than the VHCC–UG (underground) risk estimates in the original paper, and the all-cancer OR for the modified wire code was somewhat lower. The modification, although producing higher cancer-specific ORs, did not improve the relationship between wire code and measured field relative to the original five-tiered Wertheimer–Leeper code (*18*).

In Los Angeles, CA, London et al. (*11*) conducted an incidence study of childhood leukemia, similar in its basic design to the study by Savitz and coworkers, but with 24-h bedroom measurements added to the exposure methodology. Similar results emerged, with (1) residential MF strength weakly correlated

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Table I. Savitz–Kaune Wire Code

Category	*Residential Description*
HWC	<20 m from a transmission or three-phase primary distribution line
MWC	Not HWC <46 m from a transmission or three-phase primary distribution line <26 m from a spun secondary line
LWC	All other

SOURCE: Adapted from reference 17.

with wire code (which explained 8–9% of house-to-house variability; *see* reference 19); (2) a statistically significant trend of increased leukemia incidence across wire code and significantly elevated leukemia among the VHCC sample relative to the referent (VLCC + UG); and (3) weaker, less robust associations between leukemia and measured fields.

Feychting and Ahlbom (*12*) conducted a case-control study of childhood cancer in Sweden using as a study base the population of the country resident within 300 m of overhead transmission lines. Exposure metrics included spot measurements, distance from line, and MFs in the residence computed from an engineering model. The model took into account historical loads (dating back over three decades), physical dimensions of the lines, and their distance to the residences. The model thus represents a refined wire code that classifies homes on line ampacity and distance, but uses load records rather than line gauge for ampacity, and has a continuous (field strength), rather than a nominal (HCC, LCC) scale. The model served as Feychting and Ahlbom's primary exposure index. They also produced data showing that the correlation between spot measurements and fields calculated from contemporary loads was better than the correlation of the same measurements with fields calculated from historical loads. Leukemia was positively associated with fields calculated from the historical model and with proximity to the line, but not with spot measurements. None of the exposure metrics were positively associated with brain cancer. Feychting and Ahlbom concluded that these data supported a potential etiologic role for the TWA MF, because transmission lines were the main source of exposure, and the associated fields are steady over time (i.e., relative to those from distribution lines or appliances).

In terms of exposure assessment, these studies suggested that the wire code captured some element of the residential MF associated with the electric power transmission and distribution system. Furthermore, if the wire code represented TWA MF quantities, then perhaps it was a more reliable estimator of past field exposures than were the contemporaneous measurements. On the other hand, the possibility could not be dismissed that the wire code represented some other aspect of the MF environment (e.g., intermittency or transients) or that the wire code was associated with an unidentified risk factor (e.g., references 20 and 21).

To more accurately evaluate the source of MFs in residences and address some of the exposure-related questions spawned by the epidemiologic studies, the Electric Power Research Institute (EPRI) supported a nationwide engineering survey of just under 1000 residences drawn randomly from the service territories of 25 EPRI-member utilities (*22*).

The basic scenarios developed in the 1000-home survey for residential MF exposure are illustrated in Figures 4–6. Figure 4 illustrates that currents contributing to MFs occur on adjacent transmission lines; distribution primaries, secondaries, and their neutrals; as well as in alternate ground paths, particularly conductive water service. In addition, appliances constitute a potential source of

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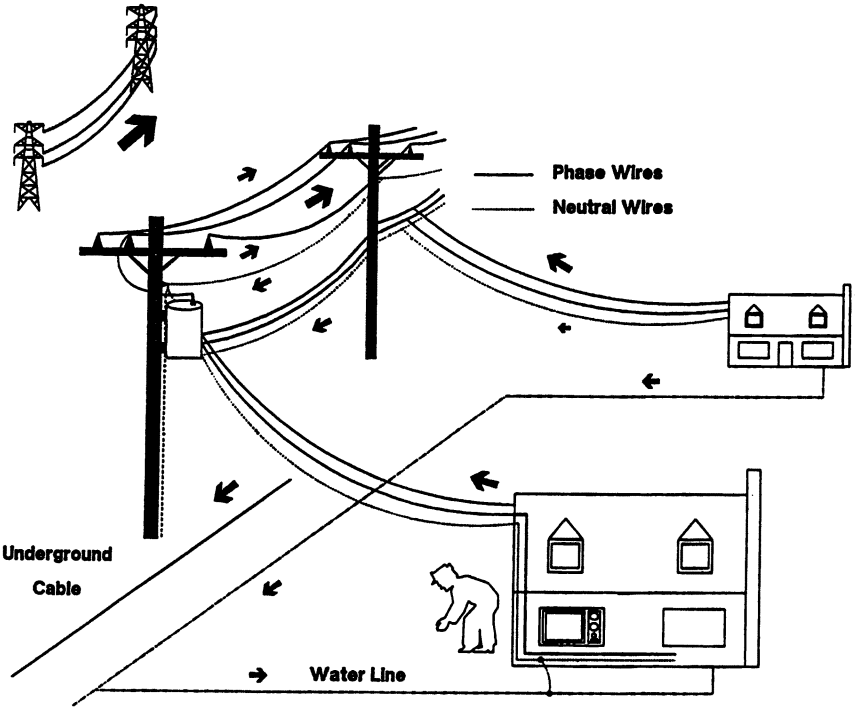

Figure 4. Possible sources of 60-Hz magnetic fields in residences: transmission, overhead distribution (primary, secondary, and net current), underground distribution, grounding system, unusual wires, and appliances. (Reproduced with permission from reference 25. Copyright 1989.)

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exposure, but because fields from most electric devices (e.g., rotating machinery) decay as the inverse of the distance cubed, appliance exposure expressed in terms of TWA may be relatively small (*23, 24*). Figure 5 shows in greater detail how spent load current splits at the service entrance to return via the utility neutral conductor or seek an alternative conductive pathway in the ground, usually the water system. Alternative pathways create net currents (shown as I_G), that can contribute to residential fields; if all load current (I_L) returned to the utility neutral (I_N), the net secondary current would be zero, in which case ground-related MFs would be minimized. Finally, home wiring that produces large current loops, as exemplified by the multilocation switch shown in Figure 6, can be responsible for fields at least as large, and possibly larger, than those from power lines.

In the 1000-home survey, data for the power-line field were obtained by deploying stand-alone recorder (STAR) MF meters at three strategic locations within the residence. The STARs measured and stored the MF every minute for

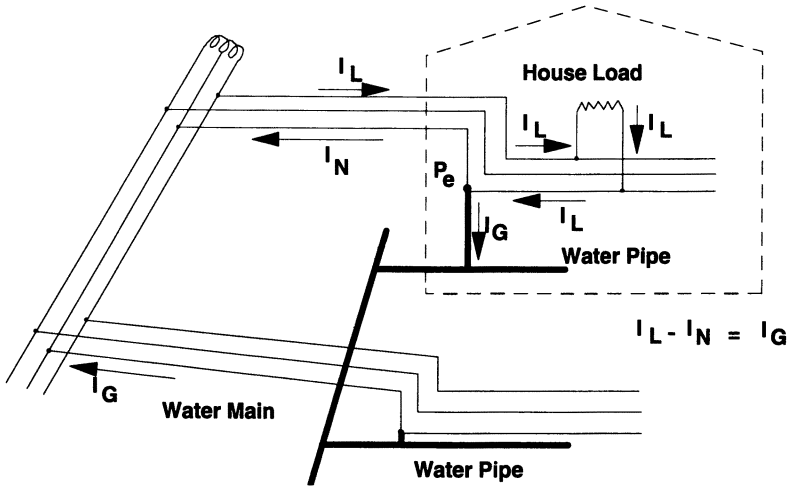

Figure 5. Example of grounding system in which a ground current, I_G, may flow. I_L is load current; I_N is neutral current; P_e is the residence's electric power service entrance. (Reproduced with permission from reference 25. Copyright 1989.)

24 h. Their data, taken collectively, provided information about the spatial and temporal characteristics of fields from external MF sources (i.e., transmission and distribution lines). Fields due to net currents fall off as $1/d$, (where d is distance), and fields due to balanced currents on multiphase (two- or three-phase primaries and two-phase secondaries) systems fall off approximately as $1/d^2$. The mathematical model Zaffanella (*22*) developed for the 1000-home survey to calculate the residential MF due to power-line currents took these relationships into account. An additional meter recorded net current in the service drop to monitor ground current. The net current data enabled Zaffanella to use another computational model, which estimated the spatial distribution of the residential MF due to ground current.

Some of the more important data from the 1000-home survey, summarized in Figure 7, shows that the median field (over space and time) within residential environments is influenced primarily by the power line and secondarily by the ground. However, the 5% "exceedance" field (the field exceeded 5% of the time over the space of the residence) is influenced about equally from both sources, and the ground-current-related MFs extend over a greater range than the power-line fields. These data suggest that if relevant biological effects (i.e., those that could lead to health effects) scale with field magnitude and are primarily sensitive to TWA exposure, then power-line fields may command priority attention. However, if fields that intermittently peak above certain thresholds are relevant to health effects, then the ground-related fields would be as important as the power-line-related fields.

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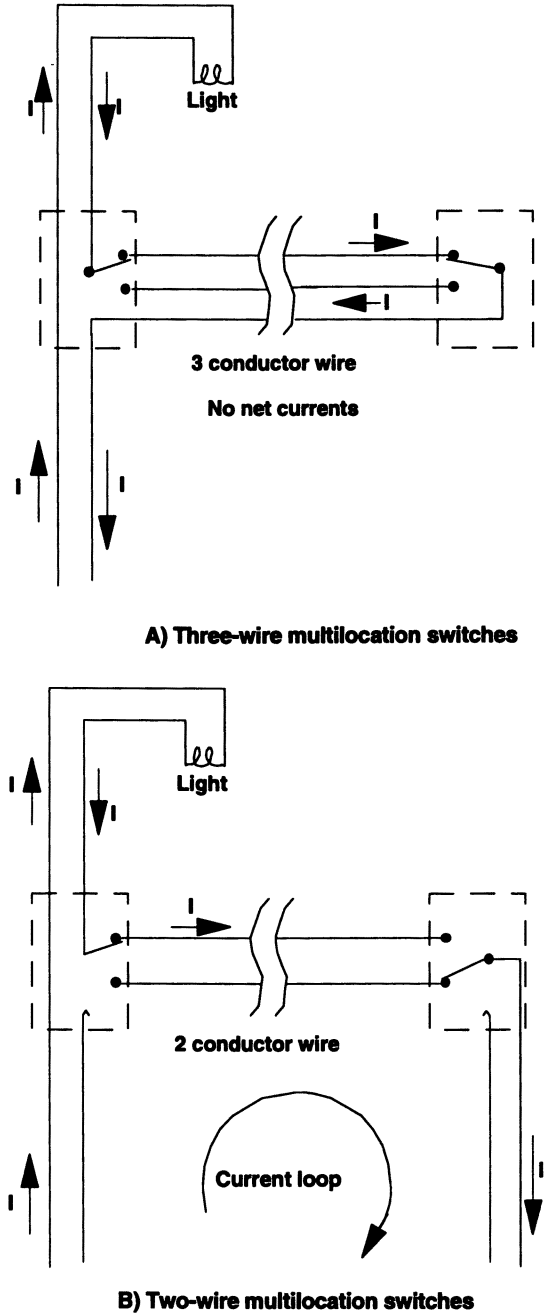

Figure 6. Examples of a three-way switch. A does not generate magnetic fields; B generates magnetic fields. (Reproduced with permission from reference 22. Copyright 1993.)

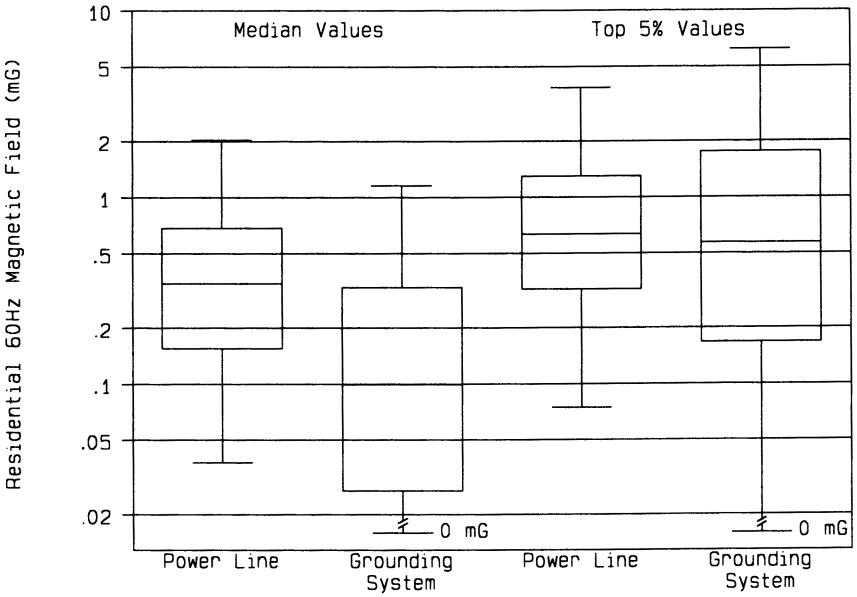

Figure 7. Distributions of median and top 5% values of power-line and grounding system residential fields, as measured in EPRI high-voltage transmission research center (HVTRC) 1000-home nationwide survey. The top 5% value of a residence's field is defined here as that exceeded in 5% of the measurements inside the residence. Distributions are represented by "box and whisker" plots, showing the fields exceeded in 5% (upper whisker), 25%, 50%, and 75% (box), and 95% (lower whisker) of the measured residences. (Reproduced with permission from reference 22. Copyright 1993.)

The 1000-home survey also collected extensive data on the relationship between MF quantities and house attributes, both electrical (e.g., indoor wiring) and nonelectrical (e.g., house age and floor space). Figure 8 shows the distribution of the average spot measurement across the residences within each wire code category. Although the fields tend to increase from LCC to HCC categories, the range of 5–95% field values span over an order of magnitude within each wire code category. For the data shown, the wire code accounts for 11.3% of the variance of the logarithm of the average spot-measured field. Figure 9 shows how the percentage of residences in each wire code with average spot-measured fields ≥2 mG increases across the wire code spectrum. Two milligauss was selected as the cutoff point for this chart, because in several epidemiologic studies this value was used to discriminate among exposure groups. The data in the chart suggest that, if personal exposure correlates with measured spot field, then wire code would misclassify MF exposure for 5% or fewer in the UG and VLCC categories (i.e., assign a low exposure classification when the MF is actually above the cutoff point) Approximately two-thirds of those in the highest

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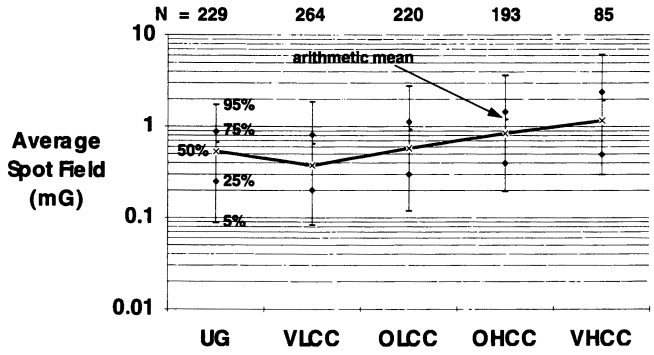

Figure 8. Distribution of average spot-measured field by Wertheimer–Leeper wire code, as measured in EPRI HVTRC 1000-home nationwide survey. N *is the number of residences. (Source: database of reference 22. Copyright 1993.)*

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category (VHCC) would be misclassified (i.e., assign a high exposure classification when the MF is actually below the cutoff point). Thus, despite the markedly increased prevalence of relatively high field levels among VHCC (and to a lesser degree, OHCC) houses, epidemiologists must deal with the exposure misclassification problem to identify valid relationships between health outcomes and MFs.

The questions of how wire code, MF levels, and personal exposure are interrelated and how measurements vary over time have been the subjects of a limited number of investigations. In the largest of these, the EMDEX Project Residential Study, Bracken and co-workers conducted multiple visits to the homes of employee volunteers representing 39 electric utility organizations

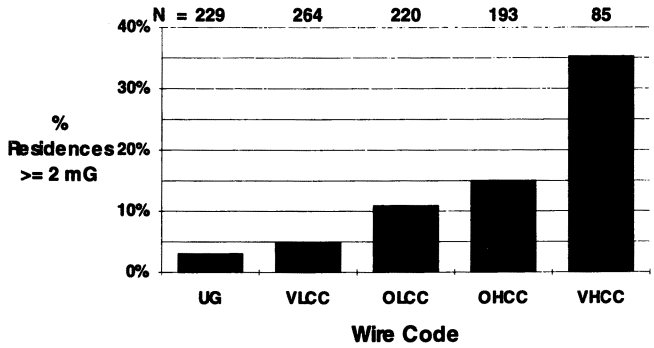

Figure 9. Fraction of residences with fields ≥2 mG by Wertheimer–Leeper wire code, as measured in EPRI HVTRC 1000-home nationwide survey. N *is the number of residences. (Source: database of reference 22. Copyright 1993.)*

from 1990 to 1992. The project takes its name from the EMDEX, a compact, lightweight device that measures and stores the three-axis ELF MF at a user-determined rate (*26*). The EMDEX downloads to a microcomputer for subsequent data analysis. The measurement period of each visit lasted for approximately 3 days and included spot measurements, personal exposure measurements, and fixed site measurements whenever the EMDEX was not worn.

Data were collected on 396 residences, visited an average of 3.9 times each, with an average of 136 days elapsed between successive visits. Figure 10 shows the distribution of personal MF exposure means per residence. Personal TWA exposures were higher in HCC categories. The plot, in general, mirrors those Bracken et al. (*27*) reported for the long-term and spot measurements, as well as the charts for the Savitz (*14*) and 1000-home survey (Figures 3 and 8, respectively). Overall, wire code explained 11% of the variance in mean personal exposure per residence. Within wire code categories, spot measurements and long-term area measurements both were moderately to well correlated with mean TWA personal exposure (Table II). Previous studies (*23, 28*) also indicated statistically significant correlations between measured fields and personal TWA exposures within residences.

The EMDEX-derived data allowed the examination of other possibly relevant exposure parameters. One of these was the mean first difference, which is the average of the absolute difference of successive EMDEX readings (every 10 s) over the course of a visit. The first difference may reflect a measure of intermittence, that is, the frequency and extent with which the steady-state field changes within a residence. The possible relevance of measures of intermittence depends on the results of laboratory studies of cells and whole animals in which the response to such fields can be studied more carefully. Nonetheless, the study did not report a strong relationship between wire code and the first difference (Figure 11).

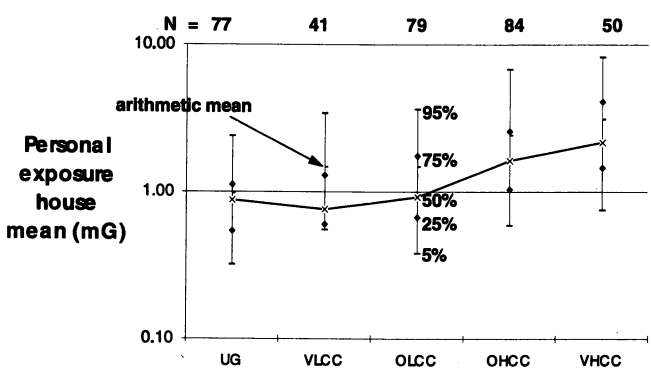

Figure 10. Distribution of personal exposure means per residence, by Wertheimer–Leeper wire code (EPRI EMDEX Residential Study). N *is the number of residences. (Adapted from reference 27.)*

Table II. Relationship Among Measures in EMDEX Residential Project

Wire Code	Number of Residences	*Pearson r Correlation of log Mean Personal Exposure With:* log Spot Measurement	*Pearson r Correlation of log Mean Personal Exposure With:* log Long-Term Measurement	*Spearman r Correlation of log Mean Personal Exposure With:* log Spot Measurement	*Spearman r Correlation of log Mean Personal Exposure With:* log Long-Term Measurement
UG	77	0.81	0.70	0.77	0.73
VLCC	41	0.66	0.54	0.63	0.52
OLCC	79	0.67	0.64	0.73	0.66
OHCC	84	0.78	0.82	0.74	0.79
VHCC	50	0.85	0.93	0.84	0.92

SOURCE: Adapted from reference 27.

The final example drawn from the EMDEX Project Residential Study addresses the temporal stability of measurements and exposure. As shown in Figure 12 for roughly 75% of the cases, the between-visit difference in the TWA personal exposure quantity was 1 mG or less (top curve), and for roughly 85% of the cases, the between-visit difference in the average long-term measurement was 1 mG or less (bottom curve).

Dovan et al. (*29*) also examined the long-term stability of spot measurements from a subset of 56 homes drawn from the study by Savitz et al. (*10*) already discussed. Across a 5-year period, they reported a correlation between spot measurements of greater than 0.7, and this finding suggested that spot measurements are relatively stable over significant periods of time. Kaune and Zaffanella

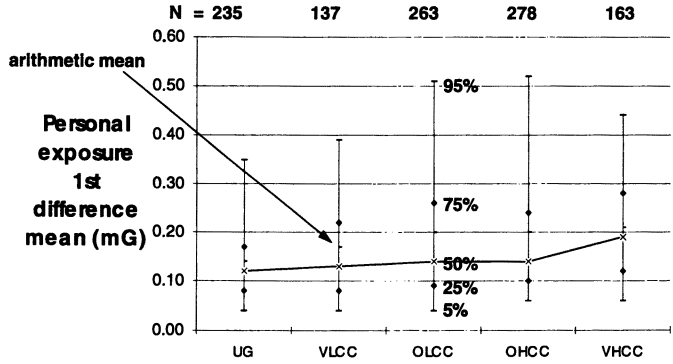

Figure 11. Distribution of the first difference of personal exposure by Wertheimer–Leeper wire code (EPRI EMDEX Residential Study). N *is the number of visits. (Adapted from reference 27.)*

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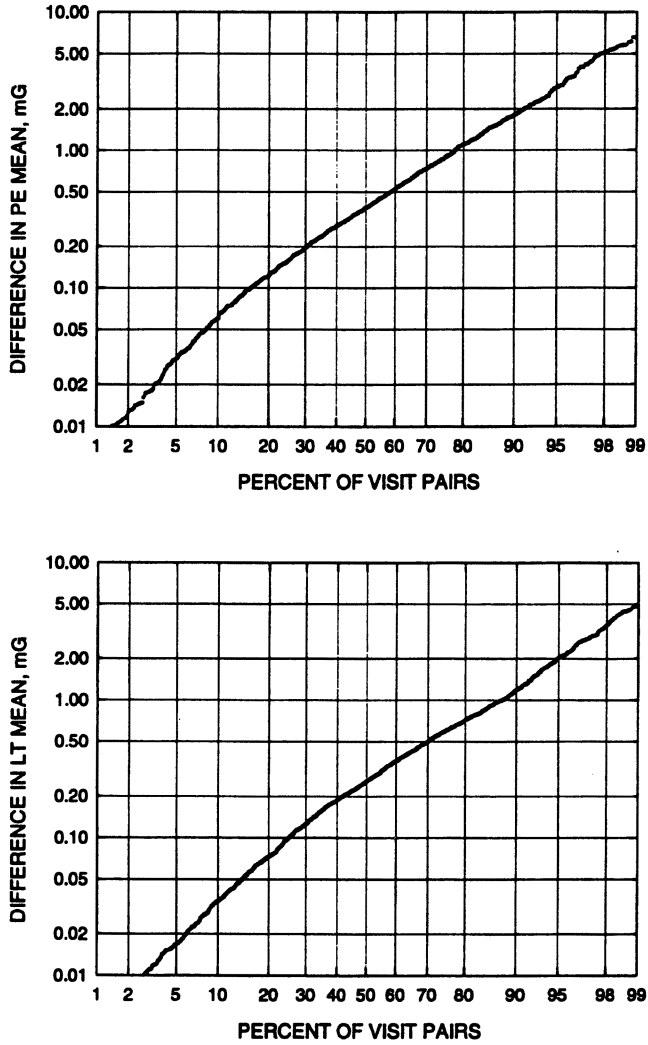

Figure 12. Cumulative frequencies. Top: Differences of personal exposure (PE) means between visits to the same residence. Bottom: Differences of long-term (LT) mean-measured field between visits to the same residence. (Source: reference 27.)

(*23*) measured residential fields and TWA personal exposures in two visits spaced about 6 months apart for a group of 30 children. Personal exposure was measured with an AMEX-3D, a small (wristwatch-sized) device that integrates the MF and reports a TWA, but provides no time history (*30*). They reported that (1) spot-measured fields for the two visits to a house correlated well with one another; (2) spot-measured fields for one visit correlated well with personal ex-

posure for the same, as well as for the other visit; and (3) interestingly, personal exposures between visits were not well correlated. Kaune and Zaffanella (*23*) proposed that personal exposures have stable and unstable components that compromise their predictive abilities for exposures at other points in time. However, spot measurements capture a stable component of exposure, which improves their predictive capability for personal exposures and other spot measurements.

This discussion has traced the evolution of residential MF exposure assessment from the development of the Wertheimer–Leeper wire code and its refinements, through the characterization of residential fields and their source apportionment, to the description of the relations among wire code, stationary measurements, and exposure, as assessed by personal monitoring. Most of the available personal monitoring data document exposures within the residence only and do not take into account exposures in other environments (e.g., at school or shopping). Kavet et al. (*28*) reported an average nonresidential TWA exposure of about 2 mG for a group of about 40 adults. Exposure away from home, including occupational exposures (to be discussed), will tend to attenuate exposure gradients attributed to either wire codes or residential measurements, to the extent that such exposures are nondifferential with respect to the residential exposure surrogates.

To date, understanding of residential exposure assessment is based on an evaluation of the wire code, which is a static quantity, or summary measures of residential fields, such as spot measurements or average long-term (1–3 days) field, which tend to represent measures of central tendency, most notably, TWA values. The number of possible exposure metrics is virtually limitless, and until biological research points us in a definite direction (or directions), the TWA exposure is likely to serve as a conservative default metric. Spot-measured fields, long-term area measurements, and TWA personal exposures are correlated weakly with wire code (as already discussed). TWA metrics have not been as strongly associated with health outcomes as the wire codes have been, but they have provided data suggestive of relationships.

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Occupational Exposure

More than 50 studies have addressed the question of whether MF exposure in the workplace leads to an increased risk of cancer (*1, 31*). The cancers of major concern include leukemia, brain cancer, and breast cancer (male and female). In addition, about 15 studies considered the potential relationship between pregnancy outcome (either spontaneous abortion or congenital malformations) and MF exposure resulting from video-display terminals (*32*).

The MF occupational and residential studies share some of the same generic issues with respect to exposure assessment. First, both have relied, at least in their formative stages, on relatively crude surrogates of exposure, the residential studies using wire codes and the occupational studies using job title. Second,

almost all of the MF epidemiologic studies used retrospective designs, which require reconstruction of MF exposure histories that could extend decades into the past. Third, epidemiologists do not have the information with which to structure exposure metrics that derive from a valid biological rationale. In an epidemiologic sense, these issues boil down to one of exposure misclassification. Misclassification of exposure, in the absence of other biases, generally leads to a more randomized distribution of exposure throughout a study population. This situation leads a priori to reduced statistical power to detect true associations, and a posteriori to a bias of the risk estimate toward the null.

Occupational studies concerned with MF exposure have predominantly been population-based; that is, their study sample was drawn from the general population. Documentation of a subject's exposure history, usually based on job title history, was accomplished with various techniques (to be discussed). Several MF studies, though, have been industry-based, that is, based on populations employed within specific sectors of an industry. Information for such studies was usually available from company files that contained data not available in the public record. These data can be quite detailed, including individual job history and industrial hygiene data on exposures and work practices. Inherently then, the industry-based study may offer a more powerful design compared to a population-based approach.

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Population-Based Studies

Nonetheless, the population-based approach provides an important exploratory role for framing hypotheses about the relationship between specific exposures and disease that industry-based studies may examine in greater detail. Also, population-based studies are relatively inexpensive (because the data are on record), can be completed in a relatively short time, and can include very large populations. Over the past 15 years, methods to more accurately characterize exposures for population-based samples have been developed and their performance evaluated. In 1980, Hoar et al. (*33*) introduced a "job exposure matrix" or JEM for linking descriptive information on an individual's industrial and occupational job codes to specific exposures to chemicals and physical agents. Hsieh et al. (*34*) refined this approach by grouping jobs with common exposures into "clusters". To a limited degree, these methods have been used by others in addressing MF exposures (e.g., references 35 and 36), and JEMs have more recently been used for industry-based studies of MF and cancer (*37*).

Siemiatycki et al. (*38*) used a large population-based case-control data set with information on 160 chemical exposures in the workplace to evaluate the statistical efficiency of five basic approaches (Table III) to exposure assessment for population-based occupational studies. These included the use of four basic techniques: (1) retrieval of routine records (e.g., death certificates), (2) interview of subject, (3) JEM, and (4) use of expert panel to assign exposure estimates to specific jobs.

In Electromagnetic Fields; Blank, M.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1995.

Table III. Main Distinguishing Features of Five Types of Case-Control Study Design

Design Name[a]	*Exposure Data Collection*	*Exposure Variables That Can Be Analyzed*
REC ONLY	Abstract main job title from routine record	Job titles
REC + JEM	Abstract main job title from routine record Apply job exposure matrix	Job titles Substances
INT ONLY	Interview to obtain lifetime job title history	Job titles
INT + JEM	Interview to obtain lifetime job title history Apply job exposure matrix	Job titles Substances
INT + CHEM	Interview to obtain lifetime job title history Subject-by-subject exposure assessment	Job titles Substances

[a]ABBREVIATIONS: REC ONLY, routine record only; REC + JEM, routine record plus job exposure matrix; INT ONLY, interview only; INT + JEM, interview plus job exposure matrix; INT + CHEM, interview plus chemist evaluation.
SOURCE: Reproduced with permission from reference 38. Copyright 1989 The Johns Hopkins University School of Hygiene and Public Health.

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Using interview combined with expert judgment as their "gold standard", Siemiatycki et al. (*38*) determined that study designs based on routine record retrieval only, record retrieval plus JEM, and interviews only provided poor efficiency (as measured by the power to detect a predetermined risk ratio with a fixed study size), and correspondingly would require very large populations to detect a moderate risk. The approach that combined interviews with JEM provided a more favorable efficiency. Bouyer and Hémon (*39*) extended this type of analysis to show a marked improvement in study power if JEM-based job categories were assigned a "probability of exposure" score on a continuous scale instead of a binary yes–no score as Siemiatycki et al. (*38*) had done.

Against this general background, the development of MF exposure assessment can be traced. The first suggestion of a possible relationship between MF exposure in the workplace and cancer originated from a series of studies in the early 1980s (published as letters to the editors of medical journals) that used a proportionate mortality ratio (PMR) approach based on routine records (*40–43*). (Even before these studies appeared, Wertheimer and Leeper (*9*) had extracted mortality data from 1950 on selected electrical occupations from a U.S. Public Health Service publication, and calculated a small, but statistically significant, increased standard mortality ratio for these workers.) The studies re-

ported that leukemias (particularly acute leukemias) were overrepresented within electrical occupations, and these findings suggested a potential etiologic role for MF.

However, MF exposures associated with electrical occupations were barely characterized at the time, and the possible roles of other potential workplace carcinogens (e.g., metal fumes and solvents such as benzene) were not evaluated. Despite these sources of uncertainty, these brief reports stimulated further investigation into the relationship between MF exposure and leukemia, as well as other cancers. Selected studies are summarized in the next section to illustrate the types of exposure assessments that epidemiologists subsequently used.

In 1985, Lin et al. (*44*) published a case-control study of brain cancer mortality among adult males in Maryland. Using industry and occupational data from the death certificates together with the expert opinions of an industrial hygienist, an occupational physician, and a radiation physicist, Lin et al. assigned each member of the study population to one of four exposure groups: definite, probable, possible, and none. The basis for the expert assignment was not given, but the study reported odds ratios that increased with the assigned probability of exposure.

Preston-Martin, Mack, and colleagues (*45, 46*) conducted a case-control study of brain cancer among male adults in the Los Angeles, CA, area. They obtained occupational history data through personal interviews with the cases and controls. Odds ratios were computed on the basis of time spent as an electrical worker (0–5, 5–10, >10 years); two definitions of electrical work were used, based on previous papers in the epidemiologic literature (*47, 48*). The primary basis for these categorizations was not given, but is assumed to be intuitive because of the absence of extensive measurement data for the occupations studied. Mack et al. (*46*) reported an excess risk of glioma, particularly astrocytoma, for those in the >10 years exposure category.

In response to concerns about male breast cancer from studies with relatively small case loads (*49, 50*), Demers et al. (*51*) conducted a case-control study on cases incident across all the surveillance, epidemiology, and end results (SEER) registries in the United States, which account for about 15% of the population. They conducted their analysis using questionnaire data from an in-home interview that included information on the start and end dates of the two longest-held occupations, and occupational classifications based on Lin et al. (*44*) and Milham (*47*). After dividing the study population according to age at first exposure and time period of exposure, Demers et al. reported excess risk for the subgroup that was less than 30 years of age at first exposure, with exposure of 30 or more years prior to diagnosis. The highest risk was seen for electrical trades and related occupations.

Spitz and Johnson (*35*) and Wilkins and Sinks (*36*) used JEM to study potential associations between parental occupational exposures and neuroblastoma mortality and brain tumor incidence in children, respectively. Spitz and

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Johnson obtained paternal job title data from birth certificates of the cases and controls. They observed an increased odds ratio for paternal employment in Hsieh's job cluster No. 7 (*34*), which contains electrical tradesmen. Curiously, this job cluster was not assigned an MF exposure rating by Hsieh et al. Spitz and Johnson (*35*) also assembled job titles according to the "exposed" job classifications listed by Wertheimer and Leeper (*9*). Again, increased odds ratios for neuroblastoma were observed. Wilkins and Sinks (*36*) classified job titles on the basis of parental interviews, and used Hsieh cluster No. 3 to represent MF exposure, as Hsieh had done. However, the jobs listed in this cluster, although showing an association with brain cancer, were not intuitively associated with electrical environments. Cluster No. 7, which is more likely related to electrical work, showed no association with cancer. Such results support the need for improved exposure assessment frameworks for MF epidemiologic studies.

Typically, population-based studies will consider the risk of more than one cancer in relation to a set of job titles or other exposure variables. In a case-control study of leukemia and brain cancer, Loomis and Savitz (*52*) used the U.S. Census Bureau's occupational classification system to code industry-occupation records from adult mortality data tapes of the National Center for Health Statistics (NCHS). The study was based on 16 states that code job information from death certificates (routine records) and report them to NCHS. They assigned cases and controls based on "exposed" job titles from previous publications (*41, 44, 47*) and reported no association of job title with leukemia, but a positive association with brain cancer.

In Sweden, Törnqvist et al. (*53*) conducted a follow-up leukemia and brain cancer incidence study that linked (via individual identification numbers given at birth) job titles coded from census records with medical registries. They chose for study those electrical occupations already associated with excess risks in previous studies. They also enlisted expert opinion from industrial hygienists and reported some job-related MF measurements, but the input from these sources did not appear to play a role in the analyses. Although elevated risks were reported within specific jobs for each cancer, a consistent pattern was not apparent.

Guénel et al. (*54*) conducted a follow-up study of the Danish population to evaluate the associations between MF exposure expressed as experience in electrical occupations and incidence of a variety of cancers in men and women. Census records provided industry-occupation data on the cohort, and the occupations were classified as "exposed" on the basis of the investigators' analysis of published papers with MF measurement data. The resulting classifications included the following: no exposure above threshold (set at 3.5 mG), probable intermittent (defined by the investigators as exposure for "a few-minute periods") exposure above threshold, and probable continuous exposure above threshold. They reported increased leukemia risks for men with continuous exposure. No excess risks were noted for brain cancer (both sexes) or breast cancer (both sexes).

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In Electromagnetic Fields; Blank, M.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1995.

Finally, Floderus et al. (*55*) conducted a population-based case-control study of leukemia and brain cancer incidence among Swedish adult males. Using occupational history data collected by questionnaire, Floderus et al. took 1-day measurements for job sites that corresponded to the longest-held job 10 years prior to diagnosis for cases and index date for controls; thus, electrical occupations were not specifically targeted. More than 1000 measurements were collected, and the measurement data (field recorded every second) allowed the investigators to structure four different exposure metrics for each job type: average field, median field, standard deviation of the field, and percent of time above 2 mG. They reported an increased risk of chronic lymphocytic leukemia that showed a distinct dose–response trend with average field level, with weaker associations for the other metrics. No association with acute myeloid leukemia was seen, and the results for brain cancer were suggestive, but inconsistent. Adjusting for the possible confounding effects of such factors as smoking and exposure to benzene and ionizing radiation did not alter the results.

Population-based epidemiologic studies concerned with MF have used a variety of generic exposure assessment techniques, as described in Table III. Most assessments were based on qualitative evaluation and categorical assignment of study subjects based primarily on job title—as derived from routine records or interview—but some studies also used the time dimension to address potential latency and exposure-time relationships. Until the study by Floderus et al. (*55*), no study had rigorously attempted to determine the MF field environment of the occupations of study subjects.

Industry-Based Epidemiologic Studies and Measurements

Epidemiologists involved with industry-based studies have the same basic methodologies available to them as discussed for population-based studies, but, as mentioned, they often have access to more detailed job histories and industrial hygiene data specific to the scenarios studied.

Stern et al. (*56*) conducted a case-control study of leukemia mortality at the Portsmouth, NH, Naval Shipyard. Job histories were constructed from personnel records, and a regression model for electricians, based on accumulated time on the job, resulted in an increased risk associated with such work. Radiation (for which dosimetry data were available) and solvent exposure did not confound the result.

Matanoski et al. (*57*) used AT&T employee records together with MF measurements taken in situations characteristic of telephone electrical work to conduct a case-control study of leukemia. They analyzed the data by job title (e.g., central office technician, cable splicer, and nonline work), by several exposure metrics based on the job-specific measurements (mean and peak fields), and by 10- and 15-year lag periods (exposure during the lag period, which directly precedes diagnosis, is thus excluded from etiologic consideration). According to

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the investigators, their results suggest excess risk of leukemia for long duration of employment in environments with intermittent (i.e., switched) fields.

With the most sophisticated JEM used to date for assessing MF exposure, Sahl et al. (*58*) studied mortality due to leukemia, lymphoma, and brain cancer among employees of Southern California Edison Company (SCE). The study incorporated both cohort and case-control analyses, with a large variety of exposure metrics from (1) a crude assignment of workers to either a catch-all category of electrical worker or other, to (2) "usual" occupation (e.g., electrician or line workers), to (3) time accumulated within specific jobs, to (4) integrated MF exposure using a variety of parameters, based on a measurement program conducted at SCE specifically for the study. Field levels were recorded for 14 jobs, five of which are shown in Table IV. All parameters shown in the table were used in the case-control analyses. In addition, various combinations of latency (or lag) and exposure windows were used to examine possible associations with median fields, and the fraction greater than 10 and 50 mG. The authors reported no associations of cancer with any of the exposure metrics used.

In follow-up analyses of the aforementioned data set, Sahl et al. (*59*) used a principal component analysis to show that exposure parameters could be bundled into groups (or factors) within which the parameters were highly intercorrelated. Thus, the geometric mean, median, and the fraction of readings exceeding 5 and 10 mG formed one such group. Discriminant analysis revealed that this group of exposure parameters also discriminated among occupational categories (Figure 13). Of course, we lack an understanding of whether one or more of the exposures responsible for distinguishing among jobs may have biophysical relevance.

MF exposures have been more intensively characterized for the electric utility industry than for any other industry. Using the EMDEX measurement technology, Bracken (*7*) measured personal exposure for 1882 utility worker volunteers, representing 55 utility organizations (59 sites), in the United States, Canada, New Zealand, and Ireland. Data were collected for 4411 workdays and

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Table IV. Measurements at Southern California Edison for Selected Jobs

Job	*Days Measured*	*MF Mean (mG)*	*MF Median (mG)*	*99th Percentile (mG)*	*Fraction >10 mG*	*Fraction >10 mG*
Technical support	57	2.3	0.7	27.9	0.040	0.002
Clerical	55	1.8	1.0	16.8	0.017	0.000
Electricians	83	30.1	3.7	384.6	0.299	0.074
Line workers and splicers	210	10.3	0.7	179.9	0.088	0.027
Plant operators	91	10.8	2.2	121.6	0.141	0.024

NOTE: 99th percentile is the field exceeded in 1% of the measurements.
SOURCE: Adapted from reference 58.

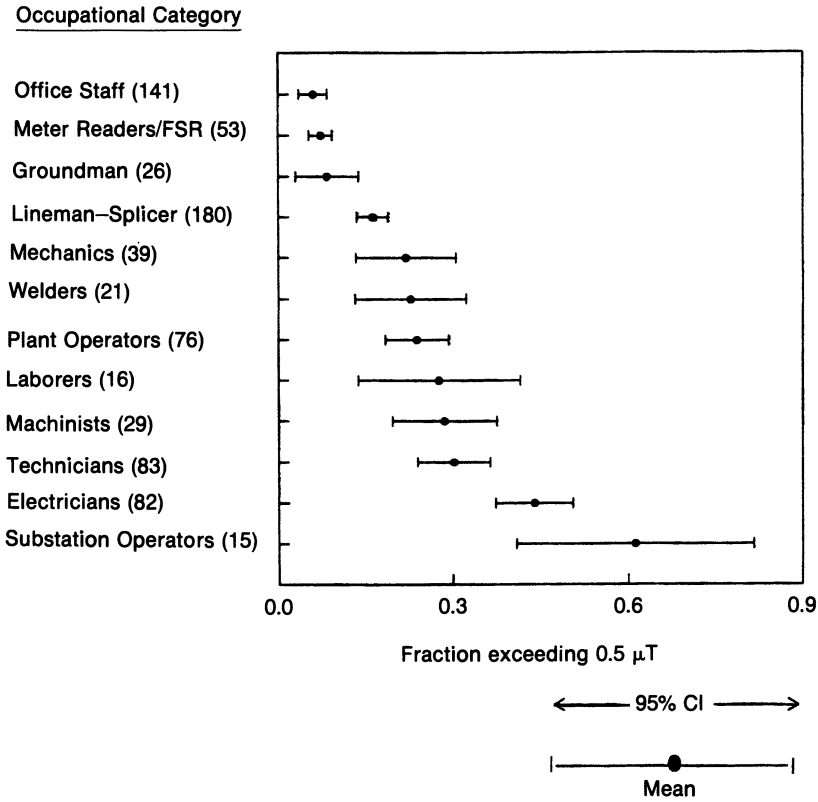


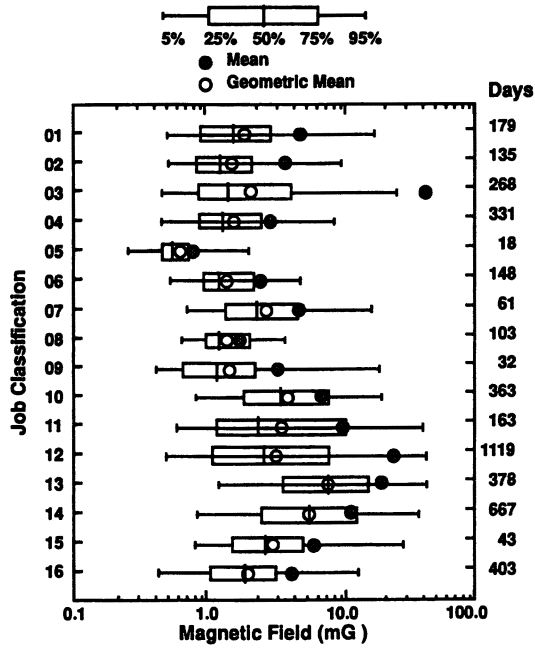
Figure 13. Means and 95% confidence intervals for the fraction of field levels exceeding 5 mG (0.5 μT). The number of days of collected data is in parentheses. Office staff includes clerical, technical support, craft supervisors, and administrative and managerial staff. The fraction of field levels exceeding 5 mG is the proportion of all measurements that are greater than 5 mG. (Reproduced with permission from reference 59. Copyright 1994 Wiley-Liss, Inc.)

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch011

1512 nonwork periods, with the EMDEX recording the MF every 10 s. Figure 14 shows the distribution of workday mean exposures by job classification. For reference, the distribution for Job No. 5, clerical without computer, is similar to that found for home environments. The highest levels of workday exposure means were associated with substation workers and electricians, but even within these groups, the range of exposures for the 5th to 95th percentiles spanned well over an order of magnitude.

Figure 15 shows the distribution of the 10-s measurements by the environment the worker occupied. The chain of electric power delivery, from generation to distribution, is represented; the highest fields are associated with substation environments However, the 95th percentiles of exposures for transmis-

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch011



Key to job classifications:

01 Managers & supervisors w/o computer
02 Managers & supervisors w/computer
03 Professional & technical occupations w/o computer
04 Professional & technical occupations w/computer
05 Clerical occupations w/o computer
06 Clerical occupations w/computer
07 Support service occupations
08 Outside customer service occupations
09 Drivers & equipment operators
10 Generation facility operators
11 Generation facility mechanics
12 Electric power line installers, repairers, & inspectors
13 Substation operators & inspectors
14 Electricians (electric system)
15 Welders
16 Other construction, field & craft occupations

Figure 14. Distributions of magnetic field workday means for job classifications. Arithmetic mean values can be dependent on a few very high readings and thus may not be indicative of measures for the group as a whole (EPRI EMDEX Occupational Study). (Reproduced from reference 60.)

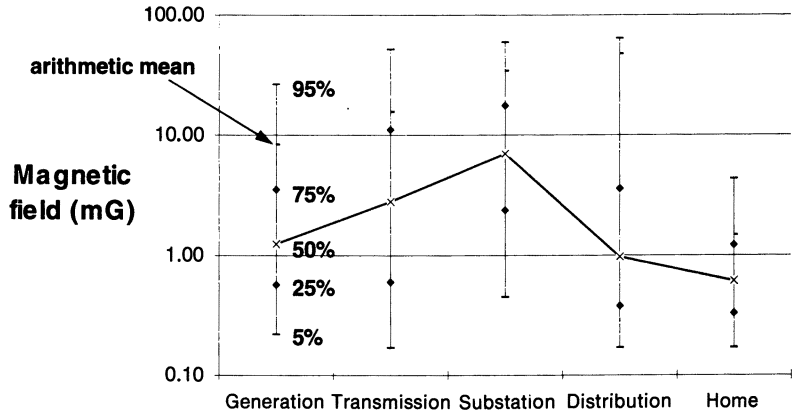


Figure 15. Distribution of 10-s EMDEX measurements by occupied environment (EPRI EMDEX Occupational Study). (Adapted from reference 60.)

sion, distribution, and substation locations are equivalent. Figures 14 and 15 also show that the distributions are far from normally distributed, as seen by the large divergence of the mean from the median. Rather, the distributions are closer to log-normal, as evidenced by the closer correspondence of the geometric mean to the median, and the more symmetric appearance of the log distributions around the 50th percentile.

Savitz et al. (*61*) used this occupational database to analyze the correlations among a variety of exposure parameters (arithmetic and geometric mean, 20th and 90th percentile, median, and percentage of time above 2- and 20-mG thresholds). Correlations were computed within individual worker days and within 16 job title groups. Within job title correlations between parameters were very high (e.g., Pearson $r = 0.94$ and Spearman $r = 0.86$ for correlation of geometric mean with percentage of time above 2- and 20-mG thresholds), and tended to be larger than within-worker correlations. The data suggest that a single measure of central tendency is a practical index of upper percentile (90th or higher) or threshold exposures, particularly when exposure is assessed at the level of job title.

Previously, Deadman et al. (*62*) conducted a smaller scale survey on 20 electric utility workers and 16 office workers whose MF exposures were measured once per minute for 7 days. The results were basically similar to those of the EMDEX project. Exposures associated with electric utility operations were significantly greater than office and nonwork exposures. For work periods only, the geometric mean of the exposure of electrical workers was 10 times that of office workers; for the entire week (including nonwork), the geometric mean was 3.5-fold higher. In a follow-up analysis, Armstrong et al. (*63*) observed high correlations ($r > 0.8$) "between the time-weighted arithmetic mean (TWA) and indices that explicitly emphasize short but high intense exposures, such as peak

values and time above thresholds." Thus, TWA quantities measured in a utility occupational setting may represent other exposure metrics related to extreme values as well.

Bowman et al. (*64*) evaluated possible MF exposure within electrical occupations with a series of spot measurements taken at 114 work sites; their results suggested that MF exposures for electrical jobs most likely exceed those received in homes or offices. To address the concerns about MF exposure and potential chemical confounders within electrical occupations, Bowman et al. (*65*) conducted a MF measurement and expert industrial hygiene survey in three sites: Los Angeles, CA, Seattle, WA, and New Zealand. In this study, the selection of electrical occupations was based on those that Milham used in a 1982 PMR study (*40*). The jobs considered were as follows:

- electrical and electronic engineers
- electrical and electronic engineering technicians
- electricians and electrician apprentices
- electric power line and cable workers
- television and radio repairers
- motion picture projectionists
- power station operators
- telephone line workers and splicers
- welders and flame cutters

In addition, a variety of nonelectrical occupations (e.g., carpenters, janitors, and sextons, and clerical workers) formed a comparison group. Final MF exposure values were tabulated by weighting task-specific measured fields by the fraction of time spent by a worker in a given job category on each task. Chemical and other exposures were qualitatively ascertained through interviews with long-term employees and occupational health staff.

Some of the MF exposure results are summarized in Tables V and VI, which show that, compared to nonelectrical workers, electrical workers had significantly greater mean MF exposures and spent a significantly greater fraction of their time above different field thresholds. A retrospective model based

Table V. Task-Weighted Mean Magnetic Field Exposure (mG) ± SD

Occupation	*Los Angeles*	*Seattle*	*New Zealand*
Electrical	9.6 ± 1.3	27.6 ± 6.0	10.0 ± 2.5
Nonelectrical	1.7 ± 0.1	4.1 ± 0.5	2.1 ± 0.2
p	<0.0002	<0.0002	<0.0012

SOURCE: Adapted from reference 65.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch011

Table VI. Percent of Work Time Above Threshold ± SE (Los Angeles, CA)

Occupation	*>2.5 mG*	*>25 mG*	*>250 mG*
Electrical	34.5 ± 1.7	5.4 ± 0.7	0.6 ± 0.1
Nonelectrical	12.9 ± 1.3	0.4 ± 0.5	0.0 ± 0.0
p	<0.0004	<0.0004	<0.0004

SOURCE: Adapted from ref. 65

on task weighting of jobs from 15 to 20 years in the past did not produce appreciably different results. Although the industrial hygiene survey did not reveal that exposure to other potential workplace carcinogens was associated with MF exposure, the question of possible confounding exposures within electrical occupations deserves more intensive scrutiny.

Two MF exposure studies that were attached to larger electric utility epidemiology studies have been reported recently. In France, Guénel et al. (*37*) reported the development of a JEM for MF exposure. They outfitted 184 workers at four fossil-fuel-burning and four nuclear power plants with MF personal exposure monitoring devices, and collected 776 workdays of exposure data. They evaluated variation within worker, between workers in the same job and plant type (i.e., nuclear and fossil), between plants, and between plant type. They determined that most of the between-worker exposure variance was explained by the specific plant site, rather than by a combination of occupation × plant type. However, because the survey covered only 8 of a possible 50 French power plants in the epidemiology study, they concluded that a JEM using plant as the prime exposure predictor was not practical, and they recommended an approach that included plant type and occupation.

In the United States, Savitz, Loomis, and colleagues are conducting a retrospective cohort cancer mortality study of 135,000 employees from five utilities. A preliminary study of 134 employees using an EMDEX to monitor workplace exposure validated expert classification of various utility jobs into high-, medium-, and low-exposure rankings (*66*). The exposure rankings were validated for arithmetic means, 90th and 95th percentile, and time spent above 3 mG. Subsequently, Loomis et al. (*67, 68*) developed a system for collapsing the multitude (about 25,000) of historical job titles at the five companies into 28 occupational categories (OCs) that would be amenable to analyzing for the relation of cancer to MFs, as well as other occupational exposures. These OCs were split, for sampling purposes, into high-, medium-, and low-MF exposure strata, again based on expert opinion (*67*). The high-MF exposure category included electricians and electric-power-line workers, the medium category contained craft occupations in power plants and substations, and the low-exposure group included office and service workers. Almost 2900 records were recorded for

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch011

slightly fewer than 2200 workers with the AMEX 3D (which provides a TWA exposure score) (*68*). The results are summarized in Table VII, which shows the exposure distinction among the three rankings, generally validating the expert judgment.

In an effort to identify the most efficient strategy for assigning MF exposure in the epidemiologic study, Kromhout et al. (*69*) analyzed the components of exposure variation in this data set attributable to within-worker, within-group, and between-group variance. Exposure groups were defined in five ways: (1) three a priori groups, as shown in Table VII; (2) 28 occupational categories as mentioned; (3) five utility companies, each its own group; (4) 120 groups constructed from a company by job category matrix; and (5) five a posteriori groups, collapsed from these 120 groups, based on four percentile cutoff points (25%, 50%, 75%, and 87.5%) from the AMEX measurement data. Kromhout et al. constructed a homogeneity score, ε, which equaled between-group variance divided by the sum of within-group plus between-group variance; ε varies from 0.0 to 1.0, 1.0 indicating maximal homogeneity of exposure. As Table VIII shows, ε was, not surprisingly, maximized with the a posteriori grouping. This observation led the investigators to structure the final JEM around these five groups. Using utility company as the grouping unit produced a total lack of homogeneity, again not surprising in light of the great diversity of personal exposure profiles given the diversity of personnel and their tasks.

Finally, Kromhout et al. (*69*) compared the components of exposure variance of the 28 OCs in the five-company study with the job classifications for the EMDEX occupational exposure study, described earlier (reference 7, 16 groups shown in Figure 14). Compared to the EMDEX occupational study, the percentage of total variance due to within-group differences in the five-utility study was considerably lower (16.3% vs. 42.4%), and the percentage of total variance due to within-worker differences was considerably higher (68.2% vs. 40.6%); the percentage of variance due to between-group differences was about the same for both data sets. The great diversity of group definitions in the EMDEX study, which characterized 55 companies, probably contributed to the relatively high within-group variance. In either case, these and other data sets discussed represent valuable sources of data on occupational exposure to MF.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch011

Table VII. AMEX Exposure Data (in milligauss): Five Utilities

Ranking	*Arithmetic Mean (95% CI)*	*Geometric Mean (95% CI)*
Low	2.6 (2.2 – 3.0)	1.5 (1.4 – 1.6)
Medium	5.3 (4.6 – 6.1)	2.2 (2.0 – 2.3)
High	10.2 (8.5 – 11.8)	3.4 (3.8 – 4.2)

SOURCE: Adapted from reference 67.

Table VIII. Exposure Homogeneity by Type of Occupational Grouping

Grouping	*Number of Groups*	*Homogeneity Index[a] (ε)*
A priori	3	0.29
Occupational category	28	0.49
Company	5	0.02
Company × occupational category	120	0.56
A posteriori	5	0.59

[a] $\varepsilon = {}_{BG}S^2/({}_{BG}S^2 + {}_{WG}S^2)$; where S is variance, BG means between groups, and WG means within groups.
SOURCE: Adapted from reference 69.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch011

Conclusions

Everyone is exposed to ELF MFs. Because epidemiologists cannot depend on the availability of an absolutely unexposed group to serve as an exposure referent, MF exposure assessments are under greater pressure to measure characteristics that differentiate among exposure groups. To accomplish this end, epidemiologists have mostly used relatively crude surrogates, such as wire code and spot measurements for residential studies and job titles for occupational studies, although a new generation of studies in the electric utility occupations is relying increasingly on personal exposure monitoring. The difficulties attendant to ubiquitous exposure are compounded by our poor understanding of the exposure metrics most relevant to the study of specific health outcomes.

Various studies summarized in this chapter suggested possible relationships of MF exposure to cancer attributable to dimensions of exposure that go beyond crude surrogates: historical TWA exposure based on an engineering model (*12*); probability of exposure irrespective of time (*44*); length of time on job (*46, 56, 58*); age at first exposure or latency (*51, 57, 58*); and various MF parameters associated with relevant job sites, including TWA, median, time above threshold, and different exceedance values (*55, 57, 58*). These studies (together with many others not mentioned) have not produced a consistent or clear picture of the relationship of MFs to adverse health effects. We have not identified the prediagnostic time frames that are critical or the exposure metric during those time frames that perturbs, if at all, the appropriate biological system(s).

In fact, epidemiologic results could conceivably produce false leads concerning critical time frames for MF exposure. For example, MFs are not considered to be genotoxic agents (*70*), and if they were carcinogenic (for example, as

a cancer promoter or co-promoter), one might expect their critical biological effects to occur in the later stages of carcinogenesis. However, the results of Demers et al. (*51*), as discussed, suggest that the risk of male breast cancer was related most strongly to early adult (putative) MF exposure that occurred 30 or more years prior to diagnosis. In a similar manner, Matanoski et al. (*57*) reported that risks peaked when a 15-year lag period was interposed. The further back in time needed to estimate exposure, however, the more likely it is that work practices were different with respect to both MF and other potentially carcinogenic exposures. Consequently, the interpretation of such studies, vis-a-vis temporal aspects of MF exposure, must be undertaken with caution.

Ideally, laboratory studies are well suited for exploring questions about MF exposure metrics that may be associated with adverse health effects because of the control over exposure conditions afforded to the investigator. However, a reasonably clear picture of exposure–dose relationships and dose–effect relationships resulting from identifiable biological mechanisms has not emerged (*71*). This state of affairs has been further complicated by the lack of replicability of a number of findings, sometimes even within the same laboratory.

To conclude, a great many approaches have been attempted to characterize personal exposure to MFs in the residence and workplace. Without the biophysical underpinnings for understanding the relations of exposure to dose at target tissue and of dose to biological effects potentially relevant to health effects, most epidemiologic and exposure studies have defaulted to an approach based largely on TWA exposure quantities. An exploration of other potentially relevant metrics has become possible with the advent of personal exposure devices that log a detailed exposure history over the course of a day or more. Nonetheless, the available technology has driven the selection of metrics rather than biophysical principle. Investigators have succeeded, in some measure, in describing the relationships between various exposure surrogates and personal exposure, particularly TWA quantities. Though displaying some predictive value with respect to personal exposure, key surrogates, such as wire codes and job title, have introduced into the epidemiologic studies exposure misclassification, whose effect has not been well estimated. The misclassification has arisen as a result of the inexact correspondence of surrogate category to contemporaneous exposure and the misclassification due to temporal extrapolation. The issue concerning MF and health is important enough to keep working to reduce errors associated with MF exposure assessment.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch011

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RECEIVED for review June 19, 1994. ACCEPTED revised manuscript April 17, 1995.

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Electromagnetic Fields and Cancer: Laboratory Studies

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch012

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Convincing evidence from a large number of laboratories indicates that exposure to extremely low frequency (ELF) magnetic and electric fields produces biological responses in animals. However, no animal studies clearly demonstrate deleterious effects of ELF fields, although several suggest potential health impacts. A major current emphasis in laboratory research is to determine whether the reported epidemiological studies that suggest an association between electromagnetic field (EMF) exposure and risk of cancer are supported in studies using animal models. Several approaches are outlined in the experimental approach to this question. From the perspective of laboratory animal studies, this chapter will discuss studies investigating the potential relationship between ELF magnetic and electric field exposure and the risk of cancer.

THE INTERACTION OF AN AGENT WITH HUMANS is of prime importance and concern, but many areas of biological investigation are more efficiently and appropriately conducted with various animal species. Animal studies provide an integrated system that can be used in studies in which experimental variables can be controlled, specific hypotheses can be explored, and exposure can be precisely assessed. Given the uncertainty and the relatively low power of electro-

0065–2393/95/0250–0225$12.00/0

magnetic field (EMF) epidemiologic studies to ascertain the relationship between EMF exposure and possible adverse health effects, animal studies are especially important in evaluating any potential association between EMF and cancer.

However, animal studies for risk assessment purposes have limitations that must be addressed. Extrapolation of experimental results from laboratory animals to humans remains somewhat tentative, both because of important biological differences between humans and animals and because the mechanisms (and hence the biologically relevant exposure parameters) by which the effects may arise are often unknown. Furthermore, studies using laboratory animals are typically carried out with high doses throughout the lifetime of the animals. The relevance of these exposure conditions to those generally experienced by humans is uncertain.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch012

Animal Models

Several different approaches and animal models can be used in laboratory cancer studies. The selection of a specific model depends largely upon the hypothesis chosen to evaluate a particular underlying mechanism. For example, if one desires to test an agent (e.g., EMF) for its potential to be a complete carcinogen (an agent that by its application alone causes a cancer to develop), 1½ to 2 years of exposure of mice or rats to the agent is necessary. During that time it is important to keep exposure to other possibly confounding agents to a minimum. In this regimen the animals are observed during the major portion of their lifetime, and the number, type, and time of development of tumors are the critical end points. This type of study should include several dose groups and requires a relatively large number of animals, particularly if the natural incidence of a tumor type is low. As one would surmise, studies evaluating complete carcinogenicity are expensive because of both the length of time and the number of animals involved. It is also an open question as to whether such studies can yield meaningful results when studying EMF. This problem arises because of the inadequacy of present knowledge on what aspects of the EMF signal may be biologically active.

Because carcinogenesis is a multistep process, another approach is to assume that the agent of interest acts either as an initiator or a promoter for which a two-phase protocol is required for testing. *Initiation* is defined as a genotoxic event in which the carcinogen interacts with the organism to directly affect the DNA. *Promotion* is operationally defined, associated with the experimental protocol, and occurs when the promoting agent is applied subsequently to initiation and generally over a protracted period of time. Promotion is associated with a number of subcellular events that are generally nongenotoxic and is responsible for the conversion of initiated cells to cancerous cells. To evaluate EMF as

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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) over a long term. If EMF were to be investigated for possible promotional effects, the animals would be treated with a known initiator (e.g., 7,12-dimethylbenz[*a*]anthracene, DMBA), and subsequently exposed to EMF over a long term (months). These initiation–promotion approaches have the advantage of using fewer animals, a shorter time, and less cost. However, a given model is usually limited to evaluating a specific type of cancer and may provide only general information on possible biological mechanisms of the agent of interest (EMF) and cancer development.

Spontaneous Tumor Development and Complete Carcinogen Studies

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch012

Few truly life-long animal studies examining EMF as a complete carcinogen have yet been fully reported, although several are under way (in the United States, Italy, Japan, and Canada). Japanese workers reported (*1*) preliminary results of a life-span study to determine the spontaneous carcinogenic effect of 50-Hz sinusoidal AC (alternating current) magnetic fields in rats. Groups of 48 Fischer-344 rats of each gender were exposed to flux densities of 5 mT, 0.5 mT, or sham fields for 2 years. Exposure to the fields did not increase the incidence of total tumors, nor was the incidence of leukemia or brain tumors altered.

In a multigeneration study (*2–4*), CFW mice were exposed to 60-Hz AC magnetic fields at 25 mT (250 G) for three generations. Animals from the second and third generations were conceived and raised in the fields. Preliminary results from two sets of animals suggest a significant relationship between exposures to the magnetic field as a function of time and malignant lymphoma. Of 55 exposed animals, 45% developed malignant lymphoma by the age of 140–273 days; none were found in the controls (*3*). In another group of animals, 78% (32) of the exposed animals had premalignant changes or malignant lymphoma compared to 6% (2) of the controls when evaluated at 363–418 days of age (*4*). This work has appeared only in abstract form, and the exposure intensities were extremely high. However, if the results are indeed related to the fields rather than artifacts from the high fields (i.e., heat, noise, vibration, etc.), then these data could provide a starting point for understanding dose–response relationships for magnetic field exposure and health-related end points.

Additionally, several studies that were designed to evaluate EMF as a promoter of cancer contained control groups that were exposed to EMF without being initiated with a carcinogen (e.g., chemicals or radiation). Three such studies contained parallel groups of animals to investigate EMF as a possible complete carcinogen. These studies include an investigation of lymphoma development in mice (*5*), a mammary tumor-promotion study in rats (*6*), and a

mouse skin tumor-promotion study (*7*). A major deficiency of using such "add-on" studies to evaluate complete carcinogenicity is the small group sizes involved.

In the lymphoma study, Svedenstal and Holmberg (*5*) investigated the influence of pulsed magnetic fields on the lymphoma development in the radiation-induced lymphoma mouse model and on spontaneous lymphoma development in a life-time study. Mice (227 total) were divided into four groups and exposed to (1) 5.24-Gy X-ray, (2) pulsed 20-kHz magnetic fields (vertical, sawtooth shaped with a flux density of 15 μT), (3) both X-rays and magnetic fields, or (4) no treatment. No significant differences were found in the induced or spontaneous lymphoma incidence as a result of magnetic field exposure.

The Beniashvili et al. study (*6*) found an increase in mammary gland tumors in rats exposed to 20 μT for 3 h/day (7 of 25 animals) compared to no tumors in 50 unexposed animals. However, the number of animals studied is extremely small for this type of study, and no conclusions can be drawn from these results. The skin tumor study reported no increase in tumors with long-term exposure to magnetic fields (500 or 50 μT). Both studies are discussed in more detail in the following section.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch012

Initiation–Promotion Studies

Leukemia Studies. Clinical progression of leukemia was investigated in 8-week-old female mice (10 per group) that were implanted with P388 leukemia cells and subsequently exposed to 60-Hz sinusoidal magnetic fields in Helmholtz coils for 6 h/day, 5 days/week until death at flux densities of 1.4, 200, or 500 μT (*8*). Neither incidence nor clinical progression of the leukemia in the implanted animals was altered by the magnetic fields at any intensity studied.

Mammary Cancer Studies. Beniashvili et al. (*6*) induced mammary tumors in rats with nitrosomethylurea (NMU) at age 55 days (five groups of 50 animals). Following administration of the NMU, two groups of the rats were exposed to a 50-Hz magnetic field for 30 or 180 min each day. Two other groups were exposed to a static magnetic field for either 30 or 180 min/day. The results of this experiment are striking, as animals exposed to either the variable or the static field for 180 min/day had a higher incidence of mammary tumors as well as a greater number of tumors. In addition, the latent period was decreased in the exposed animals. No tumors developed in 50 control rats over the 2-year study period, whereas seven appeared in 25 rats exposed for 3 h/day to the 50-Hz magnetic field (without NMU). Such results suggest a possible promoter effect of EMF in rat mammary carcinogenesis.

In two preliminary studies using 60-Hz electric fields (40 kV/m), Leung et al. (*9*) reported that rats initiated with DMBA by gavage at 54 days of age had slightly more mammary tumors per tumor-bearing animal at either 18 or 23

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weeks of exposure than did similarly initiated animals that were not subsequently exposed to the field. A statistically significant difference could not be demonstrated in either individual study for this response, nor was there evidence of an increase in tumor incidence in either study. However, by pooling the data of the two studies, the authors found a statistically significant increase in the number of tumors per tumor-bearing animal. Even though the two studies were not identical replicates, the results suggest a weak promotional effect of electric fields.

Subsequently, a series of mammary initiation–promotion studies were conducted by Mevissen et al. (*10*). Female rats were given 5 mg of DMBA by gastric intubation and then sham-exposed; exposed to a 50-Hz, 30-mT field; or exposed to a 15-mT DC (direct current) field. All animals received three additional doses of DMBA at weekly intervals. Reference controls and sham-exposed controls had an average mammary tumor incidence of 66% and 61%, respectively. Exposure to the 15-mT DC field did not alter either the incidence or number of tumors per animal. The tumor weight, however, was significantly increased in the exposed animals. Animals that were exposed to the 50-Hz, 30-mT field did not exhibit a significant difference in tumor incidence, but the exposed animals had twice as many tumors as the controls. When this experiment was repeated using a smaller number of animals, no differences were found between exposed and controls. A third experiment in which DMBA-treated rats were exposed to a gradient (0.3–1.0 μT) 50-Hz field showed no differences between exposed and control animals. This study is handicapped by a small number of animals in each group.

Loscher et al. (*11*) performed an experiment using DMBA to initiate mammary tumors in rats and reported a significant increase in mammary tumor induction in the rats exposed to a magnetic field. All rats received four weekly doses of 5 mg of DMBA beginning at 52 days of age. A group of 99 rats was exposed, after DMBA treatment, to 50-Hz magnetic fields at a flux density of 0.1 mT for 24 h/day for 3 months. Another group of 99 was sham-exposed. After 3 months of exposure, mammary tumor incidence was about 50% higher in the exposed group (51 tumors) compared to the sham-exposed (34 tumors). This difference was statistically significant ($P < 0.05$). The tumors were also larger in the exposed group ($P = 0.0134$), but a difference was not found in the number of tumors per tumor-bearing rat.

Loscher and colleagues followed up their initial studies with two further mammary carcinogenesis studies. In one study, groups of 36 rats were treated with DMBA and subsequently exposed or sham-exposed to magnetic fields for 13 weeks (*12*). The gradient magnetic fields in this study, of 0.3–1 μT, were considerably lower in intensity than in the other study reported (*11*). The authors reported that although a detailed evaluation of mammary lesions showed a slight increase in the incidence of hyperplasias and tumors in the magnetic field exposed animals, the changes were not statistically significant and therefore not supportive of the hypothesis that EMF exposure may be associated with promo-

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tion of mammary cancer in animals. In the second study, large groups of animals (99 animals per group) were exposed to 10-μT fields for 13 weeks (*13*). A non-significant tendency to decreased tumor latency was observed in the exposed animals; however, no differences in overall tumor incidence were reported. These apparent differences in results from the same research group (i.e., from the DMBA mammary cancer study described) 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 the experiments.

Skin Tumor Models. Skin tumor promotion, after initiation with DMBA, was examined in mice exposed to a 2-mT, 60-Hz continuous magnetic field, 6 h/day, 5 days/ week for up to 21 weeks (*14*). Groups of 32 female mice were initiated with DMBA (10 nmol) on the dorsal skin. The mice were then exposed to the magnetic field with or without TPA promotion (1 μg/week for 21 weeks). Because none of the exposed or sham-exposed mice developed papillomas, the authors concluded that the fields did not act as a classical tumor promoter. In a second study, two groups of 48 mice were similarly initiated with DMBA, and promoted with 0.3 μg (4.9 nmol) of TPA for 23 weeks with or without magnetic field exposure to investigate the copromotional effect of the fields. The onset of tumor development was slightly earlier in mice that were treated with TPA plus magnetic fields, compared to sham-exposed. However, at the end of the study, neither the number of animals affected nor the number of tumors per animal was different between the two groups (*15*).

A life-span skin carcinogenicity study in NMRI/HAN mice exposed to AC sinusoidal magnetic fields found no evidence that magnetic fields promoted the formation of skin tumors (*7*). Groups of 36 mice were initiated with DMBA (25 μg applied to the dorsal skin of each mouse) and exposed to 50-Hz sinusoidal magnetic fields with flux densities of 50 μT or 50 mT for 103 weeks, 19–21 h/day, or sham-exposed. These results do not support the findings that continuous magnetic fields promote skin carcinogenicity in SENCAR mice (*15*); however, the flux densities used in the SENCAR mice were much greater than that used by Rannug et al. (*7*).

The tumor-promoting effects of continuous and intermittent magnetic fields were compared in a long-term skin carcinogenesis study of female SENCAR mice after initiation with DMBA (*16*). Groups of 40 mice were treated with DMBA (10 nmol) 1 week before being exposed to the magnetic fields. Continuous and intermittent (15 s on–off) 50-Hz horizontal AC fields with flux densities of 50 μT and 0.5 mT were applied for 19 (weekdays) or 21 (weekends) h/day for 105 weeks. Appropriate untreated, DMBA-treated, and TPA-promoted control groups were included in the study design. No tumors were found in animals treated with DMBA and exposed to continuous fields of 50 μT or 0.5 mT. Totals of four tumors in four animals and 13 tumors in five animals were found in the 50-μT and 0.5-mT intermittent groups, respectively. Statistically

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significant differences were not found when the tumor-bearing animals and cumulated skin tumors of intermittent groups were compared to the control group. However, when the two intermittent groups were pooled and compared to the continuous exposed groups, a statistically significant difference ($P = 0.014$) effect was found. No hyperplastic response was associated with the magnetic field exposures. The results did not support the hypothesis that continuous magnetic field exposure promotes skin carcinogenesis in SENCAR mice at flux densities of 50 μT and 0.5 mT. However, a weak promotional effect may be associated with intermittent exposures.

The different results from the continuous and intermittent exposures are rather intriguing, but not well understood. Further laboratory experiments will provide replication of experiments and better understanding of possible mechanisms underlying the potential health effects associated with EMF.

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Liver Cancer Studies. In a series of experiments, Rannug and coworkers (*17, 18*) investigated the possibility that magnetic fields interact with known initiators or promoters of cancer to induce preneoplastic lesions in rats. As a model, they used partially hepatectomized male Sprague–Dawley rats treated with diethylnitrosamine (DENA) to initiate tumor development. They then treated the animals with phenobarbital for 12 weeks, beginning 1 week after initiation, to promote growth of enzymatically altered foci of the liver cells. In two studies, rats were initiated with DENA, then promoted with phenobarbital or with 50-Hz horizontal, magnetic fields having flux densities of 0.5 and 50 μT (Experiment 1) or 5 and 500 μT (Experiment 2). A slight increase in γ-glutamyl transpeptidase (GGT)-staining foci was reported in Experiment 1, but not in Experiment 2. In a third study, animals were exposed to magnetic fields having flux densities of 0.5 μT or 0.5 mT, both during DENA initiation and also throughout phenobarbital treatment (copromotion; *18*). The magnetic field inhibited, rather than increased, the size and number of focal lesions. These results may suggest a weak interaction of the fields on the process of carcinogenesis, but evidence of a promotion or copromotional role of magnetic fields was not demonstrated.

Conclusions

Many studies on possible effects of EMF on tumor development in animals are ongoing in several countries. However, relatively little information is yet available, and any conclusions drawn at this point would be premature. EMF as a complete carcinogen or an initiator of cancer has not been demonstrated in laboratory studies. Only one set of experiments has suggested such a link; however, those studies used extremely high field intensities and therefore results may have been confounded by problems associated with such extreme exposures.

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In the tumor-promotion studies that have been reported, results seem to be mixed. Some studies reported increased tumor development with exposure, and others showed no association. The strongest laboratory evidence for an EMF-related cancer association is in the area of mammary carcinogenesis. Several studies have been conducted, most of which suggest some increase in mammary cancer due to exposure. In these experiments, tumors must be initiated with a chemical carcinogen for the EMF to have its apparent effect. Some data support the idea that EMF may act in a possible copromotional manner, and EMF exposure alone may not be as effective in promoting cancer development. Furthermore, the association between EMF and biological effect may be greater for intermittent exposures than for continuous exposure.

Laboratory work has not yet confirmed the results of the epidemiological studies that there may be an association between EMF exposure and increased risk of cancer, and further carefully controlled and designed experimental research in the laboratory will be required to determine whether such an association is real. If further evidence suggests a relationship between exposure and potential health end points, studies in animal models will be necessary to confirm the evidence and to understand the possible mechanisms by which those effects occur. In particular, laboratory experiments allow specific hypotheses to be employed in addressing the issue of whether adverse health outcomes can result from EMF exposure.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch012

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RECEIVED for review April 6, 1994. ACCEPTED revised manuscript September 26, 1994.

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Electric and Magnetic Field Mitigation Strategies

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch013

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The emergence of new electric technologies has produced an extremely complex electric and magnetic field environment in which we must work and live. Strategies for mitigation of these fields typically vary on a case-to-case basis, depending on the regions of interest, the types of sources, the frequency, the field levels, and the attenuation requirements. At the most general level, field management is the creation, elimination, or modification of sources in order to alter the spatial distribution of electric and/or magnetic fields in some region of space. At low frequencies, management of electric fields is relatively straightforward because nearly all objects are conducting enough to terminate electric field lines. The opposite is true with low-frequency magnetic fields. Only a select set of materials, some of them relatively expensive, have the proper material attributes for effective control of magnetic fields through shielding, and active cancellation using coils that produce an opposing magnetic field is of limited use when the magnetic field has a complex structure. This chapter serves as an introduction to low-frequency electric and magnetic field mitigation and gives an overview of typical field management strategies with an emphasis on magnetic fields.

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0065–2393/95/0250–0235$12.75/0

ELECTRIC APPARATUS IS UBIQUITOUS in our modern society, and so we live in an extremely complex electric and magnetic field environment. Although no universally accepted human exposure guidelines yet exist, ongoing biological research may someday result in the establishment of such guidelines. Regardless of human exposure issues, sensitive electronics, magnetic storage media, medical equipment, and video-display terminals all have limits to electromagnetic (EM) field exposure for proper operation. Anticipation of human exposure guidelines along with any other EM interference and compatibility issues are the motivation for developing practical, cost-effective field management strategies.

Although the EM spectrum runs the gamut from 0 Hz [DC (direct current)] to above 10^{20} Hz, much of the EM focus is on power-frequency fields at 50 or 60 Hz. The power frequencies, as well as their low-order harmonics, fall well within a frequency range of 3–3000 Hz, designated as the extremely low frequency (ELF) band (*1*). In the ELF band, wavelengths are extremely long; from 100 to 100,000 km. Hence, for practical purposes, one is always in the "near field", in which quasi-static or "induction" fields dominate over the "radiation" fields. This condition means that electric and magnetic fields from power-frequency sources can be treated as being independent of each other. For a given system, the electric fields are determined by the voltages, and the magnetic fields are determined by currents. The focus of this chapter will be on low-frequency field management, with an emphasis on magnetic fields because they are the more difficult of the two to shield.

Electric and magnetic fields are formally defined in terms of the force that a charged particle would encounter as it moves through space (*2*). As such, electric and magnetic fields are vector fields; they each have a magnitude and direction associated with any point in space. The way in which the fields vary through space defines the field "structures", and field structures are a function of the sources that create them. Examples of electric and magnetic fields due to two conductors that run perpendicular to the page may be visualized through field lines, as shown in Figure 1.

Power-system voltages are sinusoidal with a fundamental frequency of 50 or 60 Hz. The loads in the system, or the impedances, determine how much current flows for an applied voltage. Linear circuit elements such as capacitors, inductors, and resistors result in currents that are sinusoidal at the same frequency as the applied voltage. Sinusoidal linear systems are handled mathematically using a phasor representation, where it is understood that all quantities oscillate at one frequency. All that is required is a phase and magnitude to fully specify the waveform of any quantity as a function of time. Thus, electric and magnetic fields created by power systems are characterized by a magnitude and phase for each of the three orthogonal vector components. The field plots of Figure 1 represent a "snapshot" in time of the field structures in a plane.

Often, however, the loads in a power system are nonlinear and the resulting currents are not purely sinusoidal; the current waveform is distorted. Examples of typical nonlinear loads are iron-core inductors or semiconductor switching circuits. Nonlinear loads result in higher order harmonics. Therefore, the

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch013

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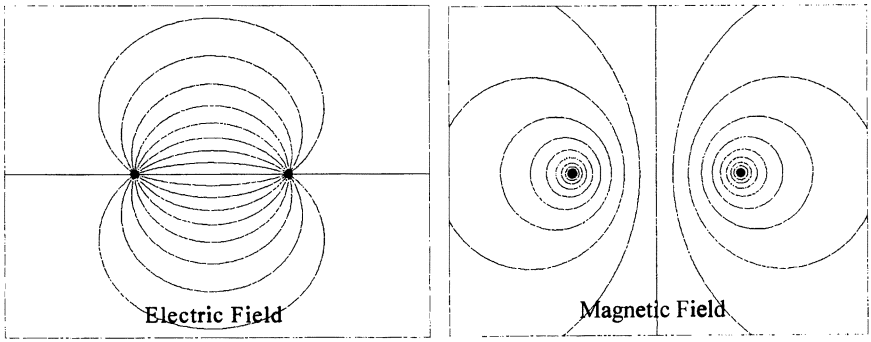

Figure 1. Fields associated with two long conductors running perpendicular to the page. The electric field is determined by the voltage between the conductors, and the magnetic field is determined by the currents. The magnetic field shown is due to equal but opposite currents in the two conductors.

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electric and magnetic fields due to a power system are present not only at the fundamental frequency but also at the higher order harmonics. This phenomenon is especially true of the magnetic fields. Figure 2 shows a periodic waveform of flux density in the *x*-direction as a function of time with third, fifth, seventh, and ninth harmonics, and Figure 3 shows the amplitudes of the corresponding frequency components.

As described, characterization of electric and magnetic fields can be quite complex. At each point in time, the electric and magnetic fields at each point in space are each specified by a magnitude and direction. In the steady state, power-system voltages, currents, and the fields they create are periodic. Thus, a field can be characterized at each point in space by the phase and magnitude of each of the three vector components at the fundamental frequency, as well as the same information about any harmonics that are present. The variation of these

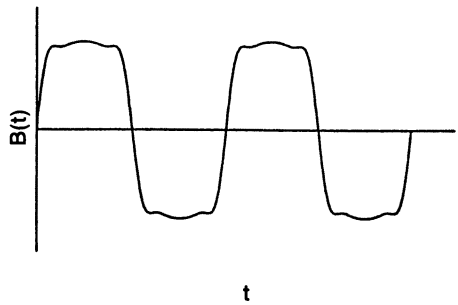

Figure 2. Periodic waveform of flux density in the x*-direction that contains third, fifth, seventh, and ninth harmonics.*

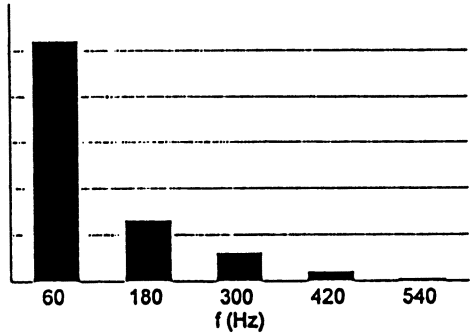

Figure 3. Magnetic field frequency components of the waveform shown in Figure 2.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch013

parameters as a function of space defines the structure of the field. In the broadest sense, field management is the creation, elimination, or modification of field sources in order to alter the field structure in some region of space, typically to reduce the field magnitudes. The rest of this chapter discusses the sources of electric and magnetic fields as well as methods for altering their structure.

Electric Fields

Electric Field Sources. The electric field strength, or simply electric field, results from the separation of charge. For a given configuration, the voltage between conductors is a measure of the amount of charge separation and hence the electric field. The usual letter symbol used in technical literature to denote the electric field is *E*.

In the context of this discussion, energized conductors are the usual source of electric fields. Electric fields are measured in volts per meter (V/m). When discussing electric fields found near high-voltage sources, such as transmission lines, the prefix "k" for kilo (1000) is often used so that the unit of measure is kilovolts per meter (kV/m) or 1000 V/m. Some scientific journals report the electric field in volts per centimeter (V/cm); 1 V/cm equals 100 V/m.

Whenever a difference in potential or voltage exists between objects, then an electric field is present. For the same geometry, a higher voltage means a higher electric field. The left side of Figure 1 shows the electric field between two wires. The electric fields are twice as large everywhere if the voltage between the two wires is increased from 110 to 220 V. As long as the leads to an appliance are energized, electric fields are present. Hence, electric fields may be present even when an appliance is not operating.

To minimize electric fields external to a system of conductors, the spacing between the conductors should be minimized. As the spacing is decreased, the electric field between the conductors increases rapidly, but the field beyond the conductors decreases. To illustrate this concept, Figure 4 shows the electric field

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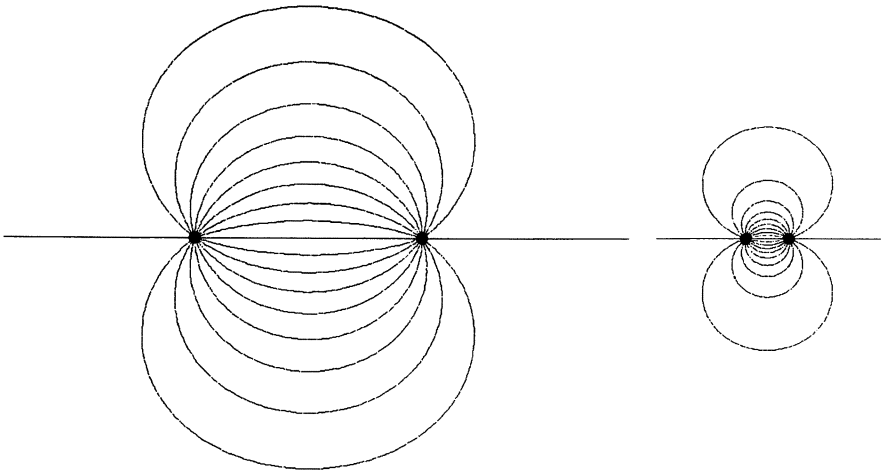

Figure 4. Electric field external to the conductors decreases as they are moved closer together. This decrease is an example of a cancellation effect because from a distance that is several times the spacing between conductors, the net charge appears to be zero.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch013

due to two conductors with wide and narrow spacing. The voltage between the conductors is held constant. The decrease in external fields is due to a cancellation effect. From a distance that is much larger than the spacing between conductors, the net charge appears as zero.

Limiting factors for conductor spacing might include dielectric breakdown, safety and reliability, maintenance, and system capacitance. For systems with uninsulated conductors, air ionizes and becomes conducting when the electric field reaches 3×10^6 V/m. Hence, some minimum practical spacing exists that depends on the voltages and the geometries of the conductors that are involved.

Electric Field Shielding. Charges in electrically conductive materials placed in an electric field will migrate to the outside edges of the material and terminate the field lines at the surface. The redistribution of charges cancels the original electric field within the material. At low frequencies, many things in our environment are conductive enough to affect the electric field in this manner. In addition to metals, which have very high conductivities on the order of 10^7 S/m, the human body, wood, and materials with some water content are conducting enough to shield electric fields.

The fact that so many physical objects distort the electric field makes control technology very easy. Placing a conductive enclosure around a volume will eliminate the electric field within, as shown in Figure 5. Charges in the conductive enclosure migrate to the surface and terminate the electric field lines.

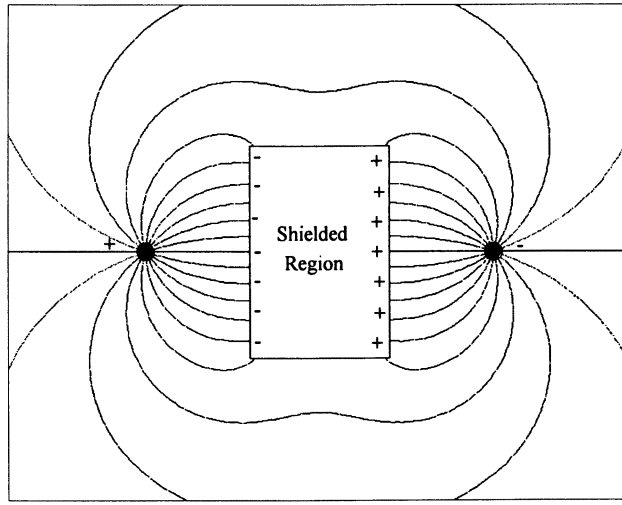

Figure 5. Charges on a conducting enclosure shield the interior by terminating the electric field lines.

This enclosure is often referred to as a Faraday cage. At ELFs, the conductive enclosure can be as simple and inexpensive as a wire-mesh screen.

The Earth itself is conducting enough to be considered an equipotential surface in many cases. In fact, voltages on conductors are often referenced to "ground" potential, the voltage between the conductor and Earth. Figure 6A shows an energized conductor above the Earth and the resulting electric field. Any conductor that is grounded to Earth provides some shielding in the region between the energized conductor and Earth, as drawings B and C of Figure 6 illustrate. Partial shielding is provided by a grounded conductor as in Figure 6B, and complete shielding is provided by a grounded enclosure as in Figure 6C.

In summary, conducting objects are effective in shielding electric fields. The subject can be shielded, as in the extreme case of live-line maintenance personnel who work on energized, extra-high-voltage conductor systems; or the source can be shielded, as in a pad-mount transformer, in which a grounded metal shroud is placed around the transformer.

Magnetic Fields

Magnetic Field Sources. Magnetic fields are produced by moving electric charges, which in general implies an electric current. Any wire carrying an electric current is a source of magnetic fields. Magnetic fields are specified in terms of magnetic flux density by the international unit of tesla (T). For the power-frequency fields typically considered, the microtesla (μT), or 10^{-6} T, is the practical version of the international unit. The popular usage in the United States is milligauss (mG), a holdout from the English cgs (centimeter-gram-second) system of units; 10 mG equals 1 μT.

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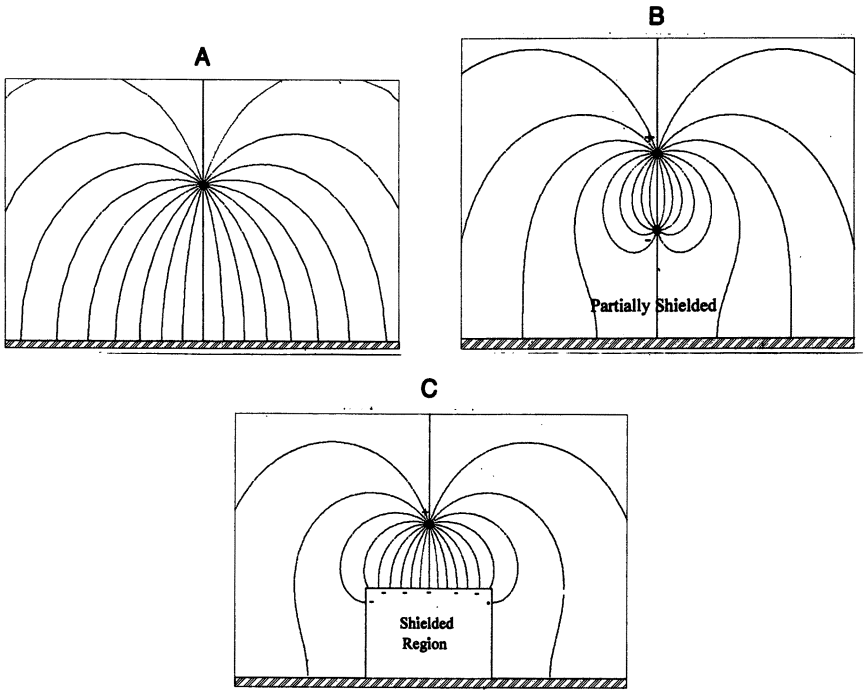

Figure 6. Examples of (A) the field lines of an energized conductor over a ground plane, (B) the shielding effected by placing a grounded conductor near the energized conductor, and (C) the shielding due to a grounded enclosure.

A long wire creates a magnetic field that is proportional to the current in the wire, *I*, and inversely proportional to the distance from the wire. However, a single isolated wire is atypical because currents flow in closed paths (unless electric charge is being accumulated). Residential single-phase wiring, for example, consists of a 110-V "hot" wire and a neutral wire in which currents flow in opposite directions. The neutral wire can be thought of as the return path for the current, although the sinusoidal current changes directions twice every 1/60th of a second. In three-phase power transmission or distribution systems, three wires carry equal currents that are 120° out of phase, but for balanced conditions, the sum of the three currents is zero at any instant in time.

The magnetic field from cables with balanced currents decreases more rapidly than for an isolated wire because the magnetic fields due to opposite currents tend to cancel. At a distance much larger than the spacing between the wires, zero current appears to be in the cable. The key to reducing magnetic fields is to keep the spacing between the source and return currents as small as possible. This concept of minimum spacing holds for three-phase systems as well, but for the higher voltage systems, a limit to spacing exists, as described in the section on electric fields.

On low-voltage systems, the wires are typically spaced very close to each other, and spacing is not an issue. In many cases, however, the grounding of

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electric systems at multiple locations for safety considerations provides alternate return paths. When this condition occurs, a portion of the return current flows in a path that can be much farther away than the intended return path, which results in higher magnetic fields. One example of unwanted currents flowing on an alternate path is shown in Figure 7. A residential service drop consists of three wires: two hot wires, one at +110 V and one at −110 V, sometimes called the hot legs; and a neutral conductor. The voltages on the two hot legs are equal and opposite with respect to the neutral conductor. When loads in the house (shown as *Z* in Figure 7) on the two hot legs are unbalanced, the difference in current is supposed to return on the neutral cable. For safety reasons, the National Electrical Code requires that a ground connection be made from the neutral bus of the service panel to the metallic water system of the house. Often, the water system is a low-impedance electric path between homes in a neighborhood. It represents an alternate path for return currents by virtue of the ground connections from service panel to water pipes in every home. Depending on the impedances involved, a significant fraction of return current may flow on the water pipes; the result is large current loops and larger residential magnetic fields.

The water pipe ground connection is, however, extremely important from a safety standpoint. It eliminates the possibility of electric shock that can occur when an energized conductor accidentally contacts the metallic water system. Ongoing research has developed and is testing methods for eliminating ground currents without compromising the safety of the electric system.

To summarize, the first part of magnetic field management is understanding the magnitude of fields that different sources create and placing them accordingly or limiting the proximity of other objects to the source. The second part of magnetic field management is understanding how the spatial distribution of currents affects the magnetic fields. This part of field management involves the limiting of large current loops by minimizing the spacing between wires that carry the source and return currents and the elimination of unwanted currents flowing in alternate paths that result in large current loops.

Magnetic Shielding Basics. As described in the introduction, magnetic fields have a structure in space, and shielding can be defined as changing or altering the magnetic field structure to lower the field magnitude in a region of space. An alternative but equally valid view of shielding is producing additional magnetic field sources that lower the field magnitude in a region of interest through cancellation. This alternative view is as valid as the first definition because the only way to alter field structure is through the creation of additional sources (assuming the original sources cannot be mitigated). In the process of shielding, one needs to understand the full impact of the shield design on the magnetic field structure; when fields are lowered in one region, the fields may be increased in another region.

Quantifying Shield Performance. In some technical literature the shielding factor is defined as the ratio of the field outside the shield to the field

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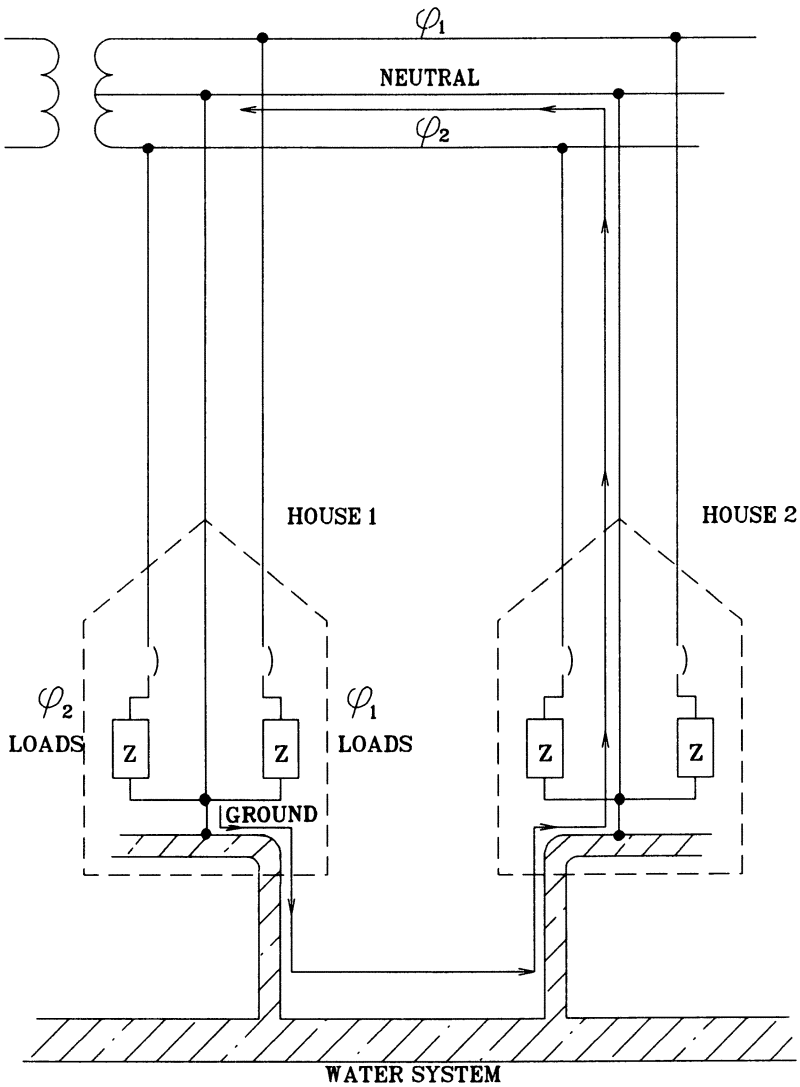

Figure 7. Alternate return path for current returning to the distribution transformer. Instead of returning on the neutral conductor of the service cable, some currents flow across the ground connection to the water pipe, out onto the water system, and back through the ground connection and neutral conductor at other homes.

just inside the shield. This ratio is not a true measure of shielding because the shield changes the original field structure. A shield increases the magnetic field in some regions while it reduces the field in others. A true measure of shield performance is obtained by looking at a single point with and without a shield. A typical measure of shield performance is the shielding factor, the ratio of the magnetic field at a single location with a shield in place to the magnetic field at the same location without the shield.

Because the shielded and unshielded fields may be out of phase, the shielding factor is a phasor with magnitude and phase. The magnitude gives the ratio of peak fields, and the phase represents the difference in time between the peak field magnitudes. With this definition, a shielding factor magnitude of 0.01 means that only 1% of the original field exists with the shield in place, or conversely, that the shield reduces the field by 99%. A shielding factor phase of 90° means that the peak shielded and unshielded fields occur one-fourth of 1/60th of a second apart in time; 1/60th of a second represents 360° phase for a 60-Hz sine wave. Except for a limited set of ideal shield geometries, the shielding factor will vary as a function of space. Shielding factor can be confusing because a smaller number represents better shielding.

The inverse of the shielding factor magnitude represents the attenuation factor; for example, a shielding factor of 0.01 represents 100-fold attenuation. Often the attenuation is specified in decibels (dB) as:

$$\text{attenuation (dB)} = -20 \log |SF|$$

where |SF| is the shielding factor magnitude. Thus, for example, 20 dB of attenuation represents an attenuation factor of 10 and shielding factor of 0.1, and 40 dB represents an attenuation factor of 100 and shielding factor of 0.01.

Passive Shielding Mechanisms. Ferromagnetic materials are a secondary magnetic field source, in which charges at the atomic level result in magnetic fields at a macroscopic level (*3*). In most ferromagnetic materials, these very small sources are randomly oriented and not apparent until an external field acts on them to line them up in an additive fashion. Some ferromagnetic materials maintain a portion of this magnetization because of an external field and act as a field source even without the external field, as with permanent magnets.

The problem sources of magnetic fields are typically currents flowing in wires. As secondary sources, however, ferromagnetic materials can alter the original field structure in a region of space and hence are useful for shielding. Traditionally, ferromagnetic materials are thought of not as sources that result in cancellation but as materials that alter the structure of the magnetic field by providing a preferred path for lines of magnetic flux. This process is often referred to as flux shunting and is illustrated in Figure 8.

People often think of ferromagnetic materials as the only type of magnetic shield (which is usually true for DC magnetic fields), but a second shielding

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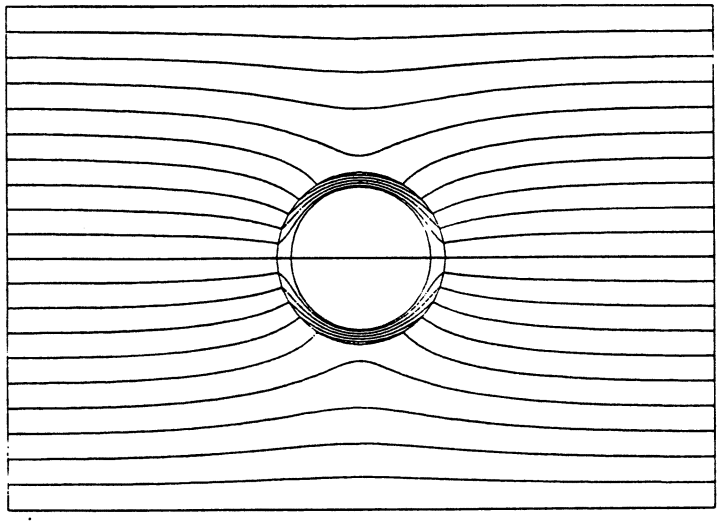

Figure 8. Example of the flux-shunting effect of a ferromagnetic material.

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effect for AC (alternating current) fields is produced by induced current, as predicted by Faraday's law. Faraday's law, one of Maxwell's equations that govern electric and magnetic fields, indicates that electric fields, and hence currents in a conductive material, are produced by time-varying magnetic fields. These currents are induced in a manner that tends to oppose or cancel the original magnetic field.

Thus the two mechanisms for shielding magnetic fields with materials are the induced-current mechanism and the flux-shunting mechanism. Both mechanisms are due to the induction of new magnetic field sources, which modify the original field structure.

Magnetic Shielding Classification

With the basic shielding concepts in mind, we can now move to a broad classification of magnetic field shielding methods, shown in Figure 9, that is useful for putting the different approaches in perspective. At the highest level, magnetic shielding can be broken into two main categories, active and passive. Passive shielding is a result of one or both of the two shielding mechanisms, flux shunting due to ferromagnetic materials or induced currents due to electrical conductivity. Passive shielding occurs in response to an applied field. Active shielding involves the control of additional field sources (currents) that attempt to cancel the imposed magnetic field in some optimum fashion.

Under the heading of passive shielding are shields made of materials that are both conducting and ferromagnetic, so that both passive shielding mechanisms are involved. An example of this type of shield is a box enclosure made of

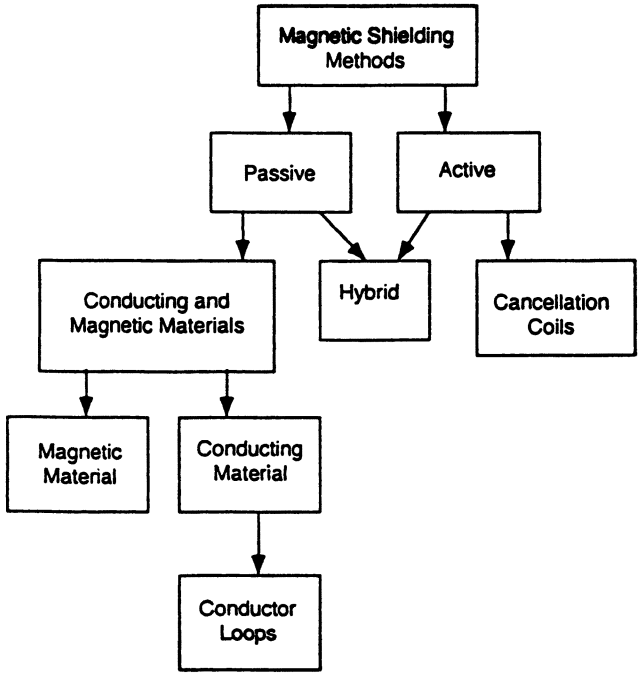

Figure 9. A classification of magnetic field shielding strategies.

steel. Steel is ferromagnetic and conducting enough that induced currents are a significant factor in shield performance. Alternatively, the enclosure could be made of both steel and aluminum.

Passive shields exist that make use of only one of the two basic shielding mechanisms. A magnetic material shield that makes use only of the flux-shunting mechanism might be made of a ferrite material that has a low conductivity. Examples of high-conductivity shields are enclosures or barriers made of typical nonferromagnetic metals, such as copper or aluminum.

Finally, under the conducting-only subset of passive shielding is the conductive-loop passive shield subset. Instead of allowing currents to flow throughout enclosures, the induced currents are limited to flow in conductive loops of wire. This type of shielding is being investigated for reducing magnetic fields near transmission and distribution power lines. An example is shown in Figure 10. Shield wires run parallel to the power lines for the distance that is being shielded and then connected at the ends to form a loop. The magnetic fields from the power lines induce current in the shield loop that tends to reduce the magnetic fields from the power line.

In active shielding, the currents in a loop are not induced but rather are controlled to have the proper phase and magnitude that best cancels the imposed magnetic field in some region of space. Often active shielding involves sensor

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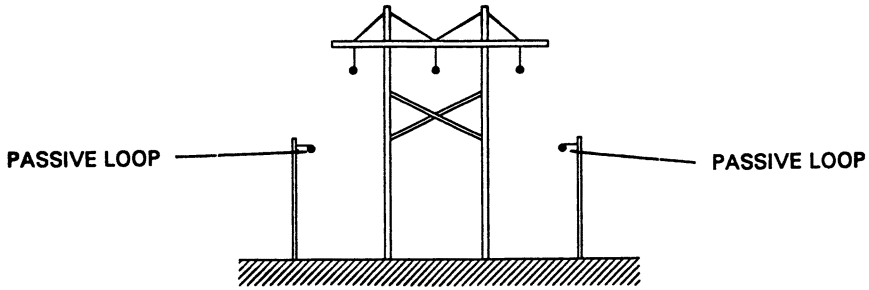

Figure 10. An example of passive magnetic field shielding using a passive wire loop. Induced currents in the loop reduce the fields beneath the transmission line.

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feedback for automatic control of the currents in the cancellation coils. Disadvantages of active shielding are that, if the currents are not phased properly, one can increase the field instead of cancel it, and building a system that cancels the field equally well in all regions of space is nearly impossible. Active cancellation and conducting-only passive shielding (nonferrous materials) represent shielding options that do not affect the Earth's DC magnetic field.

Finally, hybrid systems are combinations of active and passive shielding methods that make use of the strong points of each method to form an extremely effective shield system. A method for enhancing the shielding effectiveness of ferromagnetic shields called "shaking" can be classified as a hybrid method. Shaking is a method for improving the effective permeability of a ferromagnetic material shield through the use of coils that create a relatively strong field in the shield at another frequency (*4*). The additional field at a different frequency keeps the magnetic domains from locking on crystal defects, thus enhancing the ferromagnetic effect. The drawback is the creation of a new field at another frequency. Reference 5 describes an extremely effective hybrid magnetic shield that makes use of a number of the shielding methods.

The sections that follow discuss passive and active shielding in more detail.

Passive Shielding. Passive shielding is accomplished by placing a material shield between the magnetic field source and the region to be shielded. The shield may be as simple as a flat plate of steel or as complex as a multiple-material multilayer shield enclosure. As described earlier, the two basic shielding mechanisms are based on the shield material properties: the induced-current mechanism and the flux-shunting mechanism. In both cases, secondary field sources are the result of an applied field. These secondary field sources oppose the magnetic field in the shielded region, which results in lower magnetic fields. This restructuring of the magnetic field also results in higher magnetic fields in other areas.

Flux Shunting. Another measure of magnetic fields is the magnetic field strength or magnetic field intensity, usually given as the symbol H. Magnetic field strength is measured in amperes per meter (A/m). Magnetic flux density is related to magnetic field strength through the constitutive law

$$B = \mu H$$

where μ is permeability. In air, μ equals μ_0 (the permeability of a vacuum) and has a value of $4\pi \times 10^{-7}$ H/m; in ferromagnetic materials, μ accounts for magnetization. The permeability μ of a material is a measure of how much additional flux is developed in the material for a given applied field. The additional flux is due to the lining up of many small dipoles at the atomic level, which results in a source of magnetic fields at the macroscopic level. The net effect is that the additional sources result in some cancellation of the magnetic field in the shielded region.

The term "flux shunting" refers to the perspective that ferromagnetic materials provide a preferred path for lines of magnetic flux. Plots of the magnetic flux lines near a ferromagnetic material show that the material appears to attract the flux lines, pulling them toward itself and providing a preferred path, as illustrated in Figure 8. Hence, the view is that the magnetic flux is shunted around the shielded region. The higher the permeability, the better the path for magnetic flux lines, or the better the flux-shunting capability. Shield performance using the flux-shunting mechanism depends on the large-scale geometry of a shield, the thickness of the shield, and the ferromagnetic properties of the shield material.

Figure 11 shows two flux plots from a computational program that models the shielding of a plate adjacent to two long wires carrying current in opposite directions. In Figure 11A, the adjacent plate has no effect because it has been assigned nonconducting, nonferrous properties. In Figure 11B, the plate has been assigned a high permeability, typical of a good ferromagnetic material. The flux lines are pulled in toward the material, run vertically in the material, and then exit, thus shielding the region to the right of the plate.

Permeability is a nonlinear function of applied field. Figure 12 illustrates a portion of a typical permeability curve as a function of magnetic field. The permeability starts at an initial value, increases rapidly, reaches saturation, and eventually drops to a relative permeability of 1. Saturation occurs when the maximum amount of magnetization has been achieved. Likewise, the shielding capability of this material would start at some initial level, improve as the applied field is increased, and then deteriorate as the material saturates.

Induced Currents. Faraday's law states that time-varying magnetic fields induce electric fields. In conducting materials, the result will be induced currents. The currents are induced in a manner that opposes the changes in magnetic flux through the material. Thus, the induced currents act as a source of magnetic fields that tend to cancel or reduce the applied field in the shielded region.

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Advances in Chemistry; American Chemical Society: Washington, DC, 1995.

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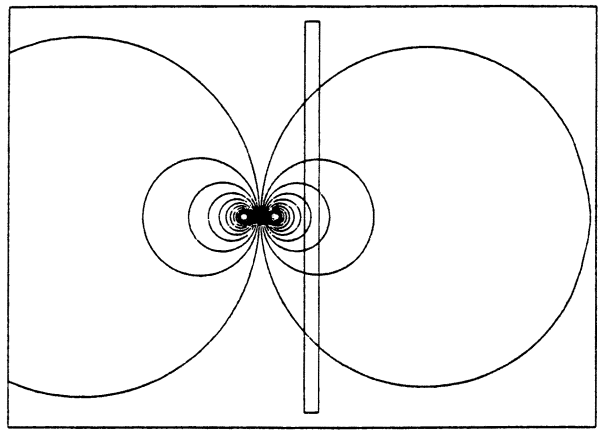

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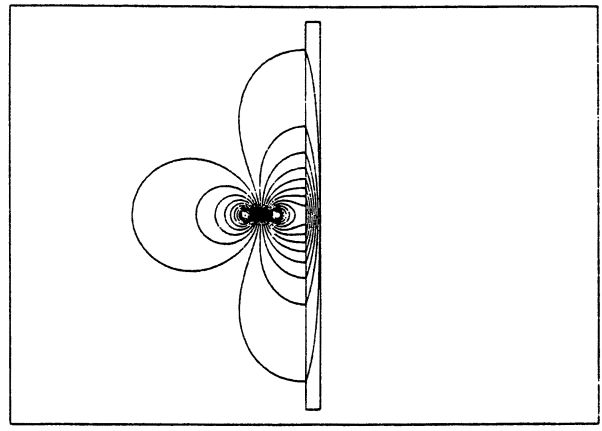

B

Figure 11. Two flux plots from a computational program that models the shielding of a plate adjacent to two long wires carrying current in opposite directions. A: the adjacent plate has no effect because it has been assigned nonconducting, nonferrous properties. B: the plate has been assigned a high permeability, typical of a good ferromagnetic material, resulting in significant shielding to the right of the plate.

The magnetic fields and induced currents in the shield decrease exponentially from the shield surface with a characteristic length called the skin depth, δ, defined as

$$\delta = 1/\sqrt{\sigma\mu\pi f}$$

where f is the frequency of the alternating magnetic field, σ is the shield material

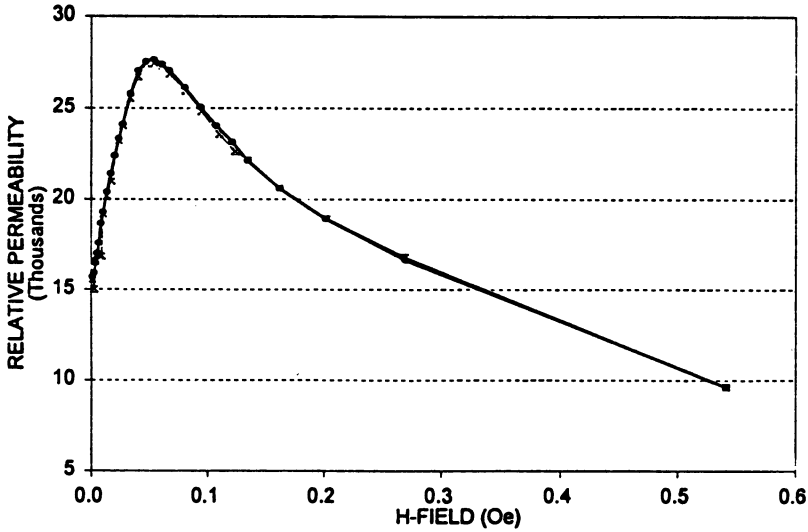

Figure 12. Permeability curve as a function of magnetic field strength, H, *in oersteds (Oe).*

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electrical conductivity, and μ is the shield permeability. The skin depth equation shows that the shielding effect of induced currents depends not only on conductivity but on permeability as well. For example, even though carbon steel is less conductive than aluminum, the skin depths are not that different because carbon steel has a higher permeability. In most cases, ferromagnetic materials made from metals such as iron and nickel are also conducting enough so that the induced currents can be quite significant.

If a shield is several skin depths thick, it can shield quite well. It is possible, however, to have significant induced current shielding when the shield is only a fraction of a skin depth thick.

Figure 13 shows two flux plots from a computational program that models the shielding of a plate adjacent to two long wires carrying current in opposite directions. In Figure 13A, the adjacent plate has no effect because it has been assigned nonconducting, nonferrous properties. In Figure 13B, the plate has been assigned a high electrical conductivity, typical of a metal such as aluminum. The induced currents flow such that the field is decreased to the right of the plate and increased to the left of the plate. The finite extent of the plate results in some field lines coming around the ends.

For the comparison of different materials, Table I shows the calculated shielding factors of an infinitely long cylinder surrounding two wires carrying current in opposite directions. Exact analytical expressions, based on first-order modified Bessel functions and their derivatives, exist for this idealized geometry. The cylinder has an inner radius of 10 cm. The high-nickel alloys provide the best shielding, but they are the most expensive, must be annealed, and are sensi-

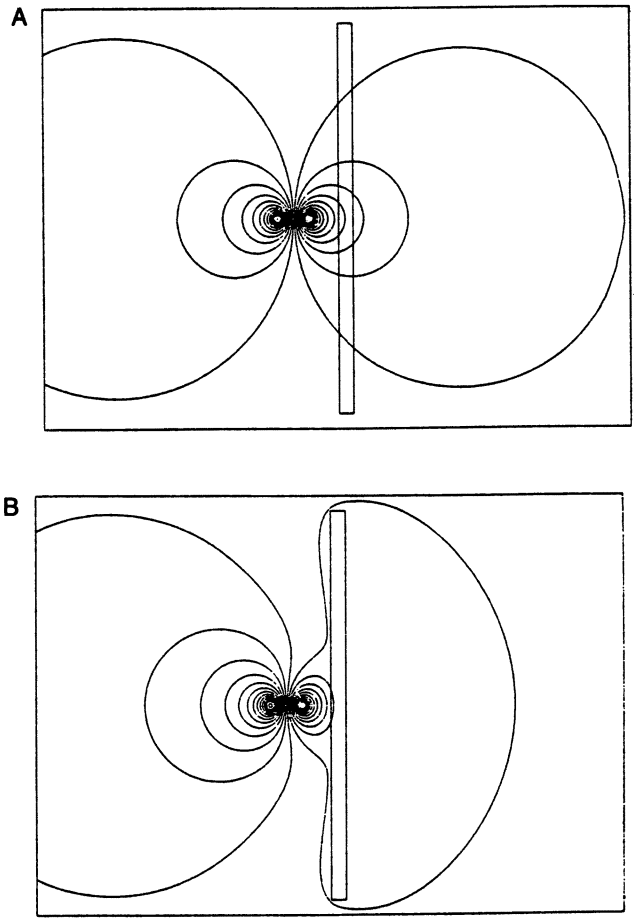

Figure 13. A: flux lines due to two wires carrying equal currents in opposite directions adjacent to a nonconducting plate. B: the same wire sources, but the plate is now conducting, and induced currents shield the region to the right of the plate. Due to the finite height of the plate, some flux comes around the ends.

Table I. Calculated Shielding Factors for an Infinitely Long Cylinder with Inner Radius of 0.1 m as a Function of Three Thicknesses and Various Materials

Material	*5 mm*	*2 mm*	*1 mm*
Aluminum	0.2529	0.5493	0.7964
Copper	0.1425	0.3410	0.5878
Carbon steel	0.0841	0.3337	0.5246
48% Nickel		0.0013	0.0128
80% Nickel		0.0001	0.0030

NOTE: Absent entries signify a shielding factor $<10^{-4}$, which would appear as all zeros to four significant digits.

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tive to shock and stress, which degrade the shielding performance. In actual application, the shielding factor will be a function of position due to end effects and possibly due to the method used to connect cylinders end to end. For a given material, not only the thickness but also the radius of the cylinder has an effect on the shielding factor.

As with the cylindrical shield, the large-scale dimensions of a plate shield (i.e., length and width) and location of the shield with respect to the magnetic field sources have an impact on the shielding factor. The two graphs in Figure 14 illustrate these two issues by way of results from a two-dimensional simulation

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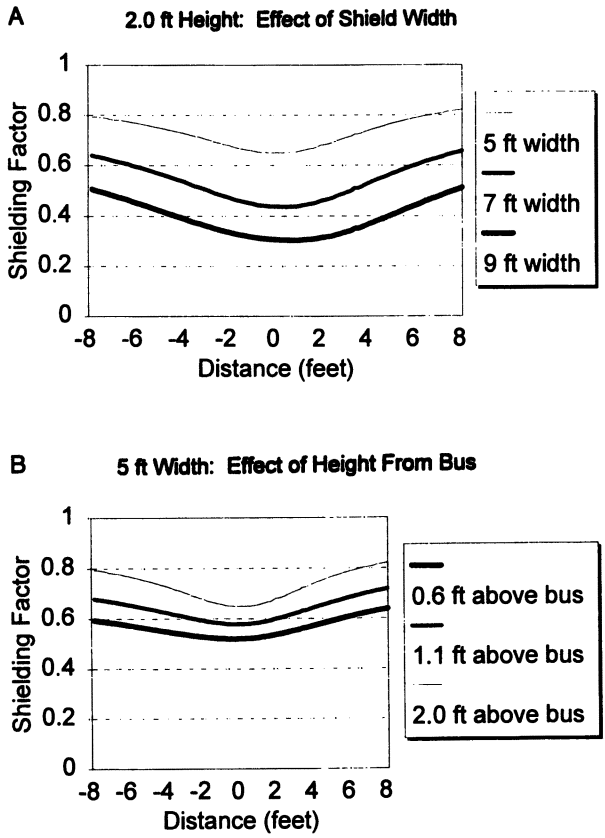

Figure 14. Two graphs of shielding factor curves from a two-dimensional simulation that illustrate (A) the dependence of shielding on the large-scale dimensions of a shield and (B) the dependence on shield location for a plate above a three-phase bus with 1.5-foot spacing between conductors. The carbon steel plate was 0.05 inches thick. The curves show the shielding factor along a line 7 feet above the three-phase bus.

of a plate shield above a three-phase bus carrying balanced currents. The shielding factor is evaluated along a line located 7 feet above the bus. In Figure 14A, the three lines show how the shielding factor changes as the shield width is increased from 5 feet to 7 and 9 feet. Figure 14B shows how the shielding improves as the shield is moved closer to the bus. For all cases, the shielding is most effective near the center and degrades as one moves toward the edges.

As mentioned in the shielding classification, conducting loops are a subset of conducting-only passive shielding. The induced currents, instead of flowing throughout sheets of material, flow in wires. The currents in the wire loop, and hence the obtainable shielding, are limited by the impedance of the loop, a combination of the resistance and self-inductance of the loop. Because the loop is simply a wire circuit, capacitive elements may be added that cancel some of the inductance, which results in a lower impedance and higher cancellation currents. However, adding capacitance changes the phase of the cancellation currents. For a given configuration, a capacitance exists that is a compromise between lower loop impedance and phase shift in the wrong direction. This capacitance will result in optimum shielding (*6*). As mentioned earlier, this method of shielding is being applied to transmission and distribution lines. Figure 10 shows an example of a transmission line with parallel shield wires that reduce the magnetic field beneath the wires.

The shielding available from a passive loop is quite limited when compared with a material enclosure. Enclosures can reduce the fields by several orders of magnitude, but a passive loop typically only approaches 1 order of magnitude reduction or less.

Multiple-Layer Shields. Traditionally, nested layers of magnetic materials with air gaps have been used as DC and AC magnetic shields. The layer closest to the source is made with material that has a higher saturation flux density, and the inner layers are made of high-performance ferromagnetic materials that saturate at lower flux densities.

The development of analytical models of shields consisting of multiple layers has shown that enhanced passive shielding can be obtained through the use of alternating layers of conducting and magnetic materials. For a fixed shield thickness, the combination of materials in multiple layers outperforms a single-layer shield of the same thickness made with only one or the other material by itself. Furthermore, a given shield thickness has an optimum number of alternating shield layers as well as an optimum percentage of each material.

For example, in some configurations an 8-mm shield made of two pairs of aluminum and steel (four layers, each 2 mm) will outperform a single 8-mm layer of steel or a single 8-mm layer of aluminum. A multilayer shield made of aluminum and steel may approach the performance and cost of a single layer of expensive nickel alloy material while weighing less.

The mechanical challenges of multilayer shields are not always justified, but in some cases, they may represent cost, weight, and performance improvements for a given shielding application.

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Practical Shield Considerations. An understanding of the shielding mechanisms is important in implementation of magnetic field shielding. As described earlier, induced-current shielding is dependent on the induced currents circulating over large-scale dimensions. If a shield is constructed with four insulated aluminum sheets, as shown in Figure 15, instead of one large sheet, the shielding effectiveness will be diminished. The same type of consideration holds for ferromagnetic materials, in which the shielding diminishes with the addition of air gaps between adjacent sheets of shield material.

Often it is unclear whether it is more effective to shield a room from external sources or shield the sources. In some cases the distinction is not clear, such as when a flat sheet of material is placed between source and shielded region. From the cost standpoint, one would obviously want to minimize the material and construction costs of a shield system. The distance from the source often determines the extent of the shielding required. As an example, consider the configuration shown in Figure 16, in which a flat sheet of shield material is placed between a three-phase source and the room to be shielded. The finite extent of the shield is an issue, as was shown in Figures 13 and 14, in which the field comes around the sides of the shield. The shielding effectiveness is at maximum directly behind the shield and then decreases as one moves back from the shield. The closer the plate is to the source, the smaller the shield needs to be for the same effectiveness. On the negative side, the closer to the source, the higher the losses in the shield. If ferromagnetic materials are used, then the nonlinear property of the material must be considered. In higher fields, the shield will have a higher effective permeability and will be more effective. If the source is strong enough, the ferromagnetic shield may saturate, which results in poor shield performance.

Active Shielding. Active shielding is typically what comes to mind when people talk about magnetic field management through cancellation. In fact, it is often referred to as active cancellation and not considered shielding, per se.

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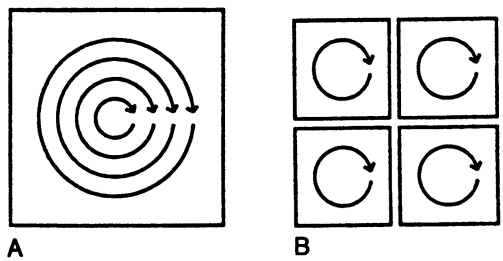

Figure 15. Shield performance is diminished in going from a single continuous sheet of aluminum (A) to four separate sheets (B) because the induced currents can no longer circulate over the large scale dimensions of the shield. Electric continuity must be maintained between adjacent sheets.

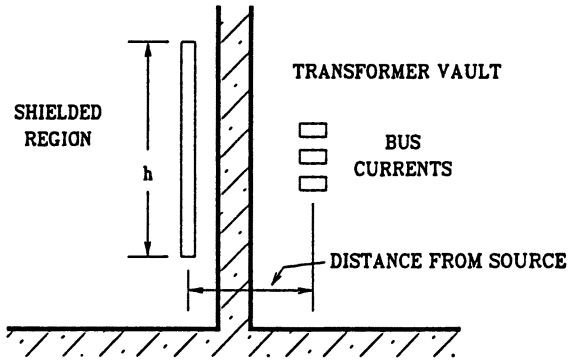

Figure 16. Flat sheet of material between a three-phase bus and a room forms a magnetic shield. The distance from the source has an effect on the required large-scale dimensions for the shield.

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An active cancellation system consists of coils carrying imposed currents. The orientation of the coils, as well as the phase and magnitude of the imposed currents, is such that cancellation of the magnetic field is achieved in the region of interest. Figure 17 illustrates the basic idea of active cancellation. An offset loop carrying current in the opposite direction creates a magnetic field that opposes the original source field, which results in cancellation that varies as a function of space. In some regions of space, the field is actually increased. In actual implementation, the sources and cancellation coils may be much more complex.

Complex multiple-coil systems are commonly used on naval vessels for "magnetic silencing" to avoid detection and magnetically triggered weapons. Active cancellation is also used in the medical industry for the shielding of magnetic resonance imaging (MRI) equipment because it is much lighter than a passive shield made of ferromagnetic material. Active cancellation is ideal for MRI equipment because details of the magnetic field sources are exactly known.

In many cases, the magnitude and phase of current in the cancellation coil are simply adjusted to best cancel the magnetic field at some point in the region of interest. However, the original source of the magnetic field may vary in time, which necessitates continuous adjustment of the current in the cancellation coil. A magnetic field sensor at some location in or near the shielded region may be used as the feedback information for a control system that automatically adjusts the current magnitude and phase in the cancellation coils. This situation is shown schematically in Figure 18. A feedback control system automatically tracks variations of the magnetic field.

On the negative side, if not phased properly, an active cancellation system can increase the field instead of canceling it, and building a system that cancels the field equally well in all regions of space is nearly impossible. Complex systems of cancellation coils are required for canceling magnetic fields that vary dramatically in space. Furthermore, the ability of a feedback system to respond

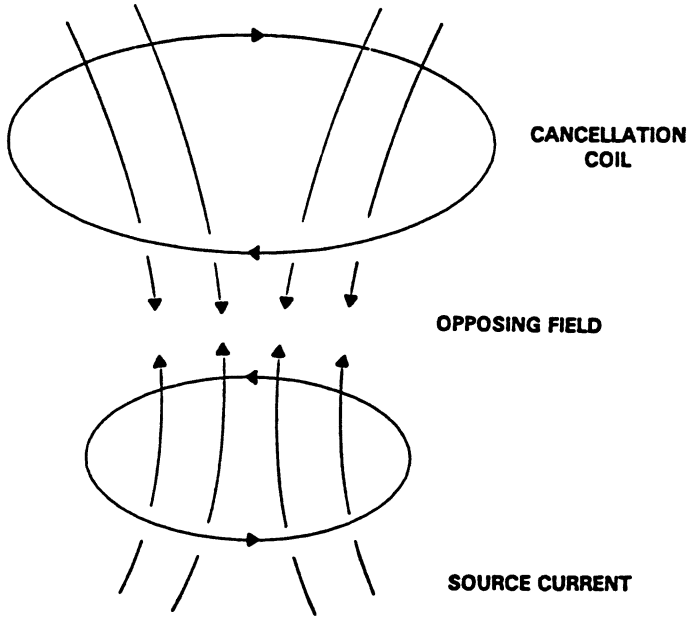

Figure 17. This two-coil setup illustrates the basic concept of active shielding. The cancellation coil above the source coil carries current so that it produces a field that tends to cancel the source field.

to quick changes in magnetic field is limited. This system limitation contrasts with the response of an induced-current shielding system, which has better performance as the speed of a magnetic field transient increases.

Conclusions

Because so many materials or objects are conducting enough to alter electric fields, magnetic field management is much more complex than electric field

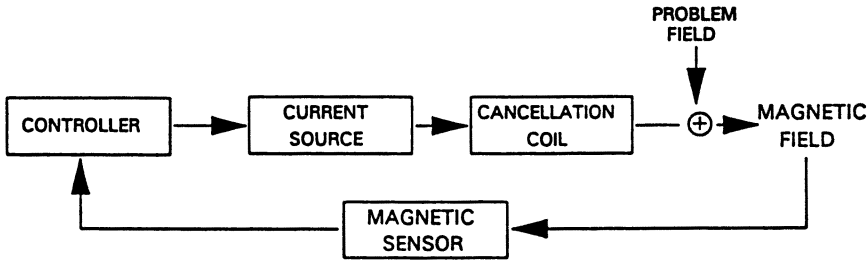

Figure 18. A magnetic field sensor provides feedback for automatic control of an active cancellation system.

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management. The appropriate field management strategy will depend on the dominant field magnitudes; the type of field, magnetic or electric; the physical characteristics of the sources; the function of the sources; the attenuation required; the region over which the attenuation is required; and any constraints on various management techniques.

For both magnetic and electric fields, the physical layout of wires is a critical consideration for managing these fields. Sources should be located at a distance from a critical region, whether it is an office or a sensitive piece of electronic equipment. Minimum spacing of wires will result in lower magnetic and electric fields. Control of alternate current paths that result in large current loops is another key area for magnetic field management.

Once options for modifying the sources are exhausted, shielding is the next step. As described previously, shielding of electric fields is relatively straightforward using conducting materials. Achieving significant magnetic field reduction using active or passive shielding is more difficult. The sections of this chapter on active and passive shielding of magnetic fields discussed the pros and cons of each of these methods, as well as some considerations for implementing either of these techniques. The keys to successful shield design are (1) identifying the key magnetic field sources, (2) characterizing the fields from these sources, (3) using accurate material properties for passive shield calculations, (4) understanding the basic passive shield mechanisms, and (5) understanding the design tradeoffs between the various alternatives.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch013

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RECEIVED for review April 25, 1994. ACCEPTED revised manuscript February 13, 1995.

14

Bioelectromagnetics in the Service of Medicine

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This chapter reviews the history and efficacy of using pulsed electromagnetic fields (PEMFs) as a surgically noninvasive technology for treating bone injury or bone disease. The primary power distribution of PEMFs is in the extremely low frequency range, but the pulse pattern used diagnostically for promoting healing of fracture nonunion differs from that used for treating osteonecrosis. Although some of the local biomechanisms stimulated by PEMFs are understood, the physical mechanisms by which PEMFs are coupled to specific biological events remains elusive and requires further research. Possible applications of PEMFs to treat other skeletal disorders, such as osteoporosis, as well as nonskeletal disorders including soft tissue and nerve damage, adult-onset diabetes, and reperfusion injuries, are also discussed.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch014

Bioelectromagnetics and Bone Repair

History. Electric stimulation of bone repair when fracture healing is slow or nonexistent has a long history. Shortly after Volta created the first battery and Galvani identified "animal electricity" at the end of the 18th century,

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three cases of fracture nonunion were treated successfully by passing "electric fluids" through needles inserted into the fracture gap. The 1812 report by Mr. Birch, a surgeon at St. Thomas Hospital in London, triggered a spate of similar treatments, and by the mid-1800s electric stimulation was considered a method of choice for slow-healing fractures. These DC (direct-current) electrodes were largely uncontrolled, and the mechanisms of action were unknown. Abuse of the method, in treating a whole range of human ills from cancer to colds toward the end of the 19th century, caused a backlash as science began its march to become the backbone of medical practice. Soon electric stimulation was looked upon as quackery.

A resurgence of interest was engendered simultaneously in the United States and Japan in the 1950s and 1960s when the validity of DC stimulation of bone healing was confirmed in animal studies (*1*, *2*). Subsequently, an ever-broadening search for mechanisms of action began at the cellular level. Simultaneously, efforts were launched to bypass the need for surgical implantation of electrodes and to place electric stimulation of fracture repair on a more practical, less risky, and less costly basis. As a result of these parallel but interlinked efforts, great progress was made in the care of fractures that fail to heal and in opening horizons to the benefits to be gained in other diseases and disorders by inducing purposeful and precise modifications in the electric microenvironments of many different cell types.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch014

At present, DC electrodes are used in the United States to treat fracture nonunion, but only as an adjunct to an open surgical procedure. Uncooperative patients, the need for better alignment, and removal of muscle or other soft tissue displaced by the original injury into the fracture gap constitute the main indications for this method. At the mechanistic level, weak DC currents (in the microampere range) mimic the injury potentials generated in bone by a fracture (*3*). These artificially induced microampere currents appear to restart the healing process by recruiting bone-forming cells in a manner similar to the original repair response (*4*).

Development of Surgically Noninvasive Method. During the search for surgically noninvasive methods to influence fracture healing, both time-varying capacitative and magnetic fields were investigated (*5*, *6*). These fields were soon recognized to carry much greater "informational" content than DC, which is either on or off, of one polarity or another, and of one amplitude or another. The frequency(ies) carried in an alternating or pulsing field appears to play a major role in determining the type(s) of synthetic and functional activities being displayed by a cell, given proper amplitude, orientation, and exposure characteristics of the applied field (*7*). Evidence of bioresponse specificity has been developed (*8–10*) in various tissue-culture, animal, and clinical settings. Saying that a patient was "electrically stimulated" is thus as nonspecific as saying a patient was given a drug.

As the press, lay, legal, and epidemiologic communities have become more focused on hazards postulated to exist in environmental magnetic and

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Advances in Chemistry; American Chemical Society: Washington, DC, 1995.

electric fields, specificity of bioresponse seems to have been ignored. The field frequency patterns, amplitudes, and conditions of exposure in the treatment of more than 300,000 ununited fractures over the past 15 years, since the Food and Drug Administration (FDA) approved the method as safe and effective, differ significantly from those of environmental fields. No untoward events or hazards were noted in these patients, and the method appears to be devoid of risk. In fact, the lessons learned from basic and clinical studies strongly suggest that the hazard issue for power lines and other field sources, if it exists at all, will not be resolved until research includes spectral analysis and exposure conditions, in addition to field strength.

Each year approximately 2 million long-bone fractures occur in the United States. Of these, 5% fail to heal normally within 3–6 months, and some never heal, ending in amputation. Recalcitrant healing stems mainly from destruction of blood supply at the time of injury, infection, inadequate alignment and immobilization, or combinations. The normal process, in which the fracture gap is bridged with bone, is interrupted at a stage when soft tissue fills the interval between the fracture fragments, which leads to pain and instability. Until this soft-tissue bridge is removed or can be induced to become bone, the patient is disabled.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch014

Pulsed Electromagnetic Fields

Care of Fractures Failing to Heal. Classically, patients with delayed union or nonunion have been repaired surgically by using bone grafts and by internal or external fixation devices made of metal. During the past two decades, since time-varying magnetic fields were first used in the author's laboratory at Columbia University, increasing use has been made of this surgically noninvasive technology to "jump-start" the healing process (*11–13*). Low-energy, extremely low frequency (ELF), pulsed electromagnetic fields (PEMFs) are applied to the fracture site for 8–10 h/day, at home, from an external coil and a small, battery-powered pulse generator. The characteristics of the electric fields (currents) induced by the magnetic field in the injured extremity essentially mimic the internal fields present in bone when it was dynamically deformed, before fracture. Normally, these electric fields are created during physical activity (e.g., walking) by mechanical deformation of bone and soft tissues, which produces a pulsatile electric polarization in these structures both from piezoelectric–electret and electrokinetic events (Figures 1 and 2) (*14*, *15*).

Biomechanisms and Coupling Mechanisms. In the 20 years of clinical use, considerable attention has been focused on the local biomechanisms of PEMF action that result in a success rate equal to that of surgical repair (*16*). Cellular responses stemming from these time-varying fields are appropriate to correct the pathology, just as antibiotic use is appropriate to control infection. Jump starting a car with a dead battery creates an operational machine; exposure of a nonunion to PEMFs can convert a stalled healing process to active repair,

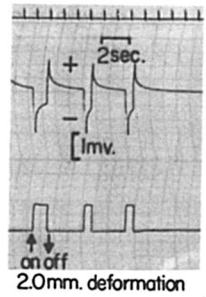

Figure 1. A dynagraph trace of strain gauge-measured deflection of a thin cantilever beam of fresh bone (bottom) and the electric polarization pattern, as measured with Ag–AgCl electrodes on its surface (top). These voltage characteristics served as a model in developing the magnetic field parameters that could induce somewhat similar waveforms in undeformed bone.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch014

even in patients unhealed for as long as 40 years! Soft fibrocartilage in the fracture gap is induced by PEMFs to calcify, thereby facilitating invasion by blood vessels that bring in the bone-forming cells needed to convert the rubbery union to a solid bony union (Figure 3) (*8*). In fact, as much is known about this process from tissue-culture, animal, and human biopsy data as is known about the mechanisms of aspirin action.

Unfortunately, on the physics side of the equation, very little is known about how such weak athermal fields are coupled to the specific cellular events that have been measured. The cascade of signal transduction processes being

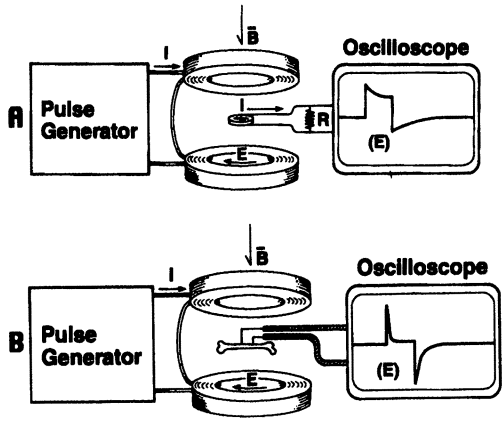

Figure 2. Diagram depicting the waveform induced by a magnetic ($\bar{B}$) field, generated by Helmholtz-aiding coils. The generator produces a single-pulse signal, similar to that used clinically. A: waveform measured with a standard coil probe in air. B: waveform measured on a piece of bone. The oscilloscopic trace in part B and elements of the top trace of Figure 1 are similar. R, *resistance;* E, *electric field;* I, *current.*

triggered by PEMFs has been traced in considerable detail from the cell membrane to the nucleus and on to the gene level, at which both selective effects on transcription and translation have been recorded (*17*). Data that have accumulated suggest direct field effects in chromosomes devoid of a cell membrane or nuclear envelope (*18*, *19*).

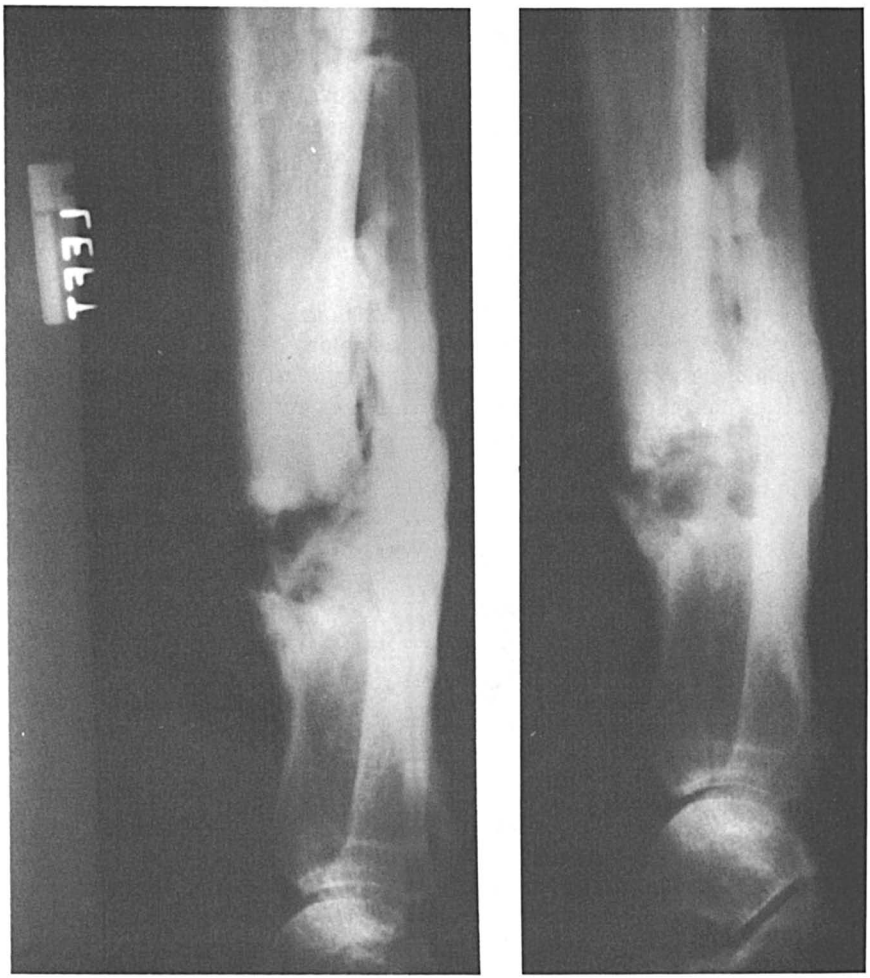

Figure 3. A lateral (i.e., side view) X-ray of the tibia (shin) in a 40-year-old patient whose fracture occurred 5 years before. In that interval two unsuccessful bone grafts had been performed before pulsed electromagnetic field (PEMF) treatment was instituted. Severe infection was present and amputation had been suggested as an alternative. B: complete bridging of the gap with bone, 6 months after coils were applied. The patient wore a cast and used crutches during PEMF treatment.

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Although considerable attention has been focused on physical mechanisms, such as ion cyclotron resonance, parametric resonance, Lorenz forces, and singlet–triplet states, among others, to explain the bioeffects of weak ELF fields, the search lags far behind those that define cell reactions. Perhaps the complexities of biology have eluded the physicist to the same degree as the complexities of physics may have eluded the biologist. For example, phased arrays of antennae, such as those in Jodrell Bank, have been used extensively by astrophysics to detect weak electromagnetic signals from the universe. In bone, a myriad of cells (osteocytes) are electrically coupled via junctional complexes to form phased arrays, far more sensitive and selective than one might judge from the analysis of a single, spherical cell awash in a sea of thermal noise (Figure 4).

Despite our extensive understanding of how PEMFs promote healing of patients with ununited fractures, many authors continue to promote the notion that "the mechanism" is unknown without defining whether they are referring to the biological or physical effects of PEMFs. Furthermore, physicians and biologists seem to have forgotten their elementary education that all chemical reactions are based on electric charge–charge interactions. This confusion has helped retard more extensive clinical use of the technology because regulators at the FDA, although they approved PEMFs as safe and effective in 1979, continue to demand an indication-by-indication approval for any new application. If the biological action of a new antibiotic proves it to be effective against the strepto-

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch014

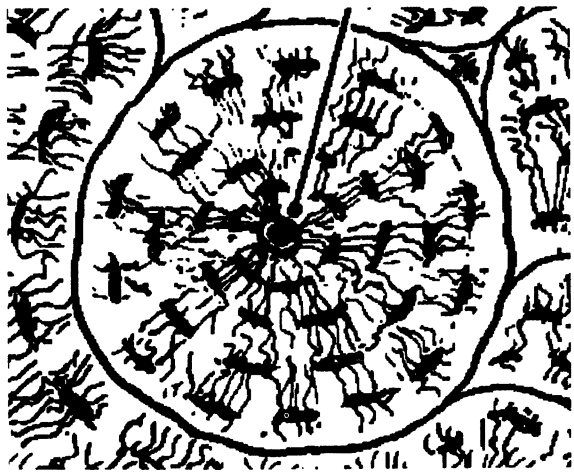

Figure 4. Diagram of bone cells, called osteocytes, embedded in an osteon (the black circle in center of the structure is a vessel). These spiderlike cells link processes throughout these 0.5–1-mm-long, cylindrical structures. The links (junctional complexes) electrically couple one cell with its neighbors in phased arrays. Such arrays are far more sensitive to weak "signals" than a single spherical cell and may explain, in part, how such stimuli are detected above thermal noise.

coccus, for example, the physician is left the decision as to when to use it, whether for a strep throat or a strep abscess; such is not the case with PEMFs.

How PEMF Treatment Is Prescribed. A PEMF unit requires a prescription from a surgeon before it is delivered for use by a specific patient. At the present time, a number of manufacturers worldwide provide equipment. In the United States, approximately 20% of the 100,000 tardy fracture unions are treated by this riskless method each year, the remainder being repaired by an operation that costs 2–3 times as much and that carries a significant complication rate. Since 1979, when PEMFs were first approved by the FDA for treating nonunions, more than 17,500 members of the approximately 20,000-member American Academy of Orthopedic Surgeons have prescribed the method, most on multiple occasions. Accumulatively, PEMFs have been used on more than one-quarter million patients worldwide. During this time, a number of double-blind clinical studies (*20–23*) buttressed earlier randomized prospective studies to show sound, statistically significant effects of PEMF in healing bone.

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Treatment of Osteonecrosis. Osteonecrosis or avascular necrosis means dead bone. The condition frequently involves the hip of young adults (average age 35–38 years) and is referred to in lay terms as a "heart attack" of the hip. It is common following a traumatic dislocation of the hip, a fracture near the femoral head (the "ball" of the joint), in kidney-transplant patients on corticosteroids, in chronic alcoholics, and in other conditions. For whatever cause, the blood supply to a weight-bearing portion of the femoral head is interrupted, and the bone dies. In the body's attempt to repair the insult, removal of dead bone outstrips the formation of new bone, thereby weakening the mechanical integrity of the head and setting the stage for collapse. Often, the condition involves both hips. If collapse occurs, disabling osteoarthritis follows quickly (in 2–2.5 years) (*24*). Unfortunately, a total hip replacement with a prosthesis at the average age of these patients practically ensures the need for one or more reoperations before the patient reaches the end of his or her life expectancy. Each replacement carries a significantly higher complication rate than the first operation.

At the time PEMFs were first used at Columbia in 1979 for osteonecrosis, no highly effective therapeutic alternative was available for this condition, short of a prosthesis (*25*). Since that time, more than 2000 patients with this potentially disabling condition have been treated. Treated early in the course of the disorder, almost all hips were protected by PEMFs against the natural progression of the process, bone collapse was avoided, and patients were restored in months to comfortable, active lives (*26*, *27*). Even when the femoral head has collapsed, and before arthritic changes have occurred, this method is highly effective in prolonging useful hip function until an age more appropriate for a replacement (we hope, only one in a lifetime). In the author's own practice, some patients with very severe joint incongruity (from collapse, prior to treatment) are fully functional after 12–14 years and show few, if any, signs of an impending degenerative arthritis (Figure 5).

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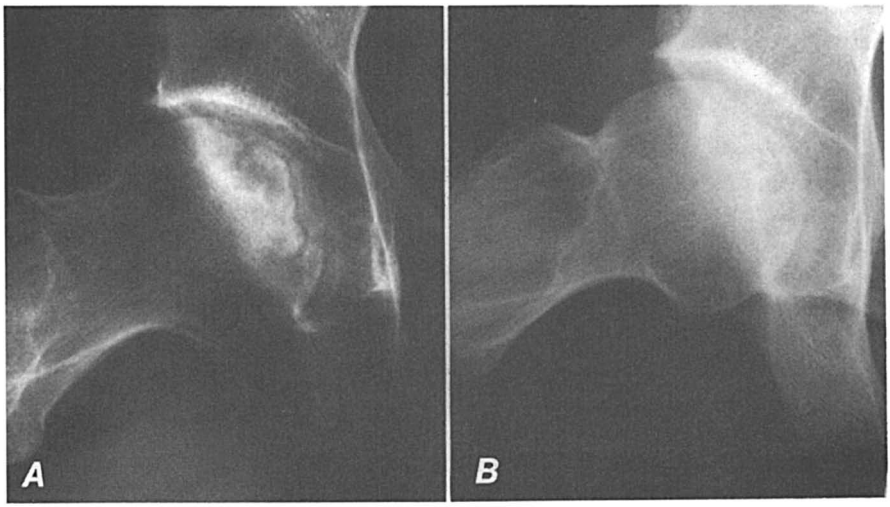

Figure 5. Side (lateral) X-ray of the hip in a 38-year-old patient with osteonecrosis probably secondary to cardiac steroid use. A: immediately before start of PEMFs, note the multiple gray lines (fractures) coursing through the "ball" (femoral head). Patient was disabled by pain. B: 1 year after PEMFs started, the patient was asymptomatic and fully active. Although some collapse occurred before treatment was instituted, it has caused no further pathologic change in the contour over the past 10 years since treatment was stopped.

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Just as in delayed fracture healing, the biomechanisms of PEMF action in this disorder are known and are appropriate to limit or correct the pathology. However, the field pattern used to treat osteonecrosis is different from that used for the nonunion and has very specific characteristics, if it is to be effective. Although PEMFs contain a broad band of frequencies (from approximately DC > 10 MHz), Fourier transforms show their major power distribution to be in the ELF range, and the spectral analysis and power characteristics of the "bone-healing pulse" are significantly different from the "osteonecrosis pulse" (*7*).

Space does not permit more than a superficial presentation of evidence here to support the statement that "different pulses produce different effects in different biotargets under differing conditions of exposure." More details are available elsewhere (*7*) that indicate that amplitude, frequency, and exposure pattern windows apparently determine if a bioeffect will occur and, if it does occur, what its nature will be. For example, both at the transcription and translation levels, data support specific and different responses to the pulse-burst and single-pulse PEMF patterns used in bone healing and osteonecrosis, respectively (*8*, *28*). Both animal and clinical data demonstrate that the waveforms and repeat patterns of the two pulse types can affect both the type of effect and efficiency in producing a given response.

Differences in bioresponses affect us in the clinic, where bone-healing pulses have little or no effect on patients with osteonecrosis. Yet both pulse pat-

terns exert an effect on blood-vessel formation (*26*, *27*). The key to this conundrum appears to lie in the type of vascular response to the two different PEMF specifications (probably because of their energy and spectral characteristics). The bone-healing pulse burst affects the early regenerative phase of vascular sprouting and increases the capillary bed, whereas the single pulse affects the maturation phase and increases the number of arterioles (*29*). This single-pulse pattern might exert a more predictable benefit in revascularization of an osteonecrotic femoral head (hip).

The 72-Hz, single-pulse pattern appears to produce two other salient bioresponses, in addition to effects on angiogenesis, that can improve the lot of patients with osteonecrosis. First, it damps bone destruction, probably by limiting the action of bone-resorbing cells (osteoclasts) (*30–32*). Second, it increases the rate of new bone formation (*31*). These several actions are important in stimulating revascularization, in rebalancing the rate of bone destruction and formation, and thereby in limiting collapse of a weakened weight-bearing structure.

On the basis of the known biomechanisms of the 72-Hz, single-pulse pattern, this therapeutic agent can be predicted to be effective not only in osteonecrosis of the hip but in other disorders characterized by substantial areas of dead bone. Radiation necrosis of bone, massive cadaver or other bone grafts, and fresh fractures with multiple fragments (i.e., segmental or comminuted) that are stripped of their blood supply by injury are all rational candidates for treatment with single-pulse PEMFs. Although no carefully constructed clinical trials have been carried out for these conditions, the author has had success in patients with each of these challenging conditions.

With the same rationale, clinical data demonstrate a high level of PEMF effectiveness in a condition of late childhood and adolescence called osteochondritis dissecans. This disorder appears to stem from the death of a segment of bone that supports the joint cartilage, and its subsequent fracture. The knee is most frequently involved, although other young joints can be a site for the condition. In the advanced case, the fractured segment of cartilage and bone can separate and become a loose body in the joint. Degenerative osteoarthritis often develops in the deranged joint, which leads to an early knee replacement in many cases (e.g., patients in their late 20s to early 30s). Results of a long-term study of PEMF effectiveness reported in June 1994 (*33*) show early healing in the patient with osteochondritis, a return to activities and avoidance of osteoarthritis, and therefore joint replacement with a prosthesis.

Future Applications of PEMFs

Osteoporosis. If altering the balance between bone formation and destruction in a positive manner is possible with PEMFs, then preventing the bone loss associated with weightlessness in space flight, from disuse, or from aging should also be possible. NASA began research support of a program at Colum-

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bia in 1976 to test the hypothesis that a bone cell responds to the "message" of an electric signal in its microenvironment regardless of the source of the signal. In other words, inductive coupling of magnetic fields could be substituted in a bone deprived of mechanical deformation to prevent bone loss (Figure 6). The result of the 3-year-long research program was positive; not only could bone loss be stopped by PEMFs, but once loss had occurred, it could be reversed (*30*). Since then, these results have been independently confirmed in several laboratories and in the clinic (*34*, *35*).

Despite mechanistic, animal, and human data supporting this approach for preventing bone loss or restoring bone mass, little of a practical nature seems likely to emerge in the near future. Astronauts do not sleep on coil bunks to maintain their bone mass while in space, probably because of the practical considerations of PEMF interference with on-board electric equipment. Furthermore, the pool of approximately 1.5 million postmenopausal women whose history of extreme bone loss has placed them at substantial risk of hip fracture does not appear likely to benefit from the technology. These fractures cost $10 billion annually in elderly osteoporotic patients, yet the specter of product liability has mitigated against pursuit of FDA approval and a market.

The press and the lay and legal worlds are clearly concerned that electromagnetic fields are hazardous to health. Despite no evidence of risk after 20

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch014

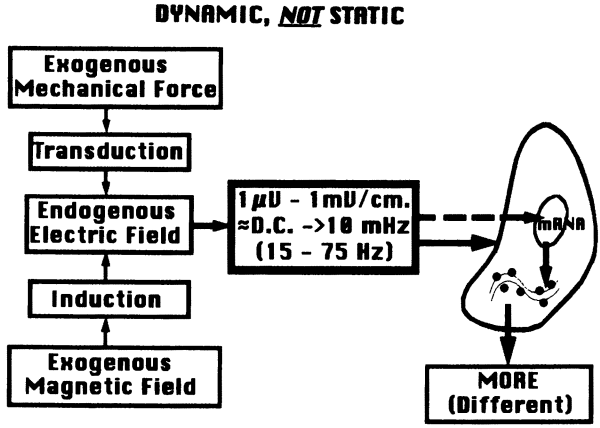

Figure 6. Diagram to link common elements of strain-induced or magnetically induced electric fields. The characteristics cited for amplitude and frequency content (in the center message box) are thought to be important in controlling bone formation and destruction, as well as their associated ancillary cellular responses. Dual arrows from the message box call attention to signaling events initiated both at the plasmalemmal level (i.e., cAMP, protein kinase C, Ca^{2+}) and within chromosomes (i.e., DNA). These messages stimulate the call to synthesize more of what it is normally producing (e.g., collagen, proteoglycan, or enzymes) or a different transcript and translation product.

years of extensive patient use and with numerous negative tissue-culture and animal-safety studies (including experimental cancer) as well as signal specificity, PEMFs continue to be lumped with environmental fields by those unfettered by scientific data. Pity the manufacturer sued by the family of a person who was treated with a coil for spinal osteoporosis and who developed spontaneous stomach or pancreatic cancer. Given the litigious environment in the United States today, along with public perception of a risk associated generally with electromagnetic fields, the potential health and cost benefits inherent in PEMF use for the treatment of postmenopausal osteoporosis will probably not be fully realized.

Osteogenesis Imperfecta. Osteogenesis imperfecta is a condition referred to by the public as "brittle-bone disease". A congenital defect in collagen cross-linking afflicts these children; growth is stunted and a low bone mass is produced. As a result of this juvenile form of osteoporosis (osteopenia), fractures are frequent, often occurring from minor trauma, such as rolling over in bed. Although PEMFs cannot (at least at this neonatal stage of development) correct the basic genetic defect, they can increase bone mass and thereby strengthen these abnormally weak skeletons and reduce the fracture rate (36). Because spontaneous malignancy is not common in these children, the potential hazards of litigation noted for adult osteoporotic patients do not appear to be a factor limiting use in this condition. On the other hand, widespread application of PEMFs for osteogenesis imperfecta in the United States is limited by FDA restrictions on PEMF use to an indication-by-indication basis. Industry says it cannot justify the sizeable expenditures required to meet FDA regulations, with no possibility of recovering costs from the small market represented by this relatively rare condition. As the mother of one such patient noted when told of this restriction: "Who will protect us against the protectors?".

Osteogenesis imperfecta, osteonecrosis, and postmenopausal or senile osteoporosis are not the only conditions in which the ability of PEMF to control bone formation and loss can be of potential benefit. Bone loss in periodontal disease and loss of ridge height in the edentulous jaw are prime candidates supported by experimental but not clinical data (*37*). The rapid destruction of bone seen in diabetics and alcoholics with nerve involvement (neuropathy) results in a Charcot joint and can be extremely disabling. Most of the skeletal damage is caused by unbridled bone destruction, which can be controlled by the early use of appropriately configured PEMFs.

Osteoarthritis. Although the biomechanisms for benefits of PEMF treatment for osteoarthritis are unknown, double-blind studies have documented significant symptomatic improvement in patients with axial (spinal) and appendicular (extremities) osteoarthritis (*38*). Field characteristics used for this condition are very different from those described in previous sections for bone healing and dead bone. A number of potential bioresponses are candidates for study, including effects on inflammation, joint cartilage breakdown and repair, and local sensory organs. Until a rational biological basis is articulated to explain the

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch014

reported clinical responses with the pulsed-field parameters being employed, however, this method will probably not enjoy widespread support in the medical community.

Soft Tissues and PEMFs. Many of the PEMF bioeffects that are useful in modifying skeletal problems could be predicted to have rational applications in skin, ligament, and wound healing, among other entities. Field and exposure patterns that increase collagen and proteoglycan synthesis and improve the growth of blood vessels can benefit various stages of the repair process in these several settings. At the clinical level, double-blind studies showed (*39, 40*) a statistically significant benefit in treating chronic refractory tendinitis of the shoulder, tennis elbow (epicondylitis), and chronic skin ulcers. Whether any of these PEMF results are linked to local elaboration of growth factors by cells in the field remains to be proved, although evidence indicates (*41*) that ion cyclotron resonance field patterns affect the synthesis and release of these factors in vitro.

The ability of PEMFs to modify cellular calcium concentrations was shown to affect secretion (*42*) and, on this basis, the technology has been used to treat adult-onset diabetes. In this condition, a coil placed over the pancreas after meals can modify blood glucose levels and reduce the need for pharmacologically based, antidiabetic agents (*43*). PEMFs also have demonstrated an ability in animals to speed regeneration of peripheral nerves after crush injury or transection (*44*, *45*). In this setting, evidence indicates that protein synthesis in the cell body (the neuron) is augmented by PEMFs, and the transport of horseradish peroxidase within the nerve fiber (i.e., axoplasmic flow) is speeded (*46*). These bioeffects may well be responsible, in part or whole, for the improvements in sensation observed in patients with diabetic neuropathy and long-standing, insensate, full-thickness skin grafts.

Tissue Electric Properties and Signal Specificity. PEMF effects on nerves and bone bring into sharp focus questions about the specificity of action of certain field parameters. For example, the single-pulse (72-Hz) pattern can be used effectively both to slow bone removal and to speed nerve regeneration. Arguably, the cellular events in these two settings are sufficiently different that they require different messages during field exposure. At least two factors deserve attention in assessing this issue. First, PEMFs contain a broad band of frequencies, some of which may affect bone cells and not nerve cells and vice versa. Second, not only are the structure and molecular content of various tissues different, but so too are their passive electric properties (e.g., dielectric constants, impedance, electret, and solid-state characteristics). This difference means that the electric voltage waveforms induced by a time-varying magnetic field differ from tissue to tissue (*47*). As a result, a nerve cell embedded in its tissue framework will be exposed to quite a different voltage amplitude and frequency spectrum than a bone cell embedded in bone, despite being exposed to PEMFs with common specifications.

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Perhaps one of the most exciting and promising applications of PEMFs, outside the skeletal system, is in their apparent ability to limit reperfusion injury, commonly attributed to production of oxygen radicals. In fact, tissue damage is significantly reduced in animal models following vascular insults such as large, full-thickness skin flaps, ligating the anterior coronary artery to produce a myocardial infarct, or ligating the anterior cerebral artery to induce a stroke (*48–50*). Although the biomechanisms behind the protective effects of PEMFs in these conditions remain to be identified, several likely avenues deserve attention. Despite their known capacity to promote capillary growth in reperfusion injury, the rate of initial tissue damage may well outstrip any improvement in revascularization, albeit rapid. If this proves to be correct and paramagnetic effects on singlet–triplet states can be confirmed, then the production of oxygen radicals at the injury site may be affected in a beneficial manner (*51*). Alternatively, modifications in nitric oxide levels and increased production of oxygen-radical scavengers, such as superoxide dismutase or catalase, deserve research support.

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Epilogue

This presentation focused on the benefits derived by society when amenable diseases and disorders are treated with appropriately configured time-varying magnetic fields. This technology carries no known risks, reduces the cost of health care significantly, and can be applied on a rational basis when known bioeffects are specifically targeted at correcting or controlling a given pathologic state. Considerable attention was focused on a biomechanistic approach because a new therapeutic agent, no matter how useful, always encounters some confusion and skepticism after its introduction. Compared to the concrete therapeutic approach of orthopedic surgery, in which the instruments are as palpable as a master carpenter's tools, PEMFs are ephemeral. They cannot be seen, felt, heard, or smelled, and medical education currently is not equipped to deal with their physical or biological aspects. Even in general medical arenas accustomed to dealing with problems considerably more abstract than surgery, drugs are palpable, but PEMFs are not.

This emerging use of selected time-varying magnetic fields as a potent therapeutic tool is yet in its infancy. One can dream about the double helix as a frequency-tuned oscillator with transcription being activated by site-specific dissociation of countercharge, but dreams must be backed by data to come true. Progress will require an interdisciplinary effort involving not only physicists, engineers, biologists, geneticists, physiologists and physicians, but particularly chemists. It is they who probably will unlock the secrets of signaling through quantum and charge–charge effects, and these secrets must become a standard part of medical education. Armed with knowledge about how it works, others can devise an expanding horizon for bioelectromagnetics in the service of medicine.

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RECEIVED for review June 19, 1994. ACCEPTED revised manuscript April 21, 1995.

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Therapeutic Aspects of Electromagnetic Fields for Soft-Tissue Healing

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Few therapies besides surgery are available to aid healing of soft-tissue injuries such as those of skin, ligament, tendon, muscle, and nerve. Noninvasive electromagnetic field therapy may offer a viable alternative. Although historically this therapy has been used to promote healing of ununited fractures, recent research indicates that this modality can now be appropriately used on soft tissue as well. We review the progress made in this area with a concentration specifically on nerve injuries.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch015

APPLICATION OF ELECTRIC FIELDS and electromagnetic fields (EMFs) to promote healing of hard tissue (bone) has been used by clinicians for almost 20 years. Only recently have these modalities been tested and employed on soft-tissue injuries (skin, nerve, muscle, and tendon) and the pain associated with injury. In this chapter we review the response of different soft-tissue models to EMFs (*see* Table I). We concentrate especially on the nervous system, which has been frequently mentioned as one of the systems vulnerable to power-line fields and cellular phones.

Some of the original in vitro (tissue-culture) and in vivo (animal) studies employed direct current (DC) or alternating current (AC) administered by electrodes immersed in culture medium, surgically implanted into tissue, or placed

0065–2393/95/0250–0277$12.00/0

Table I. Characteristics of Electromagnetic Fields

EMF Type	*Repetition Rate (Hz)*	*Peak Magnetic Field (mT)*	*Positive Induced Amplitude (mV/cm)*
Clinical PT (5-ms burst)	15	19.0	14.5
Clinical single pulse	72	3.5	15.0
PEMF (single pulse)	2	0.3	0.05–0.1
Sinusoidal	20	3.9	
	50	0.5	
	60	1.3	
	72	1.1	
	500	0.16	

NOTE: Data for electromagnetic fields (EMFs) were obtained using a probe coil (65 turns, 0.5-cm diameter). PT is pulse train; PEMF is pulsed electromagnetic field. [In vitro studies using direct current of 10–90 nA (electric current density of 13–122 nA/cm^2) correspond to an electric field of 8–72 μV/cm.]
SOURCE: Data are from references 46–49.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch015

on the skin above or adjacent to the lesion. The use of Helmholtz coils to induce EMFs for healing represents a more recent technology. The primary advantage of the magnetic coils is that they are noninvasive and can be well-adapted for treatment of soft- or hard-tissue injuries (or both simultaneously).

These modalities were initially tested in tissue-culture models by screening for adverse reactions that result from overheating (with high current levels) or production of electrochemical products at electrode surfaces. With in vitro methodologies, time of exposure, signal frequency, amplitude, and field intensities are easily determined under controlled conditions. Once these basic parameters are optimized, they can be extrapolated for use in animal preparations.

Use of Electrodes

Although stainless steel electrodes to administer DC or AC were implanted initially to promote bone repair, less reactive materials such as platinum, titanium, or tantalum replaced steel as the electrodes of choice (*1–3*). Electrodes are commonly used to promote fracture healing because bony structures are surrounded by thick soft tissues. The obvious advantage of using implanted electrodes in bony structures is that they can be inserted directly into or close to the area to promote new bone growth. In fracture healing, the bones are then stabilized with internal plates and screws or intramedullary nails, and/or the limb is immobilized by using an external cast. This relatively stable structure helps to hold the electrodes in place. For adequate healing and rehabilitation in bony injuries as well as injuries to ligaments, tendon, and muscle, early motion of the limb may be required to prevent joint contractures or tendon adhesions. Early limb motion, however, may dislodge the implanted electrodes. In skin wounds, surface electrodes are commonly used for administering current because limb movement is not usually required for effective rehabilitation.

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Use of Electromagnetic Coils

The advent of noninvasive EMF technology occurred in the 1970s and is generally used for promoting bone repair (*4–9*). Occasional use of EMFs for healing soft-tissue injuries has been reported (*10–14*). When coils are used to administer EMFs, however, it is not clear whether the response obtained is due to the magnetic field or the resultant induced electric field. In our experience using cultured neurons, promotion of growth of new nerve processes occurred whether we used electrodes to administer electric current (DC) to the neuronal cells or coils that induced electric fields via the magnetic fields (which may also contribute a growth-promoting effect). Whether these coils are employed in basic research on animals or clinically on patients, they are noninvasive and fairly easy to use.

In the following sections we present the results of experiments on two types of soft-tissue injuries: wound healing (skin and underlying tissue) and nerve regeneration. In fact, nerve regeneration is in many respects an extended wound-healing response that encompasses regrowth of nerve axons at and through the wound site.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch015

Wound Healing

After injury the healing response begins almost immediately, and it involves many cellular species that play specific roles in a time-dependent manner. To inhibit blood loss, a clot forms and the wound is subsequently debrided by inflammatory cell activity, especially by lymphocytes (*15*). This initial phase is followed by macrophage invasion, fibroblast proliferation for scar tissue formation, and subsequent development of new vascular loops to provide a blood supply to the regenerating tissue. This process of revascularization originates from preexisting capillaries; the capillary endothelial cells first proliferate and then migrate into the wound site. The influence of EMFs on the initial phases of capillary formation was investigated by Yen-Patton (*16*), who showed enhanced growth of endothelial cells that formed small capillaries in culture when the dish was exposed to 15-Hz pulsed electromagnetic fields (PEMFs). Numerous investigators (*17–19*) documented an increase in fibroblast proliferation when cultures were exposed to a number of different PEMF signals.

Skin incisions and skin flaps can be used in animals as tissue models for wound healing. To determine healing of skin flaps, assessment is made of the percentage of flap survival found in the distal portion of the flap. Flap survival is correlated directly with the blood supply, and thus treatment efficacy can be determined indirectly by measuring parameters such as blood flow and numbers of capillaries. In a study by Freedman et al. (*20*), PEMFs of 5-ms pulse bursts repeating at 15 Hz (average magnetic field of 15 G, Electrobiology, Inc., NJ) were tested on healing of dorsal skin flaps in adult rats. PEMFs applied immediately after surgery and for the next 5 days significantly decreased the percentage of necrosis relative to untreated controls. A dose response was obtained (*21*), and the greatest decrease in necrosis found was on the third day of treatment (6 h/day). A similar enhancement of flap survival was observed (*22, 23*) by using

sinusoidal electromagnetic fields (SEMFs), with an indication that the response may be frequency-specific (i.e., enhancement at 20 and 72 Hz but not at 500 Hz).

The early effects of PEMF treatment (50 Hz) on 3-cm-square skin wounds indicate that newly formed vascular networks were found 6 days after surgery only in rats subjected to the fields (*24*). This healing response extended to day 42, at which time all treated rats had closed wounds and 6% of untreated controls had wounds still not healed. In contradistinction to these reports, PEMFs (Electrobiology Inc., 15 Hz) applied to 4-cm circles of skin wounds had no effect on wound contraction and epithelialization (*25*).

Clinical application of pulsed high-frequency (27-MHz) EMFs delivered by Diapulse machines was used in patients to reduce swelling and accelerate wound healing (*10, 11, 26*). Barclay et al. (*12*) treated hand injuries with Diapulse (27.12 MHz emitting 27 and 90 mW/cm^2 in short bursts of 65 μs) for 2.5 h/day until the patients were discharged from the hospital. In 30 matched pairs the most marked effect was reduction of swelling followed by diminished pain and faster functional recovery.

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Venous Ulcers. Breakdown of skin into open sores especially on the leg may occur as a result of venous outflow obstruction. These ulcers occur most frequently in the older population and may persist as recalcitrant venous stasis ulcers. Because few if any therapies are effective in promoting healing of these wounds, noninvasive EMFs are an obvious alternative. A study by Stiller et al. (*13*) tested an EMF device on patients with venous ulcers by using a double-blind, placebo-controlled randomized design. The magnetic field amplitude was 22 G, and exposure was for 3 h/day for a total time of 8–12 weeks. Results of this study indicated that magnetic fields significantly decreased wound depth, produced a 47% decrease in wound area, and improved pain intensity scores.

The full potential for employing noninvasive EMF technology to expedite recovery and healing of skin lesions such as those from mechanical or burn injury has yet to be exploited. The advantages of local application of surface electrodes or electromagnetic coils to deliver current (and magnetic fields) cannot be overestimated.

Ligament, Tendon, and Muscle. Although few studies have been devoted to testing the efficacy of EMFs on ligament, tendon, and muscle injuries, this area is an exciting one for research. With the advent of arthroscopic surgery and increasing numbers of patients undergoing ligament and tendon repair, the use of noninvasive biotechnology to hasten return of function has potentially great benefit. For example, Currier et al. (*14*) combined electric and electromagnetic stimulation to patients following anterior cruciate ligament reconstruction to test how well this therapy could reduce muscle wasting, which is a common occurrence after such surgery. This combination, which used surface electrodes to deliver direct pulsatile current and a single coil with peak amplitudes of 1.5×10^3 G to induce current deep within the tissue, resulted in muscle

contractions lasting 10 s. After 10 weeks, muscle loss was measured and found to be significantly reduced in subjects undergoing treatment relative to control subjects. Although this study demonstrates the beneficial effects of electromagnetic stimulation on muscle rehabilitation by artificially maintaining muscle contractions, the influence of these fields on actual ligament healing has yet to be completely investigated.

A recent report on the healing of resected patellar ligaments of rabbits by Lin et al. (*27*) indicated that magnetic fields of 50 G with pulse frequency of 10 Hz resulted in the maximum healing response. These data were based on histological assessment of capillary and fibroblast numbers and on tensile strength measurements. Significantly higher tensile strength was obtained 1 and 2 weeks after resection compared to lower amplitude PEMF and to untreated controls.

The mechanisms underlying skeletal muscle activity and the influence of nerves on this activity were investigated by Lomo (*28, 29*), whose objective was to use a denervated muscle preparation to ascertain how well external stimulation could substitute for natural innervation. Lomo found that stimulating the muscles directly with surface electrodes by using a train of pulses from 1 to 500 Hz, thereby mimicking patterns of activity within the normal range, could restore nonjunctional properties of the muscle. Properties at the neuromuscular junction are, however, dependent upon both neurotrophic molecular influences and muscle activity. Preliminary studies performed in our laboratory on male rats following sciatic nerve crush injury demonstrate improved functional recovery using low-level PEMFs (2 Hz, 3 G), which promote nerve healing and regeneration without producing muscle contractions (*30*). The relative contribution of recovery due to nerve regeneration and that due to prevention of target muscle atrophy has not been studied and remains a fruitful area to be investigated.

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Nerve Regeneration

Peripheral nerves differ from central nervous structures in their ability to regenerate after injury. Moreover, nerves subjected to crush injury regenerate faster and reach appropriate targets better than nerves subjected to transection injuries. After traumatic transection injuries, the results are often devastating because the structures they innervate may become temporarily and sometimes permanently useless as a result of tissue atrophy. Time-dependent changes in integrity and function of the target tissue (or organ) are associated with the size of the nerve and the distance of the injury from the spinal cord. The closer the injury is to the spinal cord, the longer the time for recovery and the greater the likelihood of disuse atrophy of (limb muscle) target organs (*31*). Therefore, the regeneration process must begin as soon as possible to foster return of function.

In a transection injury the nerve fibers distal to the injury degenerate; new fibers derived from the proximal stump must grow out to cross the injury site to innervate the end organs. Unfortunately even with the best of surgical procedures to reapproximate proximal and distal fibers, misalignment occurs at the repair site (*32*). The slow regeneration rate and misalignment problems tend to

result in atrophy of distal end organs and little functional return. The long-term goal of our investigations is to develop innovative (bioelectromagnetic) methods to overcome the problems associated with traumatic injury and the slow regeneration of peripheral nerves.

Some of the first studies testing EMF effects on nerve regeneration used animal models with transection injuries. Wilson and Jagadeesh (*33*) reported their preliminary work on sciatic nerve with Diapulse (27 MHz, 5–120 mW/cm^2) to enhance regeneration. Conduction velocity of nerve was restored after 1 month. A more extensive study using Diapulse was conducted by Raji and Bowden (*34*) on the transected common peroneal nerve in rats. The rats were treated for 15 min daily for periods up to 2 months. Although body temperature increased transiently during the treatment periods, significant acceleration of nerve regeneration was obtained, along with an increase in size of intraneural blood vessels.

Using the transected sciatic nerve of rats as a model, Ito and Bassett (*35*) tested a single-pulse PEMF (72-Hz) signal used clinically for bone healing. After 12-h/day treatments the animals were tested for restoration of motor function; Ito and Bassett found that recovery occurred in half the time required for recovery in untreated controls. This technology was tested on regeneration of the common peroneal nerve of the cat by Orgel et al. (*36*). The leg of the cat was fitted with coils 5 days after transection and exposed to a 15-Hz pulse burst (peak induced amplitude of 15 mV, peak magnetic field of 19 mT) or a single-pulse 72-Hz PEMF (peak induced amplitude 15 mV, peak magnetic field of 3.5 mT) for 10 h/day, 6 days/week for 3 months. Those animals treated with the pulse-burst signal had a significant improvement in the number of surviving parent neurons on the operated side of the spinal cord as labeled by retrograde transport when compared with the single-pulse-treated or control animals.

We have been experimenting with the influence of electric fields or EMFs on nerve growth and regeneration for some time. Our initial studies looked at in vitro models of sensory ganglia and whether DC (*37*) and then PEMFs stimulated neurite "outgrowth", a term used for regeneration of nerve processes in culture (*38*). In the studies using DC we found that a window of 10–60 nA (13–80 nA/cm^2) applied via platinum or tantalum electrodes to culture medium containing dorsal root ganglia explants stimulated neurite outgrowth relative to controls (*38*). This enhanced growth was also found when low-level PEMFs (0.5 G, 2 Hz) were applied noninvasively to culture dishes containing similar explants (*39*). Subramanian et al. (*40*) also showed that PEMFs, 25 or 15 Hz, act synergistically with nerve growth factor to produce the longest neurites.

The animal model of peripheral nerve injury we are now using was developed from experimentation with in vitro models. A survey of PEMF signals and characteristics used in vivo to promote growth and regeneration of hard and soft tissue indicates frequency windows of specificity usually between 1 and 100 Hz (*41*). Studies of crushed nerve lesions of sciatic nerve provided evidence that PEMFs (amplitude 3 G, repetition rate 2 Hz, Bietic Research, NJ) following a crush lesion significantly stimulated the regeneration rate of rat sciatic nerve by 22% (*42*) and enhanced functional (behavior) recovery to the same extent (*30*).

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch015

Table II. Factors Used To Stimulate Mammalian Nerve Regeneration

Treatment	*Testing Modality*	*Increase (%)*	*Ref.*
PEMF (2 Hz)	Sensory	22	41
SEMF (50 Hz)	Sensory	21	45
Testosterone	Motor	26–30	50
IGF-I	Sensory	20	51
Org 2766	Sensory	20–40	52
3,5,3′-Triiodothyronine	Motor	22	53
Forskolin	Sensory	15	54
8-Bromo-cAMP	Sensory	18	55

NOTE: PEMF and SEMF are pulsed and sinusoidal electromagnetic fields, respectively; IGF-I is insulin-like growth factor I; ORG 2766 is an analogue of adrenocorticotropic hormone-(4–9); 8-bromo-cAMP is 8-bromoadenosine 3′,5′-cyclic monophosphate.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch015

In these studies the whole rat was exposed to the fields. More importantly, pretreatment of the animals before lesioning evoked the same degree of augmented response as that observed when the animal was treated after lesioning (*39, 43*). Testing this same PEMF signal and coil system using rats on the more challenging transection lesion, Zienowicz et al. (*44*) found enhanced stimulation of functional recovery (gait analysis) starting in the third month and continuing to the end of the experimental time period of 165 days.

To determine whether sinusoidal electromagnetic fields (SEMFs) are as effective as single-pulse EMFs, results using SEMFs of 50 Hz and 4 G were found to be comparable (20%) to those of PEMFs using the same crush nerve model (*45*). Pretreating the rats with SEMFs did not result in the heightened regenerative response found with the 2-Hz PEMF signal.

Table II lists the electric and chemical factors tested for their effects on nerve regeneration; these factors include PEMFs, SEMFs, hormones, growth factors, and chemical agents. This list allows a comparison to be made between the two EMF paradigms and other paradigms published in the literature used to promote regeneration. The data compiled in Table II illustrate that both PEMFs and SEMFs stimulate nerve regeneration as well and to the same extent as other factors or agents. Some of the mechanisms promoting this stimulation are therefore assumed to be similar. As with other soft-tissue models, it remains unclear whether the fields act by changing underlying mechanisms of healing or by promoting acceleration of normal regenerative processes.

Conclusion

EMF stimulation of soft-tissue healing and regeneration is still a relatively new therapeutic area of medicine. This technology employs low-level EMFs that can be administered noninvasively for extensive time periods without further surgical intervention. This therapeutic technology is especially attractive for use on most tissues, soft tissue in particular. To address such clinical possibilities, more research is needed at several different levels: those using tissue cultures to determine underlying mechanisms, those employing animal models to test for dose

response and efficacy, and those using clinical trials to determine how well humans respond to this therapy.

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RECEIVED for review April 6, 1994. ACCEPTED revised manuscript October 24, 1994.

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Electromagnetic Heating for Cancer Treatment

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Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch016

Biologists have demonstrated the cancer-killing ability of hyperthermia in combination with radiation and chemotherapy; however, clinicians have found it difficult to raise and keep the tumor temperature at therapeutic levels. During the past two decades, significant progress has been made in heat delivery, temperature monitoring, and thermal dosimetry. When electromagnetic fields are used, the energy deposition is a complex function of the frequency, intensity, and polarization of the applied field, of the geometry and size of the applicator, and of the dielectric properties, geometry, size, and depth of the tumor. Final temperatures in exposed tissues are dependent not only on energy deposition but also on blood flow and thermal conductivity of tissues. Treatment effectiveness depends on how well the tumor is heated.

PEOPLE HAVE USED HEAT FOR THERAPY since the beginning of human history (*1*). The heat sources used were natural: sunshine, fire, and hot water. The earliest recorded use of heat for cancer therapy was 5000 years ago when an Egyptian physician used a flaming wooden drill to treat a breast tumor (*1*). The heating achieved with natural sources was generally only superficial. Because changes in the vasomotor tone of the blood vessels in the skin aid in the maintenance of constant temperature in deep tissues, the usefulness of heat in therapy was limited until relatively recently. Diathermy ("through heat" in Greek) be-

0065–2393/95/0250–0287$12.00/0

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch016

came available only in the early 20th century, and it made the heating of deep tissues possible. More on the history of medical applications of electromagnetic (EM) energy can be found in references 2 and 3.

During the past two decades, interest in the use of hyperthermia in combination with other forms of therapy has increased significantly (*4–9*). Currently, hyperthermia is still an experimental treatment in the United States, and it is usually applied only to patients with advanced disease. Heating methods include whole-body heating by hot wax, hot air, a hot-water suit, or infrared radiation; and partial-body heating by radio-frequency EM fields (including microwaves), ultrasound, and heated blood or fluid perfusion. Clinical and experimental results from various countries have indicated a promising future for hyperthermia. However, the foremost problem is the generation and control of elevated temperatures in tumors.

Some reports have shown (*10, 11*) a synergistic effect of heat and radiotherapy or of heat and chemotherapy. The effective temperature range of hyperthermia treatments is very small: 41–45 °C. At lower temperatures, the effect is minimal. At temperatures higher than 45 °C, normal cells are damaged. Temperatures in tumors are usually higher than those in normal surrounding tissues during hyperthermia treatment because of a difference in blood flow. In addition, tumors are generally believed to be more sensitive to heat than normal surrounding tissues. This property is explained by the hypoxic, acidic, and poor nutritional state of tumor cells (*12*). The synergism of radiation and hyperthermia is accomplished by thermal killing of hypoxic and S-phase (DNA synthesis) cells that are resistive to radiation. Hyperthermia has been used in combination with chemotherapy because heating increases membrane permeability and the potency of some drugs.

When EM methods are used, energy deposition in tissues is a complex function of the frequency, intensity, and polarization of the applied fields, the geometry and size of the applicator, and the dielectric property, geometry, size, and depth of the tumor (*13, 14*). The material, thickness, and construction of a surface-cooling bolus between the applicator and the body also influence the amount of energy absorption. The final temperatures are dependent not only on energy deposition but also on tissue blood flow and thermal conduction. Because of the narrow effective temperature range, the tumor response rate is highly dependent on how much of the tumor is heated to a therapeutic level.

In this chapter, the biophysics of EM heating will be reviewed, and then the methods of EM heating will be discussed.

Biophysics of EM Heating

Dielectric Properties. The dielectric constant and conductivity of tissue (*15*) make propagation of EM waves in tissue different from that in free space. In general, biological tissues can be classified into two major categories. Tissues of one category have a high water content (muscle, skin, kidney, and liver tissues), which results in higher dielectric constants and conductivity than

In Electromagnetic Fields; Blank, M.;
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those of groups with low water content, such as fat and bone. Brain, lung, and bone-marrow tissues contain intermediate amounts of water and have dielectric constants and conductivities that fall between the values for the other two groups. Dielectric properties are frequency-dependent. With an increase in frequency, the dielectric constant decreases and the conductivity increases. Numerical dielectric data of various tissues have been published (*15–17*).

The action of EM fields on tissues produces two types of effects that determine their dielectric properties: the oscillation of free charges on ions and the rotation of polar molecules at the frequency of the applied field (*16*). Free-charge motion loss is due to the electric resistance of the medium. The rotation of polar molecules generates displacement current in the medium with an associated dielectric loss due to viscosity. These two effects, which produce heat in the medium, are the basis of EM energy absorption.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch016

Bioheat Equation. The rate of temperature rise, $d(\Delta T)/dt$, in tissue heated with EM energy is related to the rate of energy absorption (W_a), metabolic heating rate (W_m), and power dissipation by thermal conduction (W_c) and blood flow (W_b), as expressed in the following bioheat equation:

$$\frac{d(\Delta T)}{dt} = \frac{1}{4186c}(W_a + W_m - W_c - W_b)$$

where c is the specific heat of the tissue (*2, 18*). In a typical clinical treatment at about 50 W, the temperature rise, ΔT, will increase as shown in Figure 1. Initially there is a linear increase lasting approximately 3 min. In normal tissues, after this linear temperature increase, there is a period of nonlinear temperature rise usually lasting another 7–10 min, during which ΔT becomes important in the

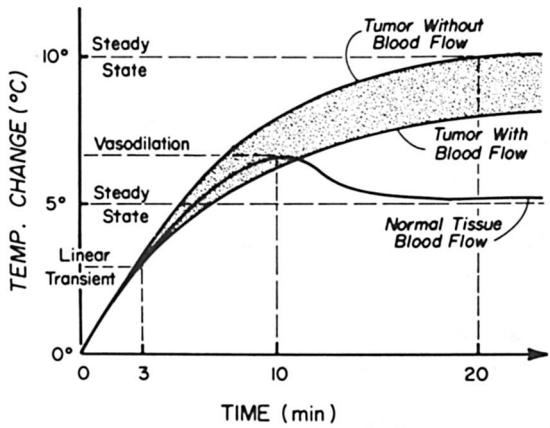

Figure 1. Temperature rise in tumor and normal tissues during hyperthermia showing the effect of blood flow. (Reproduced with permission from reference 22. Copyright 1983.)

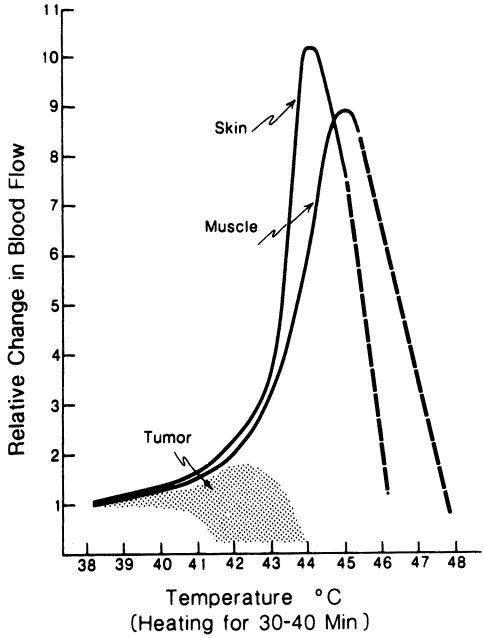

Figure 2. Relative change in skin, muscle, and tumor blood flow as a result of heating. (Reproduced with permission from reference 19. Copyright 1984 American Association for Cancer Research.)

dissipation of the absorbed energy. In tissues with a low or negligible blood flow rate ($W_b = 0$), the temperature will monotonically approach a steady-state value dictated by the magnitude of W_a, as shown on the upper curve in Figure 1, with equilibrium reached when $W_a + W_m = W_c$. In vascular tissues, a marked increase in blood flow due to vasodilation will occur when the temperature reaches the range of 42–44 °C, as shown in Figure 1, when $W_a + W_m = W_c + W_b$.

In many tumors, blood flow is vigorous at the periphery and sluggish in the center. Because the blood vessels in tumors are often fully open during ordinary conditions (*19*), in many cases no further vasodilation occurs during the heat treatment (Figure 2). After steady-state conditions are reached, the final temperature of the tumor is higher than that of the surrounding normal tissue. The shaded area in Figure 1 indicates the range of temperature rises in tumors. The lower boundary is for the periphery of the tumor and the upper is for the center, in which no blood flow exists.

The rate of energy absorption W_a must be sufficiently high so that the therapeutic level of temperature can be maintained over a major portion of the treatment period. If too low a power is used, the duration and the level of temperature elevation will not, respectively, be long enough and high enough for any benefit. With too high a power level, the temperature of the normal tissue may not be maintained at a safe level by vasodilation.

Methods of EM Heating

Microwaves occupy the EM frequency band between 300 MHz and 300 GHz, with respective wavelengths in air between 1 m and 1 mm. The most commonly used frequencies in hyperthermia are 433, 915, and 2450 MHz. They are designated ISM (industrial, scientific, and medical) frequencies in the United States and Europe (433 MHz only in Europe). Frequencies higher than 2450 MHz have no practical value as a result of their limited depth of penetration. At lower frequencies, penetration is deeper, but the applicator must be larger and focusing is difficult. A compromise must be made. Although radio-frequency (RF) energy, by International Telecommunication Union definition, lies between 3 Hz and 3000 GHz, generally for hyperthermia it means frequencies below the microwave range. The frequencies of 13.56 and 27.12 MHz have been widely used in diathermy and are now used in hyperthermia. Another ISM frequency is 40.68 MHz, but it has not been used extensively for tissue heating.

Use of other frequencies is not allowed by the Federal Communication Commission unless the treatment is administered in a shielded room to minimize interference with the communication network. In addition, stray EM radiation during treatments must be monitored. Safety standards recommended by the American National Standards Institute and the Institute of Electrical and Electronics Engineers, Inc. (*20*) and by the American Conference of Government Industrial Hygienists (*21*) are to be followed to ensure the safety of operators.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch016

Local Heating. ***External.*** The cooling mechanism of superficial tissues makes deep heating difficult by conductive methods. Two RF methods have been used to provide subcutaneous heating. First, tissues can be placed between two capacitor plates and heated by displacement currents. This method is simple, but overheating of fat, which is caused by the perpendicular electric field, remains a major problem for obese patients. Because of large differences in dielectric properties and specific heats of fat and muscle, the rate of temperature rise in planar-tissue models is about 17 times greater in fat than in muscle (*22, 23*). In addition, blood flow is significantly less in fat. Therefore, final temperatures in fat are much higher than those in muscle, and a water bolus is necessary to minimize heating of fat.

The second RF method is inductive heating by magnetic fields that are generated by solenoidal loops or "pancake" magnetic coils to induce eddy currents in tissue. Because the induced electric fields are parallel to the tissue interface, heating is maximized in muscle rather than in fat. However, the heating pattern is generally toroidal with a null at the center of the coil (*2*). Fujita et al. (*24*) recently described a paired, aperture-type inductive applicator to produce deep heating in tissue models.

In the range of microwave frequencies, energy is coupled into tissues by waveguides, dipoles, microstrips (antennas consist of a thin metallic conductor bonded to a thin grounded dielectric substrate), or other radiating devices. The shorter wavelengths of microwaves, as compared with longer wavelength RFs, provide the capability to direct and focus energy into tissues by direct radiation

from a small applicator. Engineering developments have focused on the design of new microwave applicators. A number of applicators of various sizes operate over a frequency range of 300–1000 MHz (*25–28*). Most of them are dielectrically loaded and have a water bolus for surface cooling. Low-profile, lightweight microstrip applicators, which are easier to use clinically, have also been reported (*29, 30*). Methods based on high-permittivity dielectric material, electric-wall boundary, and magnetic materials have been used to reduce applicator size and mass. These applicators are used, for the most part, for treatment of tumors a few centimeters below the skin. Pain and thermal burns usually are the major problems. Side effects of thermal blistering and burns are correlated with maximal temperatures attained during heat treatments.

Intracavitary. Certain tumor sites in hollow viscera or cavities may be treated by intracavitary techniques. The advantages of intracavitary hyperthermia include (1) better energy deposition due to the proximity of an applicator to a tumor, and (2) the reduction of normal tissue exposure compared with externally induced hyperthermia. Clinical and research studies on hyperthermia and radiation or chemotherapy of esophagus, rectum, cervix, prostate, and bladder cancers have been performed.

Microwaves and lower-frequency RF energy (2450–13.56 MHz) have been used for intracavitary hyperthermia. The main problem is that tumor temperature is unknown. Most temperatures were measured on the surface of the applicators, which can be very different from those in the tumor. Furthermore, thermocouples or thermistors were used to measure temperatures by many investigators who did not know the perturbation problem caused by the metallic sensors (*31*). One solution to this problem is to measure tissue temperature in animals and then extrapolate to humans (*32*).

Interstitial Heating. *Resistive Heating.* Tissues can be heated by alternating RF currents conducted through needle electrodes. The operating frequency should be higher than 100 kHz to prevent excitation of nerve action potentials. Interstitial techniques for radiation implants as primary or boost treatments have been practiced successfully by radiation oncologists for many years. When hyperthermia was learned to be cytotoxic and synergistic with radiation, it was natural to consider this combination with conventional interstitial radioactive implantation. Other advantages of this technique include better control of temperature distributions within the tumor compared with those of externally induced hyperthermia and sparing of normal tissue, especially the overlying skin (*33*).

Microwave Technique. Small microwave antennas inserted into hollow plastic tubing can produce satisfactory heating patterns at frequencies between 300 and 2450 MHz (*34–36*). A common frequency used in the United States is 915 MHz. A small coaxial antenna can irradiate a volume of approximately 60 cm^3. With a multinode coaxial antenna, the extent of the heating pattern can be

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch016

extended to approximately 10 cm in a three-node antenna (*37*). Because most tumors seen in the clinic are large, a single microwave antenna cannot heat the entire tumor to a therapeutic temperature. Using an array of microwave antennas is thus necessary. As in resistive RF hyperthermia, the degree of control of microwaves radiating from these antennas is important in achieving homogeneous heating. Because the antennas couple to each other, the spacing, phasing, and insertion depth affect the heating patterns of array applicators (*38–40*).

Ferromagnetic Seed Implants. Burton et al. (*41*) used thermally self-regulating implants for producing brain lesions. This technique is also applicable for delivering thermal energy to deep-seated tumors. When exposed to RF magnetic fields (~100 kHz), the implants absorb energy and become heated. But at the Curie point temperature the implants become nonferromagnetic and no longer produce heat. The surrounding tissues are then heated by thermal conduction. The influence of blood flow and tissue inhomogeneities of the tumor, which may affect the temperature distribution, can be compensated by the self-regulation of the implants, so that maintaining a temperature close to that of the Curie point is possible (*42*). Another method that exposes magnetic fluid in a tumor to an RF magnetic field (0.3 to 80 MHz) has been shown (*43*) to be feasible for inducing selective heating.

Regional Heating. ***Electric Field.*** Heating deep-seated tumors is difficult. RF energy can be deposited into the center of the body, but a large region is affected. Differential increases of blood flow in the normal and tumor tissues may result in higher temperatures in the tumor than in normal organs. However, this temperature differential cannot be ensured. Strohbehn (*44*) used the term "dump and pray" to describe the situation of putting large amounts of EM energy into the region and hoping for satisfactory results. In Japan, an 8-MHz Thermotron system uses a capacitive electric field to heat deep tumors and a water-cooled bolus to minimize the heating of fat tissue (*45*). The sizes of the two electrodes are adjusted to control the heating patterns in patients.

Most other electric field heating systems generate electric fields parallel to the body's surface. These include the annular phased-array systems (APAS) (*46*), the helix system (*47*), the coaxial transverse electromagnetic (TEM) system (*48*), the ring electrode (*49*), the segmented cylindrical array (*50*), the toroidal inductor (*51*), and the loosely coupled TEM system (*52*). The APAS, made by the BSD Company (Salt Lake City, UT), radiates 16 RF fields in phase toward the patient. This array of applicators with variations in phase, frequency, amplitude, and orientation of the applied fields can add more dimensions to controlling the heating patterns during the treatment. A newer system with eight dipoles has been evaluated in the clinic (*53*). To determine the excitation phases of an array for heating an inhomogeneous medium, a retrofocusing technique is applied (*54*). A small probe is first inserted into a tumor. A signal is radiated by the probe and received by the array of applicators outside the patient. By the reciprocity theory, conjugate fields are radiated from the applicators and focused on

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch016

the tumor. The technique was demonstrated experimentally in a water tank. A significant increase in power level, at the desired focus, was observed.

In general, superficial heating and hot spots in normal tissues are the limiting factors for effectiveness of existing treatments. Invasive techniques based on interstitial hyperthermia have been shown (*33, 55, 56*) to solve some of the deep-heating problems. However, no completely adequate deep-heating system is available. Scanned ultrasound provides an alternative method (*57*).

Magnetic Field. Magnetic fields heat tissue by induced eddy currents. The magnetic-loop applicators of the Magnetrode unit (Henry Radio, Los Angeles, CA) are self-resonant, noncontact cylindrical coils with built-in impedance-matching circuitry; they operate at 13.56 MHz and have a maximum output power of 1000 W. The RF current in the coil creates strong magnetic fields that are parallel to the center axis of the coil in which the body or limb of a patient is located. Because the magnitude of the induced eddy current is a function of the radius of the exposed object, no energy deposits at the center of the exposed tissue. However, Storm et al. (*58*) showed that the heating of tumors deep in the body was possible as demonstrated in live dogs and humans, with no injury to surface tissue. This result was apparently due to the redistribution of the thermal energy by blood flow. Nevertheless, the Food and Drug Administration has forbidden use of the Magnetrode.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch016

Whole-Body Heating. During the past 20 years, hyperthermia was used primarily for treating localized tumors. However, tumors that are resistant to conventional therapy tend to be metastatic. For these patients, local and regional hyperthermia can only be palliative. For disseminated disease, whole-body hyperthermia (WBH) in conjunction with chemotherapy and radiation was studied by many groups (*59–62*). Methods of WBH include hot wax, hot water, water blanket, water suit, extracorporeally heated blood, and radiant heat. The high morbidity and labor-intensive methods associated with WBH have caused concerns. Except for the extracorporeal blood-heating technique, which requires extensive surgical procedures, all other methods depend on conduction of heat from the body surface to the core. The target core temperature is 41.8 °C; beyond that the brain and liver can be damaged (*63*). At 41.8 °C, the metabolic rate is about double that at 37 °C, and with proper insulation, that increased metabolism can keep the body temperature elevated without additional heating. Because thermoreceptors are located cutaneously, the heating rate must be slow enough not to trigger pain or cause skin burns. The slowness of heat conduction methods causes inconvenience to patients, physicians, and the technical staff.

Volumetric heating based on highly penetrating methods is a logical alternative. To heat the whole body uniformly with EM energy is impossible. However, to heat the body regionally is possible, and the blood flow will redistribute the heat to the whole body. In the past, applications of 434- and 468-MHz microwaves were explored (*64*) in Europe for WBH, but the results did not produce any significant impact. Several regional RF systems were attempted for WBH.

In Electromagnetic Fields; Blank, M.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1995.

The BSD dipole system and the helix system (*47*) require that the body be inserted into a tunnel applicator. The BSD system also requires a water bolus in contact with the patient to provide better energy coupling and skin cooling. For hour-long use the bolus is very heavy and uncomfortable. The RF electric field system, designed by the University of California at Los Angeles group (*52*), uses three electrode plates to heat deep-seated tumors in the torso. The patient lies on a table, and the top plate is swung over the abdomen to heat the thoracic region. No water bolus is needed, and there is no contact with the patient. It is very simple to use. After 2 years of extensive phantom and animal studies, it was found (*65*) to be a very promising system for regional hyperthermia and WBH.

Discussion

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch016

In vitro, in vivo, and clinical studies have shown (*66*) that hyperthermia in conjunction with radiation and chemotherapy is effective for treating cancer. A summary of 25 nonrandomized studies, from 1980 to 1988, including 1556 superficial tumors treated with radiotherapy or radiation plus hyperthermia, shows that the average complete response rates were 34 and 64%, respectively. Clearly, hyperthermia is beneficial. However, two multi-institution, randomized phase III studies conducted in the United States, one published in 1991 (*67*) and the other to be published (*68*), did not clearly show that hyperthermia in combination with radiation can improve tumor response when compared with radiation alone. Inadequate heat delivery is considered to be the reason for failure. In contrast, three randomized phase III trials recently conducted by European colleagues all showed positive results (*69–71*). Detailed reports are not yet available to discern the difference between the U.S. and European results.

Significant progress has been made in the application of EM energy for clinical hyperthermia. Improvements in treatment equipment have been made based on medical demands, and the technological advances, in turn, have fulfilled many of the clinical requirements. Using a single piece of equipment to treat all clinical cases is impossible. After an evaluation of the location and vascularity of the tumor and adjacent tissues and the general physical condition of the patient, the hyperthermia practitioner should have the option of choosing the most appropriate equipment when hyperthermia treatment is warranted.

Hyperthermia is a complicated technique and should be applied only by individuals well trained in the use of this modality (*13*). Because of the complexity involved in the coupling of EM energy to human tumors, careful heating-pattern studies should be performed for all exposure geometries and contingencies prior to treatment to ensure the best treatment conditions for the patient. Because high-energy radiotherapy cannot be repeated after the tumor has received a maximal dosage of ionizing radiation, the physician using hyperthermia must therefore try to reach the critical tumor temperatures in optimal conjunction with the radiotherapy. Anesthetizing or tranquilizing the patient may be necessary for an effective treatment. Accurate thermometry is particularly important in all phases of clinical hyperthermia, especially when the patient is anesthe-

tized. The benefit of a good treatment outweighs minor risks. If there is no choice, it would be more beneficial for the patient to have an effective treatment with a few blisters rather than a safe but ineffective treatment. It is easier to treat the burns than the cancer.

Acknowledgments

This work was supported in part by National Cancer Institute Grants CA–33572 and CA–56116.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch016

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RECEIVED for review March 8, 1994. ACCEPTED revised manuscript January 23, 1995.

17

Tissue Electroporation for Localized Drug Delivery

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Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch017

Tissue electroporation involves (1) the creation of aqueous pathways across lipid-containing barriers such as cell membranes, layers of cells, and the stratum corneum (SC) of the skin; and (2) locally driven ionic and molecular transport through these pathways. The very localized concentration of electrically insulating lipids that is responsible for much of the barrier function of cell membranes, layers of cells, and the SC also causes an "amplification" of an applied electric field pulse, E_{pulse}*, in the sense that after a charging time a much larger electric field,* $E_{barrier}$*, appears across the barrier. This intrinsic localization at the site of the barrier is a key attribute because it results in a concentration of the physical field where it is needed to cause enhanced molecular transport. Electrical measurements before and after pulsing provide a rapid method for assessing the degree and extent of recovery of electroporation. Electroporation is fundamentally mild, leaves no chemical residues, and has the potential to deliver water-soluble molecules of a wide range of size and charge to desired sites within tissues.*

0065–2393/95/0250–0301$12.00/0

In Electromagnetic Fields; Blank, M.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1995.

Background: The Challenge of Drug Delivery

Introduction of therapeutic molecules to various sites within the body has long been a part of medicine and is recognized to be critically important. The recent success of biotechnology in producing new therapeutic molecules, many of which have short biological lifetimes, has led to major interest in drug delivery as a critical technology. Within the field of drug delivery, many new methods seek to provide both larger amounts of drugs and greater control of delivery (*1, 2*). Often drugs are taken orally, and therefore they must survive the harsh digestive process and pass from the gastrointestinal tract into the bloodstream before becoming systemically available. Direct, mechanically assisted introduction into the circulation by intravenous injection is also traditional, as are variations such as subcutaneous and intramuscular injections, which create depots within the tissue from which molecules are released into the circulation.

Iontophoresis is an electrically driven transport of drugs and is usually considered in the context of transdermal drug delivery. Iontophoresis is caused by imposition of relatively small voltages [e.g., 0.1—10 V across the stratum corneum (SC) of skin; $E_{SC} \approx 10^4$–10^6 V/m] (*3–6*). Such fields appear not to cause structural changes but instead to populate existing pathways with conducting ions. The predominant transport mechanisms appear to be electrophoresis and electroosmosis through the preexisting aqueous pathways.

A key attribute of iontophoresis is that molecular transport ceases if the stimulating voltage is removed. In contrast, electroporation often creates long-lived new aqueous pathways, so that local driving forces such as pressure differences, small voltages (therefore including iontophoresis after electroporation), and concentration gradients can produce molecular transport long after the pulses that created the aqueous pathways have vanished.

Basic Concepts of Electroporation

Electroporation involves the electrical creation of aqueous pathways ("pores") across lipid-based barriers, particularly bilayer membranes (*7–12*). Almost all of the study of electroporation has dealt with individual bilayer membranes, either in the form of artificial planar bilayer membranes or of individual cell membranes. Although not fully understood, the following concepts have significant experimental support and therefore constitute a reasonable picture of single bilayer-membrane electroporation.

Cells, living tissue, and the skin's stratum corneum all contain fundamental lipid bilayer-membrane barriers. These structures are well-known to have a large electrical resistance, which is consistent with the idea that the Born energy barrier, ΔW_{Born}, to ion entry into the membrane interior is large, $\approx 65kT$, where kT is thermal energy. If either mobile aqueous cavities or perforating aqueous pathways appear in the membrane, ΔW_{Born} is greatly reduced (*13*). Thus, the creation of new aqueous pathways across bilayer membranes is expected to

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch017

greatly increase the transport of ions and water-soluble molecules. Indeed, considerable support now exists for the hypothesis that transient aqueous pores are formed because of an increased transmembrane voltage, $U(t)$ (Figure 1). This is electroporation. Not only do these aqueous pathways greatly increase the permeability of the membrane (electropermeabilization), but the local electric field across the membrane also provides a significant local driving force for the transport of small ions and charged molecules. Presently such transport is believed to occur predominantly by electrophoresis and electroosmosis through the new pathways.

Although not yet complete, theoretical models that are based on transient aqueous pores provide reasonable quantitative descriptions of some of the basic electrical and mechanical behavior of artificial planar bilayer membranes (*14–26*), the molecular transport across cell membranes (*27, 28*), and descriptions of charged-molecule transport across the complex structure of the SC of human skin (*29, 30*). Interestingly, the dramatic changes due to electroporation are believed to involve only a small fraction of the membrane.

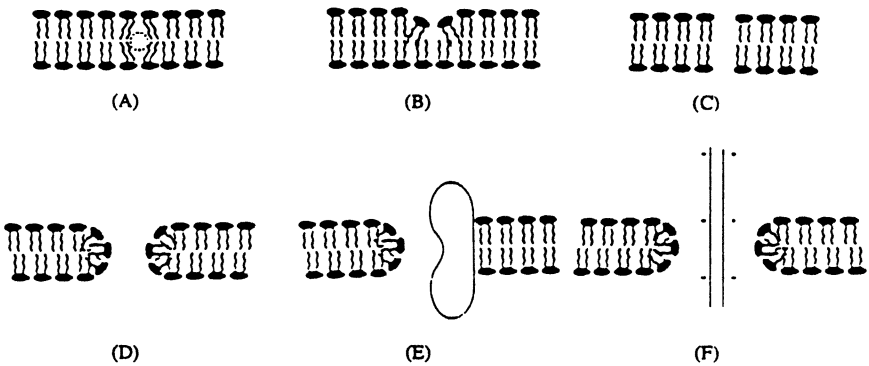

*Figure 1. Hypothetical structures for transient and metastable membrane conformations that are believed relevant to electroporation. A, free volume fluctuation (*31*); B, aqueous protrusion or "dimple" (*16, 32*); C, hydrophobic pore (*14*); D, hydrophilic pore (*14, 33, 34*), usually regarded as the primary pores through which ion and molecules pass; E, composite pore with one or more proteins at the pore's inner edge (*27*); and F, composite pore with "foot-in-the-door" charged macromolecule inserted into a hydrophilic pore (*35*). The transient aqueous pore model assumes that transitions from A → B → C or D occur with increasing frequency as transmembrane voltage* U(t) *is increased. Type E may form by entry of a tethered macromolecule while* U(t) *is significantly elevated, and then persist after* U(t) *has decayed to a small value through pore conduction. These hypothetical structures have not been directly observed and support for them derives from interpretation of a variety of experiments involving electrical, optical, mechanical, and molecular transport behavior. (Reproduced with permission from reference 11. Copyright 1993 Wiley-Liss.)*

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch017

Interpretation of experimentals with artificial planar bilayer membranes suggests that the fractional aqueous area due to pores (F_W) is $\approx 10^{-6}$ for voltage-clamp conditions (*36*). The small value of F_W is supported by a computer simulation based on a transient aqueous pore model for electroporation caused by a short pulse because this simulation predicts that for reversible electroporation $F_W \rightarrow 5 \times 10^{-4}$ for short (10-μs) pulses, but it depends on the pulse characteristics and is usually less than about 10^{-4} for reversible electroporation (*25*).

Electroporation of a fluid bilayer membrane consists of a highly interacting response to the increased transmembrane voltage that is caused by an external applied electric field pulse (*7–12*). Aqueous pathways (pores) are created by the electric field, but the creation of pores is limited because of the large increase in membrane conductance caused by the pores themselves. That is, as more pores are created and expand in size, the ability of the membrane to support a transmembrane voltage decreases because the membrane conductance rises tremendously. Thus, the process of creating pores also protects the membrane and limits the transmembrane voltage that can be achieved. During a pulse $U(t)$ tends to stabilize once electroporation has occurred, and this stabilizing also limits the net molecular transport. At the end of a pulse, pores shrink and begin to disappear.

The transient and interactive nature of the pores means that electroporation involves more than the creation of aqueous pathways; it also involves a driving force for transport through the pores, including the ionic transport that results in the membrane discharge that returns $U(t)$ to low values. The reduction of $U(t)$ in turn leads to the disappearance of pores, so that the cycle is completed as the membrane returns to its initial state.

Irreversible electroporation can also occur, and in planar bilayer membranes it is associated with rupture. In cells, irreversible electroporation is less well understood. Bounded portions of the membrane may act like planar membranes and therefore experience rupture, leaving a large opening in the cell membrane. In addition, however, reversible electroporation may occur, but the cell may be left in a chemically stressed state because of relatively nonspecific molecular exchange between the intra- and extracellular compartments. This possibility suggests that the relative size of the intra- and extracellular volumes is important, and that, therefore, electroporation of viable tissue may be less damaging than electroporation of the same cells under typical tissue culture conditions (*37*).

Mention of electroporation applications usually suggests introduction of genetic material into cells in vitro, as to date this has been by far the predominant application (*7, 9*). However, many other exciting in vitro applications have been identified (*38*), including in situ enzymology (*39*), introduction of fluorescence-labeled physical markers (*40*), and exposure of intracellular biochemical pathways to antibody-based inhibition (*41*). Even viral (*42, 43*) or microscopic inert particles (*44*) have been reported, and many other interventions based on access to the cytosol should be possible. Still other imaginative applications are

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likely, and thus the potential of electroporation for delivering molecules to desired sites within biological systems has only begun to be realized.

Tissue Electroporation

The use of electric field pulses to cause electroporation of lipid-containing barriers in tissue involves more complexity than the more familiar use of electroporation under in vitro conditions. In contrast to electroporation of single bilayer membranes (artificial planar bilayer membranes and cell membranes), tissue electroporation has only recently received attention. An important fact is that most biological barriers have evolved with lipids as a primary constituent. This means that water-soluble molecules are excluded, except for movement through specific pathways (active or passive) (Figure 2). As also noted, it also means that application of an electric field to tissue results in charging currents that bring electric charge to both sides of these barriers. The resulting separation of charge over small distances results in a large local electric field.

For example, an increase of a cell's transmembrane voltage to $U(t) \approx 1$ V results in the transmembrane electric field rising from its normal value of $E_{m,0} \approx 2 \times 10^7$ V/m to $E_m \approx 2 \times 10^8$ V/m. The electrostatic field energy is proportional to E_m^2, and therefore the electric field energy density within the membrane increases by about 2 orders of magnitude. A further consequence is that the entry of water (large dielectric constant) into the lipid (small dielectric constant) is now favored as the transmembrane voltage increases, and this tendency is the basis of aqueous pore formation. As shown in theories of electroporation, this behavior leads fundamentally to increased rates of pore formation. Thus, as the transbarrier voltage is increased, deformable lipid barriers will increasingly tend to admit aqueous protrusions, and aqueous pathway creation is favored. For this reason, electroporation can be expected to occur in all bilayer-membrane barriers in tissues.

Electrochemotherapy: Localized Tumor Treatment

Tissue electroporation has been used to provide enhanced local chemotherapy of solid cancer tumors. Often solid tumors are resistant to conventional cancer chemotherapy. Three contributions to resistance have been identified: (1) increased pressure within tumors, (2) large diffusion resistance within tumors, and (3) reduced blood perfusion within tumors (*45*). Tissue electroporation can be expected to significantly reduce physiological resistance by decreasing the pressure within tumors, increasing tissue permeability within tumors, and increasing blood perfusion within tumors. This tumor tissue response is expected, given that electroporation involves creation of new aqueous pathways across cell membranes, some of which are large. As a result, the tissue pressure should drop as outflow occurs through these pores. In addition, as the pressure decreases, tissue compression should lessen, so that diffusion resistance will decrease be-

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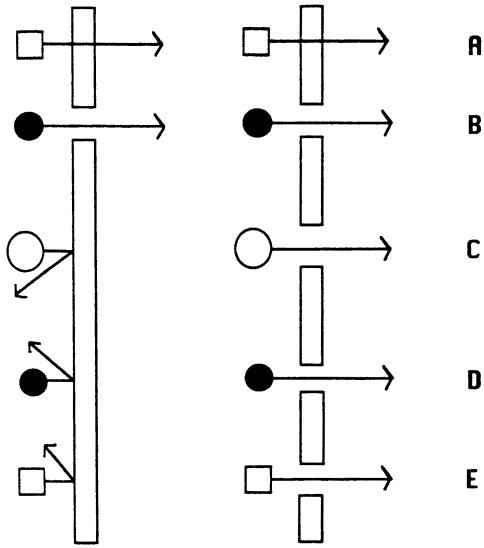

Figure 2. Schematic illustration of the enhanced molecular transport across a lipid-based barrier that is caused by the creation of new aqueous pathways. Left: Unpulsed lipid-based barrier shown with (A) a lipid-soluble molecule with some water solubility (⌊⌋) that passes through the barrier by dissolution and diffusion and redissolves in the aqueous region on the right side of the barrier. B is a preexisting aqueous pathway, which can accommodate a small charged molecule (•) but not a larger charged molecule (○); it might also accommodate a small lipid-soluble molecule that has some water solubility (not shown).

Right: Pulsed lipid-based barrier in which three newly created aqueous pathways are shown: C is larger than B, a preexisting aqueous pathway, and D is the same size as A. E provides increased transport of a lipid-soluble molecule that is also water-soluble. The amount of enhanced transport is expected to depend on the size, charge, and water and lipid solubilities of the molecules and should involve both the preexisting and newly created aqueous pathways.

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cause of both a "looser tissue" and increased cell membrane permeability. Moreover, blood perfusion should increase as the pressure drops and blood vessels and capillaries enlarge. Finally, enhanced molecular transport across the individual cell membranes of the tumor tissue should increase intracellular access of anticancer drugs at the cellular level.

The results of several research groups strongly support the efficacy of this local treatment. Most initial studies (*46–53*) used an established drug, bleomycin. Many of these studies used animal models with superficial tumors. Others have begun to examine brain cancer treatment (*54*) or to involve human subjects (*55*). In a typical application, the anticancer drug bleomycin is introduced into

the blood circulation by intravenous injection, and after a waiting time for optimal drug distribution, voltage pulses are applied locally to the region of a cancerous tumor. This treatment results in electroporation of membranes of tissue cells between the bloodstream and the tumor, and also of cells in the tumor. Overall the result is delivery of drug from the blood into tumor cells. Variants of this approach include injection of drug directly into the tissue prior to pulsing. Investigation of this application is expanding rapidly, as has been recently reviewed (*56*), and overall research and development related to this application of tissue electroporation is rapidly expanding.

Established drugs are compounds that enter cells by existing pathways (passive and active). This fact emphasizes a dilemma: Greatly reduced systemic side effects could in principle be achieved by combining local tissue electroporation with highly charged drug molecules, but such compounds are unlikely to be approved drugs. It could be argued that modification of conventional anticancer drugs should be pursued, in which, for example, charge groups are added. If intracellular enzymes are present and can cleave off the charge groups, then these charged analogs could be locally introduced by electroporation, but they would not be expected to enter cells systemically. However, the charged analogs would presumably have to pass through the full government regulatory approval process, which requirement adds a very large cost. Thus, electrochemotherapy is not likely to be as rapidly expanded to use novel (e.g., highly charged) compounds, and therefore will not make the impact that it in principle should.

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Gene Therapy: Localized Introduction of Genetic Material

Currently, a major interest is to create new therapies that are based on altering genetic material in vivo (*57*). Although the predominant approach involves the use of viral vectors for both obtaining access within cells and also providing insertion of functioning DNA, there are concerns that rare recombinant events could lead to an infectious virus. Thus, for some applications, it may be advantageous to consider localized introduction of genetic material into cells of a tissue, for example, by localized tissue electroporation.

Exploration of this possibility has already been reported (*58, 59*). For example, two types of plasmid DNA were simultaneously injected subcutaneously into newborn mice. This injection was done by folding the skin into a pleat between two electrodes and then pulsing, which caused electroporation of some cells in the subcutaneous region. Tissue samples were subsequently excised at intervals, and the primary cultures were plated in a selective medium that contained an antibiotic (neomycin), for which one plasmid codes for resistance. After several weeks of culture the stable transformants were obtained and characterized. The interpretation of these experiments is that gene introduction had been achieved in vivo.

Transdermal Drug Delivery

Transdermal drug delivery is potentially of great value. Using conventional transdermal methods, however, relatively few drugs can be delivered across the skin at therapeutic levels. Only small, lipophilic molecules such as scopolamine, nitroglycerin, and nicotine readily permeate the skin; the much larger number of hydrophilic drugs do not cross the skin in therapeutically useful amounts. Furthermore, the delivery kinetics are slow; for example, onset times of hours are typical.

The rate-limiting barrier to molecular transport is the SC, the outermost 10–15-μm-thick layer of the skin. The SC is the site of the skin's mechanical and chemical protection capability. The SC is an essentially dead tissue that can be regarded as a "brick wall" (*60*) in which the "bricks" are the corneocytes, which are flattened remnants of epidermal cells that are enclosed in an unusual protein–lipid bilayer membrane, the corneocyte envelope (*61*). The interior of the corneocytes is occupied with cross-linked keratin, and this region increases severalfold in volume as the skin hydrates. The "mortar" surrounding these "bricks" consists of multilamellar lipids, with about five or six bilayers between adjacent corneocytes (*62, 63*).

Intercellular pathways are responsible for most transdermal transport (*64*). This transport may include pathways between corneocytes within the SC, but it may also involve the so-called appendages (i.e., the sweat glands and ducts), and the hair follicles that penetrate the SC. Creation of aqueous pathways across the lipid bilayers should in principle increase the transport of water-soluble drugs through both translipid and transcorneocyte barriers and also across the layers of cells that line the appendages. Specifically, application of large electric field pulses should lead to aqueous pathway creation in both the corneocyte envelope and in the multilamellar lipid bilayers. Similarly, smaller electric field pulses should create aqueous pathways across lipid-containing barriers in the appendages. Although only a qualitative argument, the idea that new aqueous pathways should be created by large electric field pulses is supported by the fundamental tendency of lipid-containing barriers to electroporate. Quantitative prediction of skin electroporation is a daunting challenge because a series of electric field pulses appears to simultaneously create new aqueous pathways and provide a local driving force across all pathways.

The use of electric fields for enhanced transdermal drug delivery already has a long history, but only for small fields. This function is termed iontophoresis (*3–5, 65–68*), and is distinguished from electroporation. Iontophoresis is believed to act mainly on the transported molecules, involving electrophoresis and electroosmosis. Changes within the SC occur very slowly (time scale of minutes to hours) and are believed to be a secondary effect. This behavior is in contrast to electroporation, which is believed to create aqueous pathways directly, and to simultaneously provide a strong local driving force for transport through these pathways.

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The initial transdermal studies subjected skin to "high-voltage" (50–300 V) pulses and involved both molecular flux and electrical resistance measurements (*69–71*). These experiments were carried out on human skin preparations in vitro and on hairless rat skin in vivo. Permeation chambers with the donor and acceptor compartments separated by the skin were used, such that voltage pulses could be applied, electrical measurements could be made, and aliquots of solution could be removed for chemical analysis. This method allowed time-averaged molecular fluxes to be measured for three fluorescent, charged molecules, ranging from a typical "drug size" molecule (Lucifer Yellow, 457 Da, −2 charge) to a somewhat larger but highly charged molecule (calcein, 623 Da, −4 charge) to a still larger molecule (an erythrosin derivative, 1025 Da, −1 charge). The transtissue electrical resistance, R_{skin}, was measured before and just after pulsing, and this measurement provided additional evidence that significant structural changes occurred. In these experiments, a parade of exponential pulses (time constant $\tau_{pulse} \approx 1$ ms) was applied for 1 h, with 5-s intervals between pulses. Iontophoresis conditions were used for comparison, and for the same current–time integral, orders of magnitude more transport occurred for the high-voltage pulsed case. The onset for the greatly increased molecular transport corresponded to about $U_{skin} \approx 50$–100 V, which is consistent with electroporation of the approximately 100 bilayer membranes of the SC. Within this voltage range the molecular flux rose rapidly with U_{skin} but increased slowly as U_{skin} was increased further. Companion electrical measurements showed increases in skin conductance of 1–3 orders of magnitude.

The reversibility of altered transport was assessed by examining both the molecular fluxes and the skin's electrical properties. After pulsing for 1 h, the molecular fluxes usually decreased by about 90% within 30 min, and by more than 99% within 1–2 h. The companion electrical measurements showed recovery of the electrical resistance after a large drop due to the pushing. Not surprisingly, both reversible and irreversible behavior was seen, just as for individual bilayer membranes. As pulses are increased in magnitude or duration, progressively less recovery is found. This effect has been more thoroughly examined in separate studies (*72, 73*).

Other experiments used a single large exponential pulse ($\tau_{pulse} \approx 10$ ms) and used a superposed low-voltage, constant-current iontophoresis before and after the pulse (*74, 75*). These studies showed that luteinizing hormone-releasing hormone (LHRH, 1182 Da, +1 net charge) experienced a large increase in transport. Moreover, the transference number (ratio of molecular transport to ionic transport by small ions) was significantly increased, a result implying that significant new aqueous pathways were created. If only iontophoresis was used, the molecular flux increased for a particular current density, but after the single pulse, a three- to eightfold larger flux occurred. Several hours afterwards the flux diminished almost to pretreatment levels. Similar behavior was found for [arg8]-vasopressin (1084 Da, +2 net charge) and for neurotensin (1693 Da, +1 net charge).

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These and other experiments show that application of a small electric field between electroporating pulses can cause more molecular transport than either pulses or iontophoresis alone. This result is fundamentally expected if some aqueous pathways have long lifetimes. Just as the role of large electric fields is to provide a local driving force and to create pathways, the presence of a smaller field across these pathways will cause molecular transport between or after pulses. This is a general expectation: Formation of metastable pores can lead to molecular transport due to small transmembrane voltages, possibly even including voltages due to diffusion potentials that arise from slight electrolyte imbalances (*27*).

Almost by definition large changes occur in lipid-based barrier electrical conductance, $G_{skin} = 1/R_{skin}$, because of electroporation. This behavior is well-established for planar bilayer and cell membranes and should therefore be sought in skin electroporation. As already mentioned, the initial studies did find large changes in R_{skin}, and subsequent studies demonstrated that electrical measurements can provide some real-time assessment of the degree of electroporation and the kinetics of barrier recovery. This finding was more thoroughly examined in separate studies (*72, 73*).

The degree of correlation of molecular flux and electrical measurements has not yet been fully characterized. On fundamental grounds, the correlation is not expected to be complete, because the Born energy repulsion and pathway size restrictions (sieving) are predicted to exclude more charged and larger molecules from aqueous pathways that are accessible to small ions such as Na^+ and Cl^-. This behavior has been explicitly predicted for individual bilayer membranes, for which Born exclusion within a heterogeneous pore population is expected (*27*), and may therefore also occur in the multilamellar bilayer membranes of the SC.

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Noninvasive Sampling for Biochemical Assays

Truly noninvasive medical technologies are of great value because they provide interventions with benefits, but almost no risk. Two classes of noninvasive measurements can be usefully distinguished: (1) measurements that are carried out with the analyte molecule remaining within the tissue (e.g., pulse oximetry for arterial hemoglobin saturation and near-infrared spectroscopy for glucose) and (2) measurements in which the analyte is assayed outside the body [e.g., transcutaneous blood gas monitoring (*76*) and respiratory gas measurement]. Many adequate bioanalytical techniques exist for assaying the analyte outside the body (*77–80*). For example, many biosensors have been demonstrated, and their miniaturized versions are possible or were already demonstrated. But usually these sensors operate reliably only under conditions that bring a solution containing the analyte to the sensor. In this sense, the main problem is one of sampling, that is, bringing the analyte to the sensor (*81*).

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A fundamental limitation is that most biological analytes are water-soluble, do not readily cross the skin's SC, and therefore are retained within the body. The traditional method for sampling is direct and mechanical: insertion of needle into tissue or the bloodstream. Extraction of small water-soluble analytes through normally existing pathways can be accomplished by iontophoresis and has been demonstrated for the important case of glucose (*82*). This advance is important because it is a relatively simple but nonmechanical process for gaining access to an important analyte.

Medical decisions based on chemical assays traditionally involve chemical concentrations, not total amounts of analytes. For this reason, a critical question is whether the sampling process yields a representative sample, that is, a sample that has not been preferentially purified or diluted with respect to other chemical compounds, including water. Clearly, venous puncture with a macroscopic needle yields a sample that is representative of blood, because the sampling process captures a liquid volume and does not significantly involve the properties of the molecules and cells contained in that liquid.

However, extraction methods that depend on interactions of physical forces with different components of the sample have the fundamental attribute of being selective, and this attribute is undesirable. To meet the traditional requirements of diagnostic assays, sample extraction should be nonselective, that is, representative of the analyte concentration in the blood, because this value is conventionally used in making medical decisions. Absolutely representative samples are, however, not required. In fact, the selective removal of proteins from the extracted sample eliminates interference problems for some electrochemical-based sensors. The creation of new aqueous pathways through skin electroporation may therefore provide a better approximation to this requirement.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch017

Cell–Tissue Electrofusion

Electrofusion of two or more cells under in vitro conditions has been widely studied (*7, 9, 83, 84*). Extension of this process to the fusion of cells and vesicles to tissue has been reported (*85, 86*), but has not yet been extensively investigated. This approach could be applied to drug delivery by fusing drug-loaded cells or vesicles to tissues. In this case, water-soluble drugs would be introduced into the interior of those cells of the tissue that were contacted by the drug-loaded cells or vesicles. For small drugs, subsequent intracellular transport to other cells of the tissue should be governed by the extent of intracellular connections via gap junctions within the tissue.

Possible Damage and Side Effects

Any potential new therapy must be investigated for deleterious effects. With tissue electroporation for enhanced drug delivery, the issue of possible damage

to cells and tissue is therefore relevant. At the individual cell level, a large literature shows that under many pulsing conditions cell survival occurs. The use of electroporation to introduce DNA into cells under in vitro conditions is rather special. The goal is usually to obtain a small number of useful cells, without concern that many other cells are damaged. Thus, because DNA uptake in many protocols appears to increase wit pulse magnitude, conditions that are often used result in many cells (about half) being killed. This result is not a fundamental, and often cells can be electroporated with very little damage. For example, platelets can be loaded and returned to the circulation (*87*) and red blood cells prepared with recombinant CD-4 molecules can be artifically inserted into their membranes (*88, 89*), and in both cases yields are large and long circulating lifetimes are found when the previously electroporated cells are returned to the circulation in vivo. This finding is consistent with the view that insignificant damage has occurred and that electroporation is fundamentally mild (*11*).

The occurrence of reversible electrical breakdown demonstrates that electroporation is reversible at the membrane level. Clearly, however, cells can be damaged. One hypothesis is that large and relatively nonspecific molecular transport causes chemical imbalances. If the imbalance is large, the cells may not be able to recover. The ratio of intra- to extracellular volumes may be important, and therefore cells in tissues may be less susceptible to damage than the same cells electroporated under in vitro conditions, for which the extracellular volume is usually much larger (*37*). Significantly, much of the work on electrochemotherapy of cancer tumors shows that electroporation alone does not significantly damage cells (*46–49*).

In transdermal drug delivery, the idea that insignificant damage occurs is consistent with the expectation that application of electric fields results in most of the electric field becoming concentrated within the most electrically resistant regions. Within the skin, the SC is by far the most resistive region. Moreover, the SC is an essentially dead system, so that little biological damage should result if new aqueous pathways form across the SC because of electrical pulsing. Damage to underlying viable tissue is not equivalent to a protracted recovery of the SC electrical resistance after pulsing because the new aqueous pathways are believed to be created across the SC, and this does not directly involve the underlying viable cells of the epidermis.

Finally, not only have in vivo experiments with hairless rats demonstrated that some pulsing conditions provide transport of calcein into the blood without visible tissue damage (*71*), but more recent experiments have demonstrated transdermal drug delivery of LHRH across pig skin in vivo that shows by histological analyses that insignificant damage is due to electroporation (*90*). Thus, although much more study will be required, both the theoretical concepts and the experimental results to date support the idea that electroporation is basically mild and that useful operating conditions exist for which negligible damage occurs.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch017

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Advances in Chemistry; American Chemical Society: Washington, DC, 1995.

Acknowledgments

We thank T. E. Zewert, T. E. Vaughan, M. R. Prausnitz, U. Pliquett, R. H. Guy, E. A. Gift, C. Cullander, Y. Chizmadzhev, T. Chen, and V. G. Bose for many stimulating and helpful discussions and K. G. Weaver for assistance with the illustrations. This work was supported by National Institutes of Health (NIH) Grants GM34077 and ARH4921, Army Research Office Grant DAAL03–90–G–0218, and Cygnus Therapeutic Systems.

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RECEIVED for review April 25, 1994. ACCEPTED revised manuscript March 30, 1995.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch018

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The Role of Coherence in Electromagnetic Field-Induced Bioeffects: The Signal-to-Noise Dilemma

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Living cells respond to weak, low-frequency, exogenous electromagnetic fields (EMFs) that are several orders of magnitude weaker than local endogenous fields associated with thermal fluctuations. To resolve this dilemma, we propose that living cells are affected only by EMFs that are both temporally and spatially coherent. The basic idea is that a significant number of receptors must be simultaneously and coherently activated (biological cooperativity) for some minimum time interval for EMFs to affect the biochemical functioning of the cell. However, like all signal detection systems, cells are subject to the laws of physics and can be confused by noise. This concept suggested to us that a spatially coherent but temporally random electromagnetic noise field superimposed on a coherent EMF would defeat the cell's ability to respond. Because of this, induced bioeffects would be suppressed. Experimental results in mammalian cell cultures and in chicken embryos are consistent with this hypothesis. Such bioeffect inhibition by superimposed EMF noise offers a potential method for protecting living organisms against EMF-induced alterations.

THE ATTRIBUTION OF BIOLOGICAL EFFECTS at the cellular level to exposure to weak extremely low frequency (ELF) electromagnetic fields (EMFs) has remained a controversial subject despite over a decade of reports of experimental

0065–2393/95/0250–0319$12.00/0

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studies documenting the association. Theoretical arguments based on signal-to-noise considerations are in large measure responsible for the skepticism (*1, 2*). The dilemma arises because cells, existing in an electrically noisy environment, respond to external EMFs some 100 to 1000 times weaker than local noise fields resulting from the thermally driven movements of ions in the vicinity of cells. In this context Adair (*2*) has concluded that "it does not appear to be possible for weak external ELF electromagnetic fields to affect biological processes significantly at the cell level." Yet, observations continue to be published that show weak field responses (e.g., *3–5*). How can the externally impressed fields possibly influence cell behavior when cells have evolved in such a way as to function normally in the presence of the much larger local noise fields?

To address this question, Weaver and Astumian (*1*) proposed that cells integrate electromagnetic signals, effectively narrowing their acceptance bandwidth, and thus averaging out the thermal noise. However, their calculations yield averaging times much longer than exposure intervals observed to produce bioeffects. For cells of 20-μm diameter, they estimate that to achieve the required signal-to-noise improvement at 100 Hz requires averaging over 4.3×10^4 s (about 12 h); yet bioeffects have often been observed with exposure intervals on the order of (or even less than) 1 h. A simple time-averaging mechanism cannot explain the data.

An alternative explanation, also proposed by Weaver and Astumian (*6*) and elaborated by Gailey (*7*), is based on the notion that communication and cooperation among neighboring cells via gap junctions are required for an alteration of cell functioning to occur. However, EMF effects have been observed in murine fibroblast L929 cells (*5, 8*) that do not form gap junctions (*9*), as well as in transformed or cancer-derived cell lines including HL-60 (*10, 11*), Daudi (*12*), and CCM-CM3 (*13*), which are attachment-independent cells that would be expected to display few, if any, gap junctions. Multicellular cooperation via gap junction signaling is, therefore, unable to account for all the observations.

Adey (*14–17*) and Blackman (*18, 19*) conjectured that coherence in the impressed EMF plays a role in the interaction of EMFs with cells. The essence of this chapter is the examination of that role, particularly insofar as it relates to the signal-to-noise problem. Results of experiments designed to probe the relevance of both temporal and spatial coherence will be summarized. This information leads to the formulation of a hypothesis that confronts the problem of how cells detect weak exogenous fields in the presence of the large endogenous thermal noise fields.

Several experimental studies have been carried out. In our laboratory, three dealt with the role of coherence on EMF-induced effects in cultured L929 cells, specifically enhanced ornithine decarboxylase (ODC) activity; in a fourth study, the effect of EMF exposure on early developmental abnormalities in incubating chick embryos was measured. Our initial experiments involved examining the effect of introducing temporal incoherence by varying the frequency of the impressed ELF sinusoidal field at random intervals (*5*). A similar study was done in which incoherence was introduced by a variation of the frequency of the

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch018

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modulating sinusoid on an amplitude-modulated microwave signal (*20*). In both of these studies the basic outcome was that, in the absence of any incoherence, elevation of the ODC activity level resulted from exposure to the field; with incoherence incorporated into the external field, enhancement of the ODC activity was observed only when coherence was maintained for some minimum interval. In our other two studies incoherence was introduced by superimposing an electromagnetic noise signal on the impressed coherent field (*12, 21*). This exposure pattern has been replicated in a number of other studies using a variety of experimental models and biological markers. In this chapter we have focused on the impact of these studies on understanding the cellular EMF-detection mechanism, especially the process by which living cells discriminate against endogenous thermal noise fields while responding to much weaker exogenous fields.

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Experimental Results

Temporal Coherence Studies. ***ELF Fields.*** An initial study was designed to discover a possible connection between the temporal coherence properties of the impressed EMF and the response of a biological system exposed to that field. The effect on the activity of ODC produced by exposing murine L929 cells to weak sinusoidal fields formed the essence of the investigation (*5*). Cultures in midlogarithmic growth phase were subjected to 60-Hz fields of 1, 10, or 100 μT for times ranging from 1 to 8 h. Following the exposures, ODC activity was determined by the method of Seely and Pegg (*22*). The effect on ODC activity was characterized by an ODC activity ratio, R_{ODC}, calculated by dividing the ODC activity of an exposed culture with that of its matched control.

Maximal enhancement ($R_{ODC} = 2.04 \pm 0.21$) was observed for a 4-h exposure to a 10-μT magnetic field. The associated induced electric field was about 0.04 μV/cm. Comparable effects were found for similar exposures to 55-Hz ($R_{ODC} = 1.79 \pm 0.20$) and 65-Hz ($R_{ODC} = 2.10 \pm 0.35$) fields.

Incoherence was then introduced into the external field by shifting the frequency between 55 and 65 Hz. The time interval during which the signal was operated at each frequency was selected as a fixed time τ plus a computer-selected random period Δτ between 0 and 50 ms. The interval τ was varied between 0.1 and 50 s. Because $\tau >> \Delta\tau$, the coherence time is $\tau + \Delta\tau \approx \tau$. Experiments were carried out for fields with coherence times of 0.1, 1.0, 5.0, 10.0, and 50.0 s. The results, presented as the ODC activity ratio versus field coherence time in Figure 1, show that for coherence times of 10.0 and 50.0 s, the ODC enhancement is statistically indistinguishable from that obtained when a single frequency is maintained for the full 4-h exposure (essentially a factor of 2). In contrast, for coherence intervals of 0.1 or 1.0 s, no statistically meaningful alteration of ODC activity was found. A 5.0-s coherence time produced an enhancement ($R_{ODC} = 1.54 \pm 0.06$) intermediate between the control and "full-effect" values. The control-to-exposed ODC activity ratio, R_{ODC}, versus coherence time was fit to the function

$$R_{ODC} = 1 + \Delta R[1 + \exp(-t_c/\tau)]$$

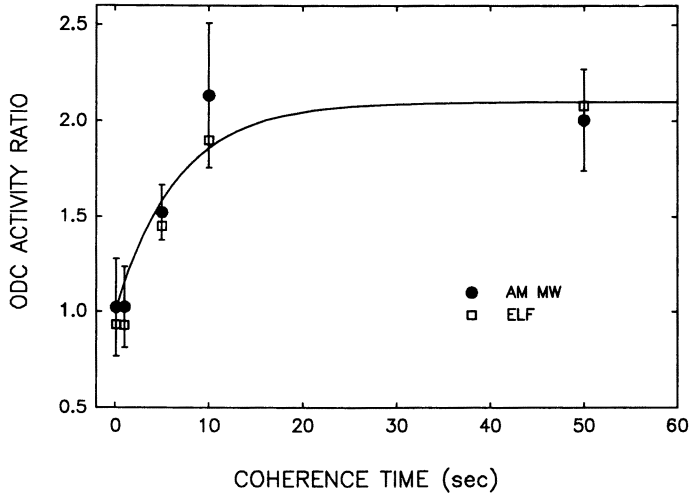

Figure 1. The ratio of exposed-to-control ODC activity in L929 cells as a function of the coherence time τ of the impressed field. Incoherence in the extremely low frequency (ELF) field was introduced by shifting the frequency between 55 and 65 Hz at intervals $\tau + \Delta\tau$, where $\Delta\tau$ is a random time interval $<< \tau$. For the amplitude-modulated microwave field (AM MW), the incoherence was introduced into the modulating sinusoid in this same fashion. (Reproduced with permission from reference 20. Copyright 1993.)

with the change in the ODC activity ratio (ΔR) equal to 1.11 ± 0.15 and the time constant associated with the cell's signal transduction mechanism (τ) equal to 8.2 ± 2.9 s.

Microwave Fields. A similar study was performed using amplitude-modulated microwave fields (*20*). In these, the incoherence was incorporated into the modulating ELF sinusoid, whereas in the ELF experiments, the frequency was alternated between 55 and 65 Hz. Initially, a series of exposures over times ranging from 2 to 24 h was conducted using 60-Hz, amplitude-modulated, 915-MHz microwaves, with modulation index m = 0.23, and an average specific absorption rate (SAR) of approximately 2.5 mW/g. These conditions produced an increase in ODC activity, which peaked after 8 h of exposure at an ODC activity ratio R_{ODC} = 1.9 ± 0.3. Using an SAR as low as 0.5 mW/g, or a modulation index of 0.60, also produced an approximate doubling of ODC specific activity after 8 h of exposure. However, no ODC enhancement was observed for cultures exposed to the unmodulated 915-MHz microwave field (R_{ODC} = 0.9 ± 0.1). The ELF amplitude modulation thus was the crucial factor in eliciting a cellular response. For 8-h exposures to microwave fields amplitude-modulated at 55 or 65 Hz, the results were essentially the same: R_{ODC}(55 Hz) = 1.9 ± 0.5 and R_{ODC}(65 Hz) = 1.9 ± 0.3.

Results of the coherence time studies are plotted in Figure 1. When the modulation frequency was shifted with a coherence time interval (τ_{Coh}) of 0.1 or

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch018

1.0 s, no significant enhancement of ODC activity over control levels was observed as indicated by R_{ODC} values of 1.02 ± 0.26 and 1.02 ± 0.21, respectively. However, with τ_{Coh} = 10.0 s, an enhancement of ODC activity was obtained: R_{ODC} = 2.13 ± 0.36. This value is (statistically speaking) the same as that found when the modulation frequency is unvarying. Increasing τ_{Coh} to 50.0 s produced an enhancement equivalent to that obtained at 10.0 s, namely, R_{ODC} = 2.0 ± 0.26. As with ELF fields, approximately twofold increases in activity were obtained with values of τ_{Coh} of 10.0 s or greater. A 5-s coherence time produced an increase that was between the control value and those obtained with $\tau_{Coh} \geq 10.0$ s; specifically R_{ODC} = 1.52 ± 0.15. When the results are fit to the same functional form as the ELF data, the parameters are ΔR = 1.08 ± 0.15 and τ = 5.3 ± 2.2 s. The differences in ODC activity ratios between τ_{Coh} of 1.0 s or less and τ_{Coh} of 10.0 s or more are statistically very significant: analysis using a *t*-test yields $P < 1.3 \times 10^{-4}$.

Several comments on these results are in order. The similarity between the cellular responses to exposure to the ELF field and the ELF-modulated microwave field, as evidenced by the similarities in the magnitude of the enhancement ΔR and the time constant τ, seems surprising, at first, because the field characteristics are so different. However, when this result is coupled with the lack of any response to an unmodulated microwave field, the obvious inference is that a mechanism exists by which the cells demodulate local EMFs (this behavior implies a nonlinear, e.g., square-law, biological EMF detection scheme). The idea is then that, once the demodulation has occurred, the cellular mechanisms that result in EMF-induced bioeffects are the same for both ELF and microwave exposure. The time constant τ can thus be interpreted as describing some fundamental response time associated with the cells' signal transduction mechanism.

Studies with Noise: Cell Cultures. ***Enzyme Activity in L929 Cells.*** The significance of the aforementioned results relative to the signal-to-noise problem is not obvious: At any instant of time, the total electric field at the surface of a cell is the point-by-point sum of the applied field and any local endogenous thermal noise fields that are present. Because the impressed electric field is a factor of 100 or so weaker than the root mean square (rms) thermal noise field, the measured time constant τ represents an interval that is insufficient for a simple signal-averaging process to reveal the presence of the weak coherent signal in the presence of the relatively intense electromagnetic noise background. As noted, the approach of Weaver and Astumian (*1*) would suggest a required averaging time of about 12 h. Nevertheless, the experiments make it clear that some process must exist that allows this recognition to occur. To elucidate some of the characteristics of this process, we exposed biological systems to bioeffect-producing fields on which controlled electromagnetic noise fields were superimposed.

Four-hour exposure of L929 cells to the 60-Hz stimulating field consistently produced an approximate doubling of ODC activity. For the work reported here a total of 73 separate experiments in which cells were exposed to the 60-Hz, 10-μT field yielded a mean ODC activity ratio of 2.0 ± 0.2. This level of en-

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Table I. ODC Activity Ratios Resulting from Exposure to 60-Hz and EM Noise Fields

Field Strength (μT)	*Sinusoidal (60 Hz)*	*EM Noise (30–90 Hz)*
10	2.0 ± 0.2	1.0 ± 0.1
100	2.0 ± 0.5	1.1 ± 0.1

NOTE: Values are exposed-to-control ornithine decarboxylase (ODC) activity ratios. EM is electromagnetic.

hancement is comparable to that previously described for similar exposure conditions (*5*). By contrast, 4-h application of the random noise fields (bandwidth 60–90 Hz) with rms amplitudes as high as 100 μT had no effect on ODC activity. These results are presented in Table I.

When a 10-μT rms noise field was superimposed on the 10-μT rms, 60-Hz field, the typical 60-Hz-induced doubling of ODC activity was eliminated. Cells exposed to this combined field displayed ODC activities that were statistically indistinguishable from those of the sham-exposed controls (*see* Table II); the incoherent electromagnetic noise field inhibited the effect of the coherent EMF. An experiment to determine the dependence on the amplitude of the noise was then performed. This experiment consisted of a series of exposures in which the 60-Hz field was maintained at 10 μT, but the amplitude of the noise field was progressively reduced from 10 μT (rms) to zero. The results of this series of measurements are shown in Table II and are plotted in Figure 2. The percent induced ODC activity is plotted as a function of the rms noise amplitude and also as a function of the ratio (S/N) of the rms amplitude of the 60-Hz signal, S, to that of the noise, N. As the noise amplitude increased, the 60-Hz field-induced ODC activity decreased. The data from this exposure series were fitted to a curve calculated from the function

$$R_{ODC} = 1 + \Delta R \frac{(S/N)^2}{\Gamma^2 + (S/N)^2}$$

with $\Delta R = 1.06$ and the constant $\Gamma = 8.7$. This function suggests that the inhibitory effect of the noise field is a function of the square of the signal-to-noise

Table II. ODC Activity Ratios from Exposure to 60-Hz, 10-μT Sinusoidal Fields with EM Noise Superimposed

EM Noise (30–90-Hz) rms Amplitude (μT)	*Exposed-to-Control ODC Activity Ratio*
0	2.0 ± 0.1
0.5	1.82 ± 0.12
1	1.49 ± 0.10
2	1.34 ± 0.07
5	1.10 ± 0.11
10	1.01 ± 0.08

Note: rms is root mean square.

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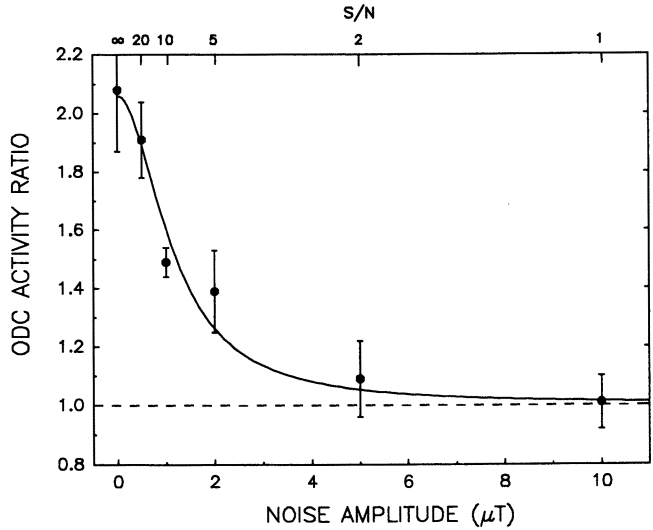

Figure 2. The ratio of exposed-to-control ODC activity in L929 cells as a function of the root mean square (rms) amplitude of the electromagnetic noise field superimposed on the 10-μT, 60-Hz coherent stimulating field. S/N designates the signal-to-noise amplitude ratio. (Reproduced with permission from reference 8. Copyright 1994.)

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ratio, S/N. When S/N was equal to 1, the inhibitory effect was essentially complete.

Enzyme Activity in Daudi Cells. A similar, if less extensive, set of experiments was performed on human lymphoma (Daudi) cells (*12*). The ODC activity ratios for cells exposed for 4 h to a 10-μT 60-Hz field, the same field with 10-μT (rms) noise superimposed, and the 10-μT (rms) noise field alone were $R_{ODC} = 1.88 \pm 0.22$, $R_{ODC} = 1.03 \pm 0.1$, and $R_{ODC} = 1.05 \pm 0.08$, respectively. The conclusions to be drawn from these data are the same as from the L929-cell data: Imposing the sinusoidal field causes roughly a doubling in the ODC activity; superimposing a comparable strength noise signal on the sinusoid essentially eliminates the ODC-activity enhancement. The noise signal by itself has no effect on the level of ODC activity.

Gene Expression in HL-60 Cells. Goodman and colleagues (*10, 11, 23, 24*) showed that transcript levels of a number of genes, including c-*myc,* β-actin, c-*fos,* c-*jun,* β-tubulin, and histone H2B, are altered by exposure to 60-Hz sinusoidal fields. In a recent set of experiments (*25*), 20-min exposure to a 60-Hz, 6.7-μT (rms) field was shown to effect a roughly 40% increase in the transcript for the protooncogene c-*myc;* the transcript level relative to control was 1.43 ± 0.16. This enhancement relative to controls was eliminated when a comparable strength noise field, that is, 6.7-μT rms amplitude, was superimposed on the sinusoidal field; the transcript level relative to control was 1.05 ± 0.07. No en-

hancement was produced by exposure to the noise field alone; the transcript level relative to control was 1.08 ± 0.09.

Relationship of Noise to Temporal Coherence. An obvious interpretation of these results is that the imposition of the noise field effectively masks the ELF field insofar as any cellular response is concerned. The idea is that superimposing noise on a sinusoid is simply a technique for causing the total impressed field to be temporally incoherent. Indeed, because the frequency spectrum of the noise includes significant amounts of energy at frequencies up to about 100 Hz, the coherence time for this signal is on the order of 0.01 s; this coherence time is much less than the (approximately) 5-s coherence requirement for field-induced bioeffects.

To test this thought, an experiment was designed that combined the noise-superposition effect with the coherence-time results given above. The coherence studies implied the existence of a time constant, τ (~10 s), that corresponds to some minimum interval over which the imposed field must be coherent if there is to be significant cellular response. The combined ELF and microwave data suggest that τ represents a fundamental characteristic of the cellular detection of EMFs. Accordingly, we performed additional experiments to explore its importance. Again L929 cells were used as the experimental model and ODC activity as the biological marker.

We hypothesized that, from the cell's perspective, the superposition for some interval of time of noise (of sufficient rms amplitude) on a 60-Hz EMF would render the total impressed field incoherent for that interval. Consequently, cultures were exposed continuously for 4 h to a 60-Hz, 10-μT field, on which was superimposed a 10-μT rms amplitude noise field that was switched on and off at 1.0-, 10.0-, and 20.0-s intervals, yielding periods of time of 1.0, 10.0, and 20.0 s when the field was coherent separated by periods of incoherence of equal duration. We expected that the possibility of inducing a bioeffect would exist only during the coherent intervals. Moreover, we anticipated that observable bioeffects would result only when these intervals are comparable to or exceed the characteristic response time τ. The results from these exposures confirmed this prediction. When noise was applied at on–off intervals of 1.0 s, the exposed cultures displayed no enhancement of ODC activity over that of the matched controls; the ODC activity ratio was 1.01 ± 0.1. However, application of noise at alternating 10.0- or 20.0-s intervals produced clear enhancement; the ODC activity ratios were 1.73 ± 0.2 and 1.69 ± 0.18, respectively. That the enhancement is given by $R_{ODC} \approx 1.7$ (rather than 2) is neither unreasonable nor unexpected because the cells were exposed to a coherent field for only 2 h out of the full 4-h exposure period.

Studies with Noise: Chick Embryos. ***Morphological Effects.*** The effects on early chick embryo development of weak EMF exposure and the mitigation of those effects by superimposing electromagnetic noise have also been studied (*21, 26*). The experiments were carried out in two groups of repli-

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Table III. Results for Embryos Subjected to PEMFs and PEMFs with Noise

Exposure Conditions	*Live*	*Abnormal*	*Percent*	P *Value*[a]
Run 1				
Sham exposed (control)	255	16	6.3	
PEMF exposed	152	29	19.1	
PEMF + noise exposed	110	8	7.3	<0.001
Run 2				
Sham exposed (control)	206	6	2.9	
PEMF exposed	203	22	10.8	
PEMF + noise exposed	181	6	3.3	<0.001

[a]*P* values were determined by paired *t*-test comparison of the number of abnormal embryos obtained for the pulse EMF (PEMF)-exposed and (PEMF + noise)-exposed conditions. Additional statistical comparisons are given in the text.

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cates (two runs), the second run being done roughly 1 year after the first. Each replicate consisted of 10, 20, or 30 eggs in each sample group. A summary of the results is presented in Table III and displayed in Figure 3. The fraction of abnormally developed embryos apparently increases when the eggs are subjected to a pulsed, external EMF. Also, this increase is apparently greatly reduced when a confusion field is present. The data were not pooled because during the nearly 1-year interval between the two runs, the supplier changed the laying flock. From the difference in the abnormal development frequencies in the control samples for the two runs, about a factor of 2, the two flocks clearly represent different experimental models.

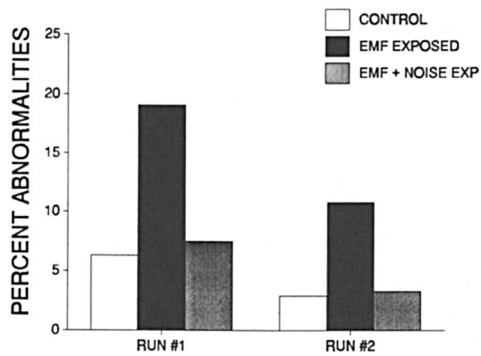

Figure 3. The abnormal development rate for chick embryos following a 48-h incubation period during which the embryos were exposed to pulsed electromagnetic fields (EMFs). The abnormality rates for sham-exposed (control) embryos and for embryos exposed to a pulsed field on which electromagnetic noise was superimposed (EMF + noise) are also given. (Reproduced with permission from reference 26. Copyright 1994.)

Because of the controversial nature of early EMF studies on avian models, in which certain groups observed effects that others were unable to replicate (*see* reference 27), a detailed chronicle of the experiments is given in Table IV. The record is a rather consistent one; no single replicate gave results that stand out as so unusual that they should be regarded as "tainted". Indeed quite a remarkable consistency is evident, with the pulsed EMF (PEMF)-exposed abnormality rate exceeding the sham-exposed rate in 20 of the 21 replicates and exceeding the PEMF plus noise (PEMF+N)-exposed abnormality rate in 17 of 19 replicates (with one being tied). The data lend themselves to various statistical analyses so that the tentative conclusions of the preceding paragraph can be subjected to quantitative scrutiny.

The overall null hypothesis that all three treatments (sham exposure, PEMF exposure, and PEMF+N exposure) yield the same rate of abnormal development was tested using the nonparametric Friedman rank test (*28*). This procedure ranks the abnormality rate for each of the three treatments (from low to high) within each replicate. For run 1, the hypothesis can be rejected with a P value < 0.001 and for run 2 with $P < 0.004$. The Friedman test can also be used to study whether differential treatment effects exist (*29*). The results are that for both runs the differences between the PEMF-exposed and the sham-exposed samples are significant at $P < 0.05$. Similarly, the differences between the PEMF-exposed and the PEMF+N-exposed samples are significant at $P < 0.05$. The differences between the sham-exposed and PEMF+N-exposed samples are not statistically significant. Paired t-tests were also used to evaluate the differences. For run 1 the abnormal development rate in the PEMF-exposed samples exceeds those for the sham-exposed and PEMF+N-exposed samples at the $P < 0.001$ level; for run 2 the same comparisons yield $P < 0.01$. Although these tests are not independent, they do serve to bear out the results obtained using the Friedman test, corrected for multiple comparisons.

The data demonstrate that imposing the PEMF on the developing embryos brings about an increase in the frequency of abnormal development (at least at the 48-h point in the incubation process). Moreover, they show that superimposing a temporally incoherent noise field on an EMF that, by itself, induces embryo abnormalities, greatly curtails (indeed essentially eliminates) its capacity for adverse developmental effects.

The conclusions that can be drawn are the following.

1. Impressing PEMFs with amplitudes on the order of 1 μT significantly increases the fraction of embryos exhibiting developmental abnormalities after a 48-h incubation period.
2. Superimposing a confusion field (a spatially coherent, temporally incoherent field) of rms amplitude comparable to the pulsed field suppresses the effect of the pulsed field on the abnormal development frequency.
3. For the noise fields considered here, the abnormality rates with no field imposed and with both the temporally coherent and noise fields are nearly the same; that is, the confusion field masks the effect of the imposed EMF even though it contains significant energy only near the fundamental frequency of the pulsed field.

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Biochemical Effects. Observations of EMF-altered morphology in the early stages of chick embryo development reflect field-induced effects at the cellular level. In the chick embryo ODC activity has been shown (*30*) to peak twice during the first 24 h of development: after 15 h (gastrulation) and after 23 h (the onset of neurulation and early organogenesis). Because ODC is a key enzyme in the biochemical pathways responsible for polyamine biosynthesis in cells and because exposure to ELF fields has been shown to enhance ODC activity, Farrell et al. (*31*) carried out a study monitoring these activity levels in developing White Leghorn chick embryos. Experiments were performed under the same three exposure conditions as were used in the morphological studies. ODC activity ratios for 15-h incubated embryos (Hamburger–Hamilton stage three; reference 32) were 2.0 ± 0.6 for the sinusoidal (60-Hz) field and 0.98 ± 0.06 for the 60-Hz field plus superimposed electromagnetic noise (30–90-Hz) field. In general terms the results reinforce the earlier conclusions: exposure to weak ELF fields modifies the biochemical pathway (characterized by ODC activity) in developing chick embryos, and this modification of embryo biochemistry is inhibited by the superimposing of spatially coherent, electromagnetic noise on the ELF field.

A similar biochemical effect was noted by Martin and Moses (*33*), who reported that exposure to weak ELF fields causes a substantial alteration of the activity of the enzymes 5-nucleotidease, acetyl cholinesterase, and alkaline phosphatase in developing embryos. They also found that when electromagnetic noise is superimposed on the applied field, the enzyme activity levels are statistically indistinguishable in the exposed and control samples. As with ODC activity, coherence in the applied field is a requirement for observing a bioeffect; the presence of a noise signal inhibits the biological response.

Discussion

Electromagnetic Noise Inhibition of Bioeffects. The results presented establish that the presence of noise comparable in magnitude to the EMF signal can nullify the bioeffects of that signal. Because no physical detection system is unaffected by noise, the apparent contradiction associated with the cellular detection of weak exogenous EMFs in the presence of much stronger, endogenous, thermally generated, electromagnetic noise had caused skepticism regarding the validity of the experimental results. However, the fact that superimposition of weak electromagnetic noise fields inhibits EMF-induced bioeffects should help to dispel such skepticism. These results provide insight into the signal-to-noise dilemma and suggest that the noise considerations up to now have underestimated the sophistication of the biodetection mechanism.

Several aspects of these results deserve some additional comment because they suggest the formulation of hypotheses to explain them. The central theme is exemplified by the results obtained in cultured murine L929 cells. Exposure to a relatively weak (10 μT) 60-Hz sinusoidal field for a period of 4 h effected roughly a doubling of the ODC activity. When an electromagnetic noise field was superimposed on the sinusoidal signal, this augmentation of ODC activity

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch018

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Table IV. Chronological Summary of the Experimental Replicates

		Control Sample			*Pulse Exposed*			*Pulse + Noise Exposed*		
Replicate No. and Date[a]		*Live*	*Abn*[b]	*Remarks*	*Live*	*Abn*[b]	*Remarks*	*Live*	*Abn*[b]	*Remarks*
1	24 Nov 91	19[c]	2		10	2		10	2	
2	27 Nov 91	26	2		9	2	1 De	16	3	2 De, 1 Tw
3	29 Nov 91	27	2	2 De	10	2		19	1	1 De
4	1 Dec 91	29	1		19	2	1 De	10	1	
5	4 Dec 91	29	2	1 De	18	3	1 De	10	0	
6	6 Dec 91	28	3		20	5		8	0	2 De
7	8 Dec 91	28	1	1 De, 1 Tw	18	3	1 De, 1 Tw	10	1	
8	11 Dec 91	30	2		10	2		17	0	2 De
9	13 Dec 91	30	1		29	7	1 De	none[d]		
10	15 Dec 91	9	0		9	1		10	0	
Run 1 totals		255	16 (6.3%)		152	29 (19.1%)		110	8 (7.3%)	

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11	9 Dec 92	19	0		20	2		17	1	1 De, 1 Tw
12	11 Dec 92	18	1	2 De	17	2	3 De	18	0	
13	13 Dec 92	18	0	1 De	19	2		20	1	
14	16 Dec 92	20	1		19	2	1 De	18	0	
15	18 Dec 92	19	0		18	2	1 De	20	1	
16	20 Dec 92	20	1		19	2		19	0	1 De
17	23 Dec 92	20	2		19	0	1 De	16	2	
18	6 Jan 93	15	0	3 De	19	1		19	0	
19	9 Jan 93	20	0		17	3	2 De, 1 Tw	none[e]		
20	12 Jan 93	20	0		19	2	1 De	18	0	1 De
21	18 Jan 93	17	1	3 De	17	4	2 De	16	1	3 De, 1 Tw
Run 2 totals		206	6 (2.9%)		203	22 (10.8%)		181	6 (3.3%)	

[a]Date on which the 48-h incubation interval was completed.
[b]Abn are embryos with developmental abnormalities.
[c]Unless noted (De is dead, Tw is twin embryo), missing embryos were broken during analysis.
[d]The noise field was inadvertently not switched on; thus there is no "pulse + noise exposed" sample for this replicate.
[e]The noise field was switched off after 30 h of incubation; thus there is no "pulse + noise exposed" sample for this replicate.

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was reduced. When the rms amplitude of the noise field was significantly smaller than the amplitude of the sinusoidal field, the reduction was small; however, as the strength of the noise field was increased, the enhancement of ODC activity was progressively diminished, until finally, when the noise was comparable to or larger than the sinusoidal field, the effect was essentially totally suppressed.

In one respect this result is not surprising: any device, even one of biological origin, that is used to detect a temporally coherent EMF signal can be rendered nonfunctional by the presence of noise. However, this mitigation of the EMF-induced bioeffect by the simultaneous application of a weak noise field (rms amplitude comparable to that of the sinusoid) is puzzling when considered in a slightly different light. As we have noted, cells exist in an environment that is naturally abundant with electromagnetic noise. The random thermal motion of ions in the vicinity of cells leads to the presence of fluctuating fields that are roughly 1000 times larger than the externally imposed sinusoidal field and noise fields: roughly 0.1 mV/cm for the rms endogenous field compared to approximately 0.1 μV/cm for the amplitudes of the induced electric field component of the externally imposed EMFs (*1, 2*). Yet, the cellular effect of the sinusoidal field is blocked by the weak external noise field, but is undisturbed by the much larger thermal noise field. Cells have apparently evolved in such a way that they treat this ever-present, random, electromagnetic background stimulation as inconsequential.

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The Requirement of Spatial Coherence. Although the mechanism of EMF–cell interaction is not known, hypotheses can be constructed to account for thermal fields being ignored by cells. The data presented here imply that cells distinguish between applied noise and thermal noise. Some fundamental difference in the properties of these two noise fields must provide the basis for such differentiation. Although both are (by definition) temporally incoherent, they do exhibit a distinct difference in their spatial behavior: at a given instant, the value (magnitude and direction) of the thermal noise field at any point is uncorrelated with its value at other locations more than a few nanometers or so distant. This property follows because the Debye screening length (roughly the range over which an ion is not shielded from other ions) in the extracellular fluid is only about 1 nm. Thus, thermally driven localized charge density fluctuations (and consequently, endogenous thermal noise fields) are spatially incoherent over distances greater than a few nanometers. By contrast, exogenously impressed fields (either temporally coherent or incoherent) always exhibit spatial coherence because their wavelengths are much larger than cellular dimensions. We hypothesize that the spatial incoherence of the thermal noise field allows cells to ignore it. Conversely, because externally imposed electromagnetic noise is spatially coherent, cells are unable to discriminate against it, and thus it is capable of confusing the biological EMF detection mechanism.

If spatial coherence is the crucial field characteristic that enables cells to respond to exogenous EMFs, then one can further infer that the biological targets

of the EMF are spatially extended or distributed. Two possibilities that should be examined are (1) that a rather large number of cells must be simultaneously stimulated or (2) that there is a collection of receptors on each cell that must be coincidentally excited to produce the observed biochemical responses; in either case some cooperativity among the entities involving inter- or intracellular signaling, respectively, must be operative.

The multicell stimulation hypothesis was developed by Weaver and Astumian (*6*) and further elaborated by Gailey (*7*). The basic premise is that communication among a group of cells is required for an alteration of cell functioning to occur. Gap junctions between adjacent cells have been proposed as a mechanism by which cells are coupled so that there is an "amplification" of the stimulating field energy. This explanation is obviously restricted to cellular systems in which gap junctions exist. For example, in the in vivo studies, field-induced changes in the frequency of developmental abnormalities and in ODC activity levels could be described in terms of such a mechanism because extensive gap junction couplings exist. However, it is not appropriate as an explanation of the EMF-induced effects in L929 cell cultures, because these cells do not form gap junctions (*9*). Further, EMF effects have been observed with transformed or cancer-derived, attachment-independent cell lines including HL-60 (*10, 11*), Daudi (*12*), and CCM-CM3 (*13*), which should exhibit few, if any, gap junction couplings. It thus seems unlikely that the bioeffects of EMFs can only proceed by interacting with aggregates of cells that communicate via gap junctions. However, other forms of intercellular signaling may exist that would allow for a multicell (as opposed to a single-cell, multireceptor) hypothesis to be preserved.

We suggested an alternative explanation that relies on the spatial coherence of the field (*8, 26*). Cellular detection of EMFs is assumed to result from the impressed fields modifying either (1) the probability of ligand–receptor binding or (2) an increase in the number of receptor binding sites available on the cell surface. Either of these would result in an increase in ligand binding and, therefore, a change in cell function. The first explanation suffers from a thermodynamic difficulty, in that it is difficult to explain how individual receptor binding would be modified by the EMF in the presence of large thermal noise fields. The mechanism of the second explanation is also not understood, but preliminary data suggest that it may occur. Liburdy and Eckert (*34*) presented initial data that indicate that the number of CD3 proteins available on the surface of Jurkat E6.1 cells is increased in the presence of an applied 60-Hz field.

The requirement that many receptors over the cell surface be simultaneously activated (known as "cooperativity") prevents random activation of individual receptors from triggering an erroneous cellular response. It would also demand that EMF activation occur only if the stimulating field is essentially the same at a number of receptor sites over the cell membrane; this requirement leads to the requirement of spatial coherence in the EMF if a cellular response is to be evoked. In this model, thermal noise fields, which are spatially incoherent, are incapable of causing any modification in cellular behavior.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch018

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Generally, one can determine the extent of cooperativity required by observing the threshold for biological response as a function of the concentration of the ligand in the vicinity of the cell. If n receptors must be stimulated to produce a response, then it is a simple matter to show (*35*) that the response varies with concentration c as

$$\text{response} \propto 1 - \frac{1}{1+(c/c_T)^n}$$

where c_T is the threshold concentration. As n increases, the threshold at c_T becomes rather large. Extending this thinking to EMF stimulation (as opposed to chemical stimulation) requires either a modification in the ligand–receptor binding or an assumption that the number of receptors available at the cell surface is proportional to the local field strength. In this case the simplest generalization of the equation for the response would be

$$\text{response} \propto 1 - \frac{1}{1+(E/E_T)^n}$$

where E is the field strength and E_T is the threshold field. Juutilainen (private communication) measured the increase in the frequency of early developmental abnormalities in incubating chick embryos as a function of the impressed field strength. These data are shown in Figure 4. A satisfactory fit to these data can be obtained for essentially any value of n larger than about 10. Although certainly not conclusive, these data are suggestive of biological cooperativity involving activation of a significant number of membrane integral protein receptors.

The foregoing discussion does not take into account the fact that multicellular organisms, including the chick embryos used in our experiments, produce endogenous electric fields as the result of the cellular activities involved in heart contraction, muscle contraction, and neuron function. Such spatially coherent, endogenous fields clearly complicate the issue of how applied EMFs are detected in intact organisms. However, the fact that the chick embryos did respond to weak, applied fields in basically the same way as did cultured cells suggests that the detection mechanism discriminates against these fields. We do not yet understand the basis for this discrimination.

The Frequency Spectrum of the Electromagnetic Noise. Comparisons of the chick embryo results with those obtained on L929 cells raise an interesting point concerning the spectral character of the noise that acts to inhibit the EMF-induced bioeffect. In the L929 cell culture experiments, imposition of a weak 60-Hz sinusoidal EMF effected an enhancement of ODC activity that the superposition of a 30–90-Hz bandwidth electromagnetic noise confusion field essentially eliminated. Qualitatively the results in developing chick embryos were similar: Exposure to a weak EMF caused an increase in the abnormal de-

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch018

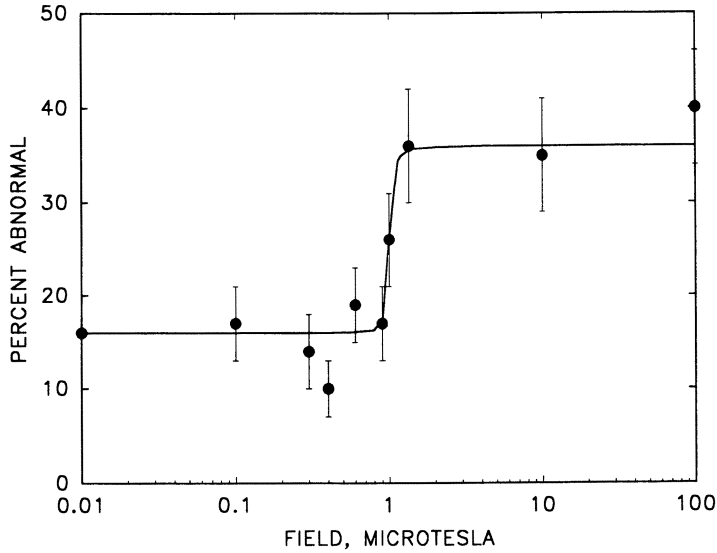

Figure 4. The abnormal development rate for chick embryos following a 48-h incubation period during which the embryos were exposed to a 50-Hz sinusoidal field plotted as a function of the amplitude of that 50-Hz field. A sharp threshold is observed at about 1.3 μT. (Data are from J. Juutilainen, private communication.)

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch018

velopment rate, which was removed with the superposition of an electromagnetic noise field. However, in this latter case the effect-producing field consisted of 500-μs pulses with a 100 pulse-per-second repetition rate. Most of the energy in such a pulsed field lies outside of the spectral range of the noise; in contrast, in the cell culture experiments the Fourier spectrum of the coherent signal (a sharp spike at 60 Hz) lies completely within the noise bandwidth. A straightforward interpretation of these results is that living cells respond to signals as a broadband detector, not distinguishing between different frequencies over a rather wide range. This interpretation is supported by the work of Juutilainen and Saali (*36*) and Juutilainen et al. (*37*), who showed that developmental abnormalities in chick embryos result from exposure to sinusoidal fields stronger than about 1 μT at frequencies ranging from 50 Hz to 100 kHz. To test the idea further, we examined the effect on ODC enhancement in L929 cells produced by a 60-Hz sinusoidal EMF by superimposing an equal-rms-amplitude electromagnetic noise field with a 90–250-Hz bandwidth. Even without any spectral overlap between the coherent and incoherent fields, the twofold increase caused by the coherent field was eliminated by the incoherent field. With both the sinusoidal and noise fields switched on, the exposed-to-control ODC activity ratio was $R_{ODC} = 1.1 \pm 0.1$. This result offers additional support for the idea of the cell as a broadband detector.

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Summary

It has been hypothesized that living cells discriminate against random thermally generated EMFs while responding to externally applied fields by recognizing the spatial coherence of the externally applied fields. Our proposal is that this behavior occurs via a cellular coincidence detection mechanism that responds only to fields that are spatially coherent over the cell membrane. The multicell detection mechanism developed by Weaver and Astumian (*6*) is also consistent with the requirement of spatial coherence in the stimulating field. In our proposed mechanism, biological cooperativity, in which a significant number of membrane-integral receptor proteins must be simultaneously activated to cause signal transduction to the cytoplasm, is the basis for the spatial coherence requirement. Because all physical detection systems are ultimately limited by noise constraints, one test of this hypothesis involves the superposition of spatially coherent temporally random noise on a PEMF that has been demonstrated to cause a bioeffect.

Experiments have been carried out using ODC activity in a variety of cell cultures and developmental abnormalities as well as ODC activity in incubating chick embryos as the endpoints. Initially, it was established that weak low-frequency EMFs caused increases in these entities following 4-h exposure for the cell cultures and during the first 48 h of incubation. Superimposing externally generated noise fields of roughly the same strength (although several orders of magnitude smaller than the endogenous thermal noise field) on the impressed fields essentially eliminated the field-induced effects. The results with the exogenous noise fields were statistically indistinguishable from the controls. Other investigators (*33, 38*) recently confirmed this bioeffect inhibition by electromagnetic noise in several other systems.

These findings support our proposal that it is their sensitivity only to spatially coherent EMFs that enables living cells to respond to weak exogenous fields while remaining unaffected by the relatively large (but spatially incoherent) endogenous noise fields that are always present. The idea that cells distinguish between exogenous and endogenous fields by recognizing the spatial incoherence of the endogenous fields offers a new consideration for explaining the signal-to-noise dilemma. Far from being "magically" exempt from signal-to-noise considerations, cellular detection mechanisms are subject to the same laws of nature as all physical systems. However, in applying these laws one must appreciate the sophistication of the biodetection mechanism and use the "correct" thermal noise (e.g., that which is coherent across the cell) in making estimates of the lower limits of detection. The masking provided by a spatially coherent confusion field could prove to be a basis for protecting humans against possible adverse health risks associated with environmental EMFs.

Acknowledgments

The authors recognize the assistance and inspiration provided by their collaborators: Ken Brown of the Biological Sciences Department, George Washington

University; Edward C. Elson, Walter Reed Army Institute of Research; and Michael Barber, Abiy Desta, Plamen Doinov, Joanne Farrell, David Krause, and Miguel Penafiel, all of The Catholic University of America. They also thank Sam Greenhouse of the George Washington University Biostatistics Center for his help with the statistical analysis of the data and Jukka Juutilainen for sharing his threshold data prior to its publication. Further, they acknowledge the Walter Reed Army Institute of Research for support of much of this work under Contract DAMD17–86–C–6260.

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RECEIVED for review April 25, 1994. ACCEPTED revised manuscript January 5, 1995.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch019

19

Electric and Magnetic Field Signal Transduction in the Membrane Na^+/K^+-adenosinetriphosphatase

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The Na^+/K^+-adenosinetriphosphatase (Na^+/K^+-ATPase), the ion-pump enzyme located in cell membranes, is a well-defined protein whose properties can be used to study the mechanism of electromagnetic (EM) field interaction with biological systems. Enzyme activity is normally inhibited by electric fields and stimulated by magnetic fields, but both fields cause large increases in enzyme activity when the initial (basal) activity of the enzyme is greatly reduced by aging, by lowering temperature, or by inhibitors. The opposing effects under optimal conditions suggest different charge movements in different parts of the enzyme. Electric fields act as if they increase ion binding at the enzyme surface, whereas magnetic fields appear to affect charges within the protein (e.g., charge transfer due to the ATPase reaction). The similar effects under suboptimal conditions suggest that the two different charge movements are coupled. The coordinated changes in charge density caused by both fields suggest ways in which EM fields affect membrane proteins in general. They also suggest ways in which the Na^+/K^+-ATPase may function as an ion pump.

INTEREST IN BIOLOGICAL EFFECTS of electromagnetic (EM) fields has been driven by epidemiological studies that report increased cancer risk associated with exposure. Changes in biosynthesis in cells following EM interaction sug-

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gest biochemical pathways that could be responsible for the association, but that area of research has not clarified the initial signal transduction step. We do not know how weak EM fields interact with molecules or how molecular reactions initiate complex cellular processes. To approach the problem of transduction of EM field exposures into molecular changes, that is, which charges are affected and how and when they move in the molecule, we have studied the effects of electric and magnetic fields on the enzyme activity of a well-defined membrane protein, Na^+/K^+-adenosinetriphosphatase (Na^+/K^+-ATPase).

Na^+/K^+-ATPase: The Ion-Pump Enzyme

The Na^+/K^+-ATPase is a ubiquitous transport enzyme whose structure and enzymatic properties (e.g., ion binding sites, ion activation, inhibitors, and temperature dependence) are well known. Its properties were first described in 1957 by Skou (*1*), who demonstrated activation by cations. The molecular structure and many properties of the enzyme have been reviewed in monographs by Lauger (*2*) on electrogenic ion pumps and by Tonomura (*3*) on energy-transducing ATPases.

The Na^+/K^+-ATPase is composed of two polypeptide chains (α and β) that extend through the membrane, and the catalytic activity (the 110-kDa α-chain) is directly affected by the ionic concentrations in contact with the two sides of the enzyme. The enzyme is activated when Na^+ ions bind to the inside surface and K^+ ions to the outside surface. Research on red blood cells established the stoichiometry of the pump (three Na^+ ions out and two K^+ ions in for each ATP split) and its reversibility (i.e., ions flowing down gradients exceeding normal reverse the action of the enzyme and cause the synthesis of ATP from precursors). When the purified molecule is introduced into an artificial bilayer (*4*), it forms a gated ion channel.

The enzyme is phosphorylated in the presence of Na^+ ions and dephosphorylated in the presence of K^+ ions. The two conformational states are E_1 when Na^+ ions (and ATP) bind from the inside, and E_2 when K^+ ions bind from the outside. The ion binding sites are not fully accessible to exchange with other ions in the medium in the two conformational states, and this property is referred to as occlusion. Also, some transitions between conformational states are voltage-sensitive (*5*). These studies have led to the formulation of cyclic schemes that alternate the two conformational states to link ion transport to Na^+/K^+-ATPase function. Post (*6*) has described the enzyme as an oscillator and has suggested that it can be forced between conformational states by imposing oscillating mechanical or electrical stimuli. Post's ideas about the enzyme mechanism were stimulated by the work of Tsong and co-workers (*7–11*), who described a ouabain-sensitive accumulation of rubidium and secretion of sodium from red blood cells stimulated by alternating currents. The Tsong group suggests that the electric field interacts directly with the enzyme, driving it through conformational states that cause ion movements. This interaction has been referred to as the electroconformational coupling model.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch019

In Electromagnetic Fields; Blank, M.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1995.

Effects of AC Electric Fields on Na^+/K^+-ATPase Activity

We have characterized (*12–17*) the effects of alternating current (AC) electric fields on Na^+/K^+-ATPase activity. AC normally decreases the rate of ATP splitting in correlation with the level of ion activation, but it can increase activity when the basal enzyme activity is greatly reduced by low temperature, by inhibitors (ouabain), or by changing the concentrations of activating cations. Figure 1 shows how the effect of an electric field varies with enzyme activity. Above an enzyme activity of 0.05–0.1 μmol phosphate·mg protein^{-1}·min^{-1}, the ratio of enzyme activity with AC to enzyme activity without AC (*E/A*) is <1, whereas below this value, *E/A* tends to be >1. The low values of enzyme activity were obtained by using different concentrations of ouabain, and similar effects were obtained by using lowered temperatures.

Because enzyme activity varies with ion concentration, by increasing at low activity and decreasing (or reaching a plateau) at high ion concentration, both inhibition and stimulation by AC electric fields suggest a field-dependent increased binding of activating cations (*12*). An increase in binding has opposite effects in different ranges of enzyme activity (*1*). At optimal activity, increased ion concentration lowers activity. When basal activity is lowered by inhibitors or low temperature, increased ion concentration increases enzyme activity. In studies in which enzyme activity varies with Na/K ratio, the effect of AC correlates directly with basal enzyme activity.

Independent evidence for an inhibitory effect of electric fields on Na^+/K^+-ATPase activity was published by Green et al. (*18*), who reported an almost twofold increase in enzyme content, measured by ouabain binding, as a result of stimulation at 10 Hz for several days. Because enzyme is synthesized in re-

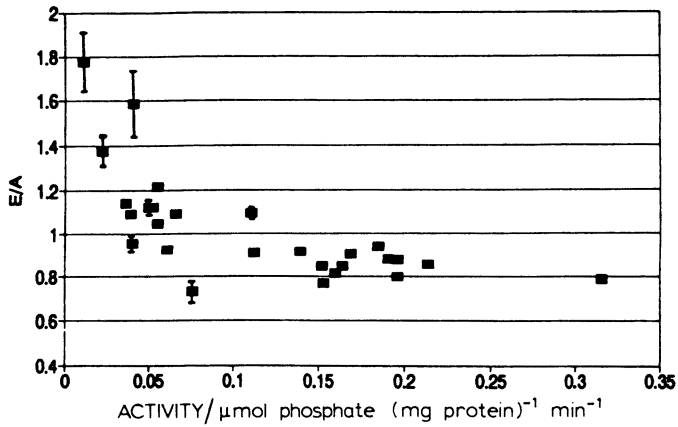

Figure 1. The ratio (E/A) of enzyme activity with AC (alternating current) to enzyme activity without AC vs. enzyme activity in units of micromoles phosphate per milligram protein per minute. (Reproduced with permission from reference 14. Copyright 1990 Elsevier.)

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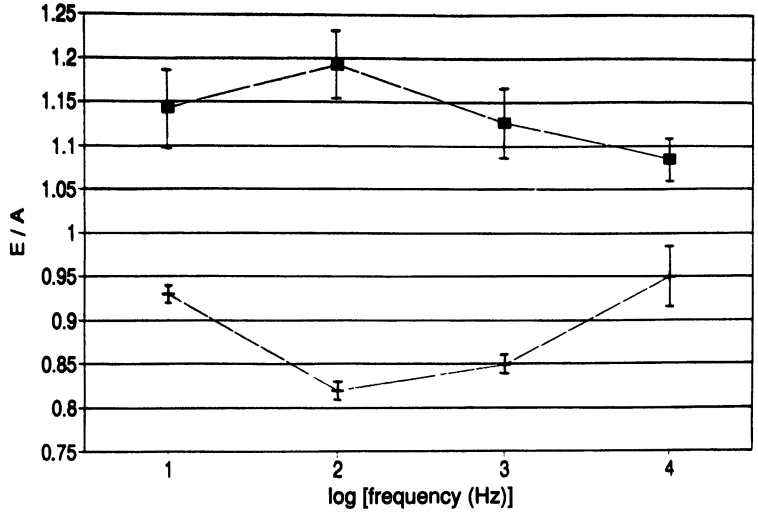

Figure 2. E/A *vs. logarithm of the frequency (hertz). Activation (■) and inhibition (+) are shown for the same enzyme preparation, and standard error bars were calculated on the basis of 10 replications per point for the activation and 13 per point for the inhibition. (Reproduced with permission from reference 15. Copyright 1992 Wiley-Liss.)*

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sponse to the need for increased ion pumping, enzyme function was probably inhibited by electrical stimulation. The investigators did not measure enzyme function directly.

The similar frequency dependence of inhibition and stimulation due to electric fields, with maximal effects at about 100 Hz (*14, 15*), are shown in Figures 2 and 3. The results suggest that both effects arise from the same mechanism. An increase in the binding of activating ions due to the field is quite likely, because the frequency dependence is similar to calculated ion concentration increases at membrane surfaces (*19, 20*). Increases in concentration would lead to increased binding, and the ion activation curves of the enzyme show that binding can lead to both increases and decreases in activity.

The ion activation model also accounts for the observed difference in optimal frequency for cation influx (10^3 Hz) and efflux (10^6 Hz) in experiments with red blood cells (*11*) (*see* Figure 4). One would expect K^+ ions bound to the outside membrane surface to be transported inward under low-frequency conditions. To affect Na^+ efflux from an intact cell, the AC signal must penetrate to the binding site on the inside surface of the membrane, an effect that is significant only at much higher AC frequencies.

Mechanism of Electric Field Interaction. The effects of electric fields can be accounted for through increases in ion activation at the surface of the enzyme. Theoretically, an alternating square wave leads to an increase in ion

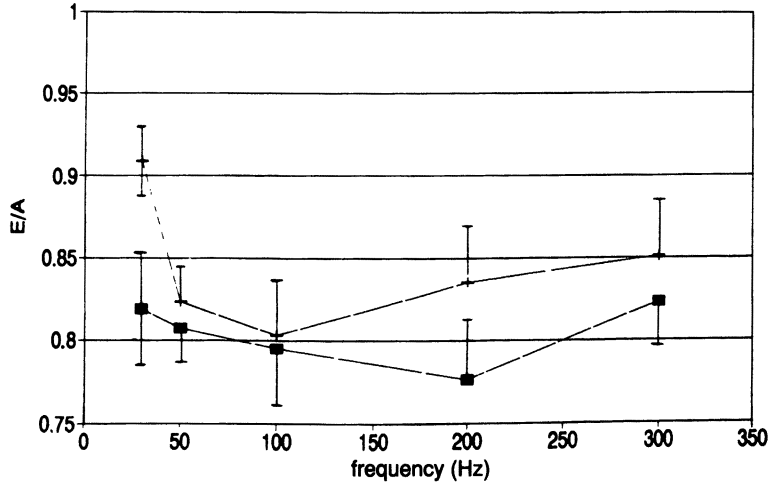

Figure 3. E/A *(for inhibition) vs. the frequency (in hertz) over an expanded scale in the region of the minimum. Data are for two enzyme preparations with standard error bars calculated for five to eight replications per point. (Reproduced with permission from reference 15. Copyright 1992 Wiley-Liss.)*

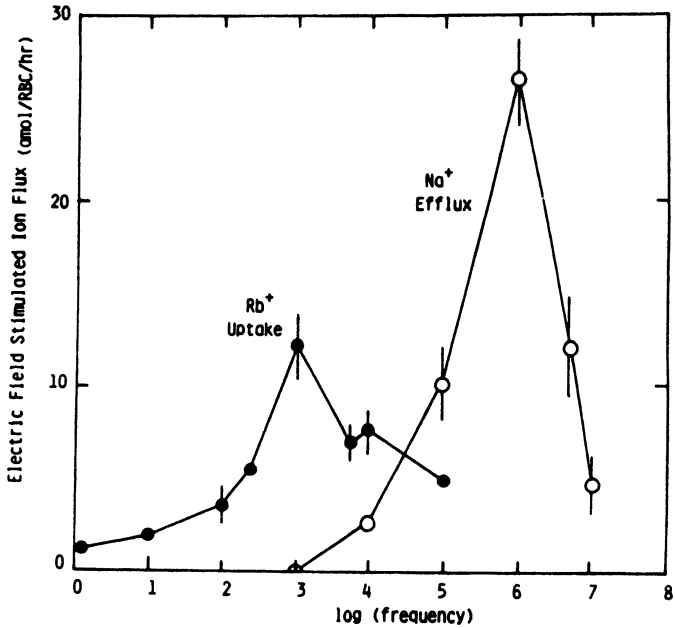

*Figure 4. Data of Tsong et al. (*11*) showing electric-field-stimulated ion fluxes (in attomoles per erythrocyte per hour) as a function of the log frequency. RBC is red blood cells. (Reproduced in modified form with permission from reference 11. Copyright 1989 Elsevier.)*

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binding (*15*), but the expected changes are far too small. Perhaps the field accomplishes the same changes in charge distribution inside the ATPase as ion binding causes on the surface. In any case, the proposed mechanism, based on increases in ion activation at the surface of the enzyme, explains qualitatively the observed dependences on electric field and on basal level of Na^+/K^+-ATPase activity through increases in cation binding at activation sites. The ion activation hypothesis can also explain qualitatively a number of other important observations that have not been dealt with by other hypotheses (*10, 11*). These include both inhibition and stimulation, the frequency dependence of both inhibition and stimulation, and the difference in optimal frequency for cation influx and efflux in red blood cells.

In summary, the proposed mechanism based on ion binding at activation sites accounts for the observed dependences on the electric field and on the basal level of Na^+/K^+-ATPase activity. The ideas are also in line with studies on the cells of slime mold (*21*), in which electric fields cause changes in the surface charge as measured by changes in the partitioning of cells between hydrophilic and hydrophobic solvents. The mechanism proposed by Tsong and co-workers (*10, 11*) does not account for the dependences of the responses in electric fields on enzyme activation and frequency.

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Effects of AC Magnetic Fields on Na^+/K^+-ATPase Activity

The effects of applied magnetic fields on enzyme activity have not been as well studied as the effects of electric fields, and reports about the effects of magnetic fields on the Na^+/K^+-ATPase have been conflicting. Batkin et al. (*22*) reported decreases in enzyme activity in mice (kidney and diaphragm) exposed to 55–60 G for 11 days, whereas DeLoecker et al. (*23*) suggested that pulsed EM fields can stimulate the ion pump in rat skin. Collis and Segal (*24*) reported that pulsed fields up to 8-G peak affect sodium fluxes in rabbit colon epithelium and that the effect depends upon the orientation of the field relative to the tissue. Interpretation of results from these studies is difficult, because the systems are complex whole tissues with intact biochemical pathways and the pulsed signals have many frequencies.

An effect of magnetic fields on Na^+/K^+-ATPase activity was suggested by comparing the different effects obtained with the same magnitudes of applied and induced currents. The threshold of inhibition at 100 Hz by applied fields was determined by extrapolation to be about 5×10^{-6} V/cm. Induced AC currents, generated with an electromagnet at 60 Hz, caused a decrease in enzyme activity similar to the electrode systems, but at apparently larger electric fields. The quantitative differences suggest that the stray magnetic fields (about 10–100 mG) in the induced current apparatus change the activity of the Na^+/K^+-ATPase in the opposite direction. We have verified a positive effect of magnetic fields on the rate, as predicted from these results.

We measured the effect of low-frequency magnetic fields on Na^+/K^+-ATPase activity, by using a new exposure system (Electric Research and Man-

agement, Pittsburgh, PA). With the enzyme under basal conditions, magnetic fields increase Na^+/K^+-ATPase activity. In the frequency range 20–70 Hz and for amplitudes of 0.02–2 G, Na^+/K^+-ATPase activity increases by about 5–10%, with optima around 60 Hz. Under suboptimal conditions (i.e., aging preparations or adding inhibitor ouabain), the effects of magnetic fields parallel the previously reported effects of electric fields. A large increase in the effect (approaching 100%) occurs below an enzyme activity of 0.05–0.1 μmol phosphate·mg $protein^{-1}\cdot min^{-1}$.

Although enzyme activity is normally inhibited by electric fields and stimulated by magnetic fields, the frequency dependence is similar for the 5-mV/m (electric) and 50-mG (magnetic) fields. The optimal frequency for enzyme inhibition by electric fields is 100–200 Hz, and the optimal frequency for increased enzyme activity in magnetic fields appears to be around 60 Hz (although the full range has yet to be determined). The overlap of the two ranges suggests action of the individual fields upon a common rate-limiting process in a closely coupled system.

The opposite effects of magnetic and electric fields on enzyme activity are in line with earlier studies on the cells of slime mold (*21*). The explanation given there for the effect of electric fields, changes in the surface charge, is similar to the Na^+/K^+-ATPase results, but the explanation for magnetic fields, a decrease in "hydrophobicity" of the surface, does not apply in terms of the Na^+/K^+-ATPase.

Mechanism of AC Magnetic Field Interactions. The data suggest that electric fields affect the enzyme in the same way as an increase in ion binding at the enzyme surface, and magnetic fields appear to increase charge flow within the protein, thereby coordinating binding sites at the two surfaces of the enzyme. Magnetic fields cannot act through the induced electric fields because the magnitudes of the induced currents are much too small and the effects are in the opposite direction.

A critical role for electrons in Na^+/K^+-ATPase function is suggested by the relation between the effectiveness of cationic inhibitors and their redox potentials (*25*). Inhibition of Na^+/K^+-ATPase activity by cations can be described in terms of competition at known sites on the enzyme. Specific inhibitors bind at known sites, and inhibition can be partially overcome by increases in the normally present cations (e.g., Na^+, K^+, and Mg^{2+}). Nonspecific inhibitors act at unknown sites, and inhibition is independent of the normal cations. Even though nonspecific inhibitors do not bind at the activation sites or on ATP, they are the most effective cationic inhibitors (e.g., Hg^{2+}, Ag^+, and Cu^{2+}), and their effectiveness correlates with the redox potential of the ions (*25*). The most effective of these nonspecific inhibitors have the lowest redox potentials, and this correlation indicates a strong ability to absorb electrons and make them less available for the ATPase reaction. The effective concentrations of the specific inhibitors do not correlate with the redox potential.

Another indication that AC magnetic fields affect the rates of reactions comes from correlations between reaction rates and optimal frequencies. The 60-

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Hz optimum for the Na^+/K^+-ATPase at 38 °C is very close to the measured reaction rate. A relation between optimal frequency and rate may account for observations in HL-60 cells, in which optimal enhancement of transcription occurs at 45 Hz, which is in the range of RNA synthesis (bases per second) rates. Also, the relation between synthesis of particular proteins in muscle and the frequency of electric stimulation, discussed elsewhere in this book, may be based on the same molecular mechanisms. These results suggest that magnetic fields could directly affect charge transfer in reactions.

Role of Surface Charge in Active Ion Transport

Much is known about active ion transport, but the mechanism is still not understood. The splitting of ATP by the ATPase provides enough energy for the transport of Na^+ and K^+ ions against their concentration gradients, but how the energy from breaking a chemical bond is transduced into a directed ion flux is not known. Perhaps the most perplexing aspect of this problem is how the widely separated sites for catalysis and ion binding are coordinated to achieve the fluxes.

Two essential molecular processes are generally believed to be involved in the active transport mechanism: conformational changes and charge shifts within the protein during enzyme activity. These processes are linked through the surface charge. The effects of changes in protein surface charge on molecular conformation can be illustrated in terms of channels, which function as molecular switches in biological membranes. The opening and closing of these cylindrical assemblies of proteins in response to electric and chemical stimuli can be described as aggregation–disaggregation reactions, in which disaggregation varies with the molecular charge. This model can describe a number of protein reactions, starting with the relatively simple disaggregation of hemoglobin tetramers with pH, to the function of voltage-gated channels during excitation and the cooling and heating observed in excitable membranes when channels open and close (*26*).

The effects of electric and magnetic fields on the ATPase have provided some insights into mechanism. Magnetic fields and electric fields appear to act at different sites on the ATPase, but both interactions affect surface charge density. Electric fields act as if they increase cation binding at the enzyme surface and lower the net negative charge. Charge shifts due to magnetic fields could lower the negative charge on one surface of the enzyme and raise it on the other. The similarity of the frequency dependence for the inhibitory electric and stimulatory magnetic fields suggests that the rate-limiting process in the enzyme links changes in ion binding to charge transfer across the enzyme.

The changes in charge due to the fields are superimposed on the ATPase reaction, and this superimposition changes the charge density at the enzymatic site. Changes in charge density due to a shift of charge between enzyme surfaces should lead to changes in ion binding at both surfaces and coordinate the two sites. Because the outer enzyme site binds K^+ ions preferentially and the inner

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site binds Na^+ ions preferentially, changes in counterion binding could create local concentration gradients of specific ions.

The interplay of changes in charge density and channel opening provides an explanation for the occlusion of ion-binding sites during part of the ATPase cycle. Conformational changes cause an ion-binding surface of the enzyme to alternately contact the aqueous compartments on the two sides of the membrane or be closed off to ion exchange. The channel model already cited suggests how a change of exposure to the different solutions on the two surfaces or a complete closure could occur. For a total of 60 charges per 100 nm^2, divided between inner and outer enzyme surfaces at 25 and 35 charges, respectively, the probability determined from the energetics of protein–aqueous interfaces is that the channel face that has the higher charge density would be open and the other face closed. A shift of 10 charges per 100 nm^2, at constant total charge, would reverse the average probabilities and cause a flipping of the enzyme from "top open" configuration to "bottom open" across the membrane. To achieve complete closure of the channel, a reaction leading to absorption of 10 charges per 100 nm^2 would be enough to close the open face of the channel. Decreases in charge density due to cation binding would decrease the number of charges that would have to be absorbed.

In summary, signaling between ion binding sites on opposite sides of the membrane and coordination of ATP splitting with directed ion transport can apparently be accounted for through the effects of changes in surface charge on the channel properties of the Na^+/K^+-ATPase molecule.

Na^+/K^+-ATPase as a Molecular Model for EM Field Effects

From studies of the effects of electric and magnetic fields on Na^+/K^+-ATPase activity, we have been able to learn about changes in molecular properties that may be characteristic of the ways in which membrane proteins are affected by EM fields. Magnetic fields and electric fields appear to act at different sites on the ATPase, but both change the surface charge density. Changes in charge density affect the electric gradient across a channel, the number of ions bound or released, and channel opening and closing, all of which affect ion transport across membranes. The changes we have described for a membrane protein may occur in other polyelectrolytes as well as in nonmembrane systems.

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RECEIVED for review February 28, 1994. ACCEPTED revised manuscript January 12, 1995.

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The Role of Cell and Tissue Calcium in Transducing the Effects of Exposure to Low-Frequency Electromagnetic Fields

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Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch020

A review of recent developments in our understanding of calcium activity within cells is discussed in the context of proposed requirements for cellular detection of low-level, extremely low frequency (ELF) electric fields within tissues. These requirements include identification of a nonlinear or rectification process, the existence of a memory process, temporal characteristics that could explain the reported frequency dependence of ELF field interactions, and a spatial integration process. Calcium channels exist in both excitable and nonexcitable cells and serve as a highly nonlinear, rectifying process, with distinct temporal characteristics. In addition, these voltage-dependent calcium channels result in calcium oscillators that are highly susceptible to weak environmental perturbations. Finally, spatially large gap-junction-connected cell ensembles commonly occur in tissues, and calcium serves both as a mediator of these interconnections as well as an important carrier of intercellular information through these channels. On the basis of the critical role calcium plays in these various physiological phenomena, the control of calcium conductivity at the cell plasma membrane is proposed as a plausible process through which ELF fields may interact with cells.

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In Electromagnetic Fields; Blank, M.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1995.

WHETHER EXTREMELY LOW FREQUENCY (ELF) electromagnetic fields are capable of directly influencing calcium activity in exposed cells has been a question inextricably linked to ELF field studies for more than 20 years. But, like most studies to date on ELF field interactions, investigations of induced calcium alterations have been phenomenological, rather than mechanistic, in nature, and have left the question of the specific role of calcium in the transduction process unresolved. The earliest studies (*1–3*) associating calcium with ELF-field-induced effects concentrated on the measurement of calcium efflux from ex vivo tissue samples. These studies have had a major impact on subsequent ELF field studies as they were the first experiments to demonstrate the possible existence of narrow frequency and amplitude ranges in which ELF fields may be capable of influencing living tissue. Yet, the difficulty in replicating these experiments and the inability to identify the origin of the calcium leaving the tissue have made interpretation of these reported calcium efflux data difficult. Interest in calcium flux experiments has been maintained in both excitable and nonexcitable cells, but to a large degree the emphasis has shifted to the study of calcium influx into cells during field exposure (*4–6*). This shift in focus is certainly an important advance, as ion influx techniques are better established and have played a critical role in understanding both nerve (*7*) and muscle cell (*8*) activity.

The emphasis on calcium fluxes, however, confounds interpretation of the physiological relevance of the reported ELF-field-induced responses, because the inward flow of calcium and the outward pumping of calcium are closely balanced to maintain free intracellular calcium levels near 10^{-7} M. Calcium signaling, in this view, arises through changes in the absolute calcium levels within the cell. During calcium level changes, diffusion-controlled calcium binding alters, either directly or through calmodulin-like proteins, the activity of the adenosinetriphosphatases (ATPases), kinases, and phosphatases, as well as the adenosine 3′,5′-cyclic monophosphate (cAMP) cyclases and esterases. Through these multiple second-messenger pathways, calcium produces the myriad responses on cell activity, including secondary alterations in the calcium fluxes. Although investigations have led (*9*) to the suggestion of calcium flux, per se, being important in certain cells because of differences in the binding kinetics of calmodulin, parvalbumin, and calbindin, changes in calcium flux clearly cannot indicate changes in either bound or free intracellular calcium levels. Slow variations in calcium fluxes may simply reflect a net downstream result of a complex signal cascade with little influence on cell activity (*10*). The demonstration of a more immediate effect on the calcium-signaling mechanism requires direct measurement of calcium levels, and ELF research has moved (*11–13*) in this direction. Preliminary reports on direct calcium-dependent fluorescent measurements appear to confirm a change in calcium levels following field exposure. However, even in these relatively rapid assays, changes in calcium levels require from several minutes to tens of minutes to become sufficiently large to reliably detect.

The reported delays in the response of calcium to ELF field exposure raise the question of whether the observed changes in calcium activity are merely

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epiphenomena of cell biochemical signaling processes, or modulation of calcium movement across the plasma membrane is an immediate consequence of ELF field exposure. Although finding the answer to this question will have little impact on studies directed toward identifying changes in the genomic and phenotypic expression of cells following field exposure, this issue does go directly to the question of the biophysical mechanism of transduction of ELF electromagnetic fields with living cells and therefore the conditions under which robust ELF field effects might be expected. In this chapter, the fundamental issues concerning ELF field detection by cells are outlined, and current advances in several areas of biology and physiology that are relevant to our understanding of signal processing by cells are reviewed. These include recent developments in electrophysiological ion channel characterizations in nonexcitable cells, gap junction physiology, network theory, and the measurement of the temporal and spatial dynamics of free calcium within cells. The conclusion reached is that although little concrete evidence now shows that transmembrane movement of calcium plays a direct role in the transduction of ELF fields to a biochemical signal, growing circumstantial evidence implicates calcium mobilization as one of the few plausible processes that could account for the remarkable ability of cells to respond to these low-level, alternating electric fields. Elucidation and characterization of the initial steps leading to calcium mobilization by ELF field exposure may therefore lead to a more complete understanding of the mechanism of ELF electromagnetic field sensitivity in living tissue.

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ELF Electromagnetic Field Detection by Cells

Environmental ELF field exposures are, by definition, those with frequency content below 3000 Hz. In amplitude, flux densities rarely exceed 10 μT, and electric field intensities are rarely greater than 1000 V/m. Despite the existence of only these two components in an electromagnetic field, exposure of biological tissues to ELF electromagnetic fields actually results in three distinct exposures. The first exposure is the magnetic field component. Biological tissue has a magnetic permeability close to 1, so magnetic flux readily passes through cells and tissues. Although the possibility exists for a direct interaction of the magnetic field through ferromagnetic particles in the cells (*14*), through paramagnetic processes such as free-radical lifetime alterations (*15*), or through "ion cyclotron resonance-like" effects (*16*), to date there is no uniform agreement that magnetically mediated biological effects occur at milligauss flux densities.

The second exposure is the electric field component. Unlike magnetic fields, the low-frequency environmental electric fields are effectively shielded out of any organism surrounded by air because of the high conductivity of living tissue. The coupling of external electric fields into tissue is no higher than 10^{-7} in the ELF range, and this coupling level prevents the induction of any electric field greater than about 0.1 mV/m in the body by even the largest environmental exposures.

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The third aspect of EMF exposure arises from the time-varying magnetic flux inducing a secondary electric field within the body tissues. The intensity of magnetically induced electric fields is proportional to the size of the tissue, the magnetic flux density, and the field frequency, so average induced electric fields as large as 1 mV/m can be induced in a human exposed to a 60-Hz, 10-μT flux density. When transitions in conductivity are considered, peak induced-field intensities can easily exceed 10 mV/m. This analysis suggests the most plausible mechanisms of interaction of environmental ELF electromagnetic fields with living tissue will be dependent on the magnetically induced electric field within the tissue.

Just as electric fields are excluded from the interior of the body, the magnetically induced electric fields within the body will be excluded from the interior of individual cells by the low conductivity of the plasma membrane. At the frequencies of interest here, the plasma membrane appears as a large impedance (membranes typically have a specific resistance of 10^3 ohm·cm^2 and a capacitance of approximately 1 μF/cm^2). As a result, any extracellular electric current will diverge around the cell and induce a surface charge on the cell membrane. ELF electric fields within tissues are therefore restricted to direct interactions with cells and involve the forces developed on the free charges or dipolar structures on or within the cell plasma membrane (Figure 1) (*17*). One active area of research is directed toward determining whether the interaction of the extracellular fields with surface charges or dipoles could be responsible for the observed cellular responses. Mechanisms of interest currently include dipole orientation (*18*), directed diffusion (*19*), and electric polarization forces (*20*). In addition, the charge induced at the extracellular surface of the plasma membrane will result in a perturbation of the transmembrane potential proportional to the extracellular field intensity and the size of the cell, and this perturbation may be the principal source of any electric field interaction. For the simplified conditions of a spherical cell exposed to a sinusoidal ELF electric field, the maximum perturbation of the intrinsic diffusion potential (ΔV_{max}) across the membrane will be

$$\Delta V_{max} = (3/2)ER \sin \omega t$$

where E is the average extracellular field intensity, R is the radius of the cell, and ω is the radian frequency of the stimulus. A typical cell has a radius on the order of 10 μm, so in the presence of a 10-mV/m extracellular field the induced perturbation of the membrane potential of the cell will be on the order of 100 nV. This perturbation is approximately 6 orders of magnitude smaller than the normal cell membrane potential.

Although this typical amplitude of an ELF-field-induced membrane potential perturbation is remarkably small, such perturbations are still above the thermal membrane noise voltage (*21*). This fact ensures that, given sufficient time to integrate this applied transmembrane "signal", it is thermodynamically possible for a cell to "detect" an extracellular ELF electric field through a transmembrane signaling process. Such a detection process would, of course, require

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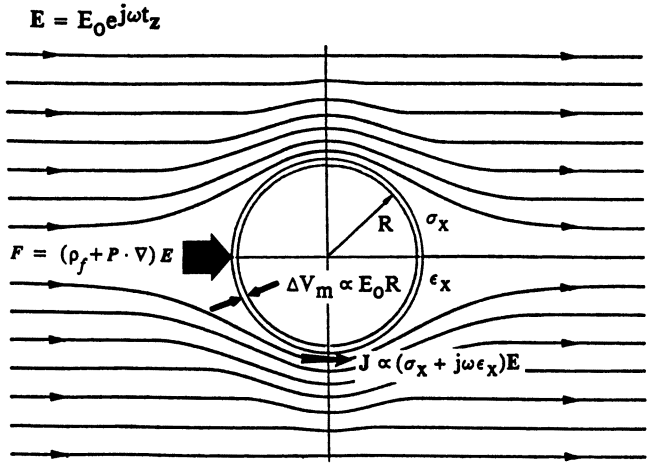

Figure 1. Schematic representation of the sites of interaction of extremely low frequency (ELF) electric fields (E) with a cell; these include both extracellular events as well as perturbation of the transmembrane potential (ΔV_m). Abbreviations: E_0, peak applied electric field intensity; F, *force;* ρ_f, *free charge density;* P, *polarization;* R, *radius of cell;* σ_x, *outside conductivity;* ε_x, *outside dielectric permittivity;* J, *current density. (Reproduced with permission from reference 17. Copyright 1992 Wiley-Liss.)*

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intrinsic memory so any incremental effect could be integrated over time. In addition, because magnetically induced electric fields are always symmetric in polarity, it is necessary to identify a voltage-dependent nonlinear aspect of the transduction process that could serve to rectify the membrane signal to permit a net accumulation of the signal with time. It would also be beneficial if an ELF transduction process could be identified that had temporal dynamics that could account for the distinct frequency dependencies reported in the ELF literature. Finally, a transduction process that incorporates spatial integration (i.e., the ability to sum the signal over a spatial distance greater than a cell diameter) will result in a larger induced signal and, hence, a greater chance of being detected by the cell population. In this context of transduction process requirements, recent developments in the biological and physiological aspects of calcium metabolism are considered.

Intracellular Calcium Dynamics

Calcium signaling processes rely on sensing changes in absolute levels of intracellular free calcium. However, the recent improvements in our ability to monitor intracellular calcium levels within individual cells demonstrate that cell responses are not necessarily a result of simple rises in free intracellular calcium concentration ($[Ca^{2+}]_i$) but may also depend on complex patterns of $[Ca^{2+}]_i$ ele-

vation in space and time. Many of the revisions in our understanding of calcium dynamics have arisen through studies of secretory cells (*22*), cells in which volume regulation is typically controlled by calcium-dependent chloride conductances and secretion is calcium-dependent exocytosis (*23*). In these cells, calcium elevations arise in response to hormonal stimulation but in a variety of temporal patterns. At supraphysiological agonist concentrations a sustained elevation in $[Ca^{2+}]_i$ is observed, whereas at physiological concentrations, oscillations or spiking activity in $[Ca^{2+}]_i$ is typically recorded. Importantly, different agonists and different agonist concentrations provoke complex and distinct oscillations or spiking patterns that may serve to characterize the stimulus.

These dynamic calcium responses have led to the suggestion that calcium oscillations may provide a digital encoding of the stimulus type and strength through frequency modulation (*24*). This hypothesis is supported by the observation that, although calcium spike frequency can be readily altered, peak calcium levels are much less variable. In cultured hepatocytes, a 10-fold variation in frequency (approximately 1 per 20 s to 1 per 200 s) is accompanied by a less than twofold change in amplitude (*25*). The actual amplitudes of these calcium spikes have been somewhat more difficult to attain. Aequorin, a calcium-dependent luminescent photoprotein, is available as a reliable indicator of calcium up to 500 μM. Although somewhat difficult to use, it can be incorporated into cells to demonstrate that calcium spikes can reach peak levels of over 10 μM (Figure 2). Conversely, fluorescent dyes are easier to utilize and provide a

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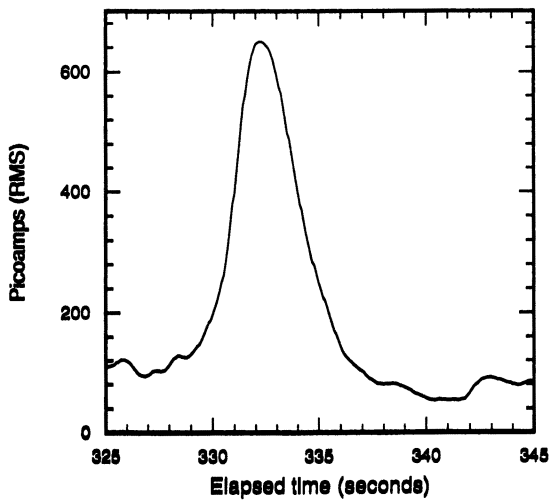

Figure 2. Calcium transient optically recorded from a single nonexcitable cell (rat osteosarcoma ROS 17/2.8) within an ensemble of aequorin-loaded cells. Typical transient time course is 3–5 s with the peak calcium levels exceeding 10 μM (T = 37 °C, pH = 7. 5). Baseline intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) of the cell ensemble is ~120 × 10^{-9} M. (Ordinant is root mean square photomultiplier tube current.)

more intense optical signal and so have been ideal for studying the temporal patterns in calcium spiking. However, fluorescent dyes strongly buffer calcium and therefore do not report $[Ca^{2+}]_i$ accurately above 1 μM.

An equally important observation regarding calcium dynamics is that the calcium spikes do not represent a uniform elevation in calcium throughout the cell but arise as a reaction–diffusion wave traversing the breadth of the cell. In most cells that have been examined, the velocity of the induced calcium waves averages 15–30 μm/s, a velocity that permits the wave to traverse the typical cell within several seconds (*26*). These observations on the wave nature of the calcium transients have motivated the development of several mechanistic models that attempt to describe the regulation of calcium-transient behavior. In the calcium-induced calcium release (CICR) model, free calcium triggers the generation of inositol trisphosphate (IP_3), which is capable of releasing additional calcium from intracellular stores to propagate the calcium wave. In fact, two variants of this model exist, one in which calcium is the only diffusing reactant and one (the IP_3–calcium coupling model) in which both calcium and IP_3 can diffuse (*27a*). These two models are not strongly dependent on plasma membrane calcium fluxes. However, investigations have confirmed (*27b*) that in some cell types, when the intracellular calcium pool is depleted, or when the IP_3 calcium release mechanism is blocked (e.g., with heparin), calcium waves may still be triggered. These observations have resulted in the development of an alternative model of calcium control, calcium-induced calcium entry (CICE), wherein an influx of calcium opens adjacent calcium channels and thus ensures wave propagation. This model requires an extracellular source of calcium, and although calcium spiking has been demonstrated in the absence of extracellular calcium, demonstration of a sustained response always requires an extracellular calcium source. The CICE model of calcium signaling would be strongly modulated by alterations in plasma membrane calcium permeabilities.

The potential importance of calcium spiking with respect to ELF-field-induced effects does not arise so much through functions existing primarily in excitable or specialized secretory cells but from far more ubiquitous roles. One of these roles involves cellular division. The role of calcium as a second messenger during mitosis has previously been proposed (*28*), but recent work has demonstrated (*29*) a causal relationship between the $[Ca^{2+}]_i$ transients that occur during the cell cycle and progression through the cell cycle. By blocking mitogen-induced $[Ca^{2+}]_i$ elevation, investigators have shown cells to be reversibly blocked at the G_1–S transition, and this behavior demonstrates the calcium transient to be a necessary, although not sufficient, condition for cell cycle progression. These data will undoubtedly stimulate renewed interest in reports (*30*) that imply a critical permissive role for calcium at other points in the cell cycle, for example at the G_2–M checkpoint (signaling completion of DNA repair) and on entry to anaphase (signaling complete chromosome alignment). The critical timing associated with the progression of the cell cycle and the importance of calcium transients in permitting progress through the cycle suggests that even modest alterations in calcium permeability may sufficiently perturb spike timing to alter the normal cell cycle progression. The growing number of reports (*31a,*

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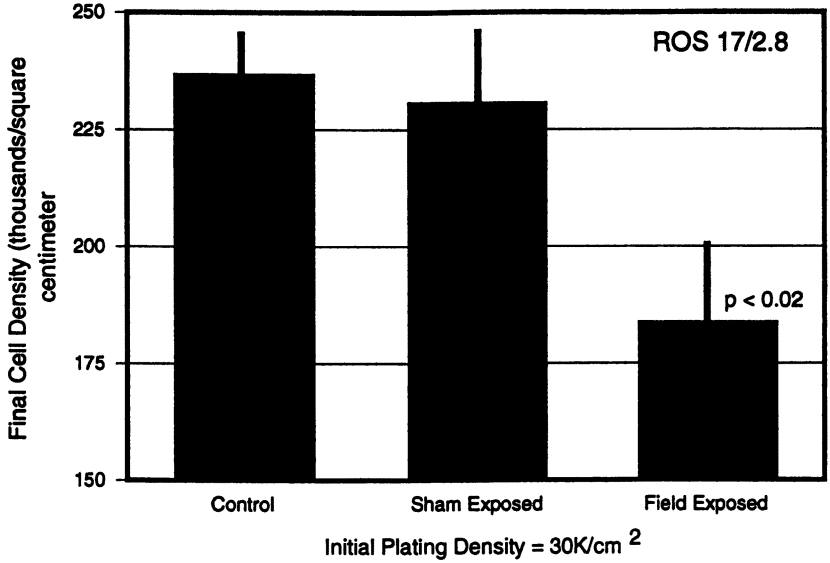

Figure 3. Inhibitory effect of 72-h ELF (30-Hz) field exposure (6-μV/cm rms) on the normal proliferation of ROS 17/2.8 osteosarcoma cells in monolayer culture. (Adapted from reference 31.)

31b, 31c) associating ELF exposure to modifications of growth rate in a wide variety of cells (cf. Figure 3) lend credence to the possibility that even a small direct influence of ELF fields on calcium activity could lead to significant long-term effects.

Calcium Channels in Nonexcitable Cells

The ability of ELF fields to directly affect calcium permeability at the plasma membrane presupposes the existence of voltage-dependent calcium channels in the cell membrane. Yet, although the majority of reports of ELF-field-induced effects have been on nonexcitable cells, electrophysiological demonstrations of calcium channels have been largely restricted to nerve and muscle cells. The development of patch-recording techniques (*32*) significantly advanced our ability to investigate ion channels in smaller, nonexcitable cells. Correspondingly, the development of perforated patch-recording techniques (*33*) has dramatically improved the ability to record the extremely small calcium currents that exist in small nonexcitable cells without the problem of loss of the intracellular regulatory components by dialysis. Investigation of nonexcitable cells has shown (*34, 35*) that, unlike nerve and muscle cells in which the transmembrane calcium conductances are dominated by high-voltage activating L- and N-type calcium channels, nonexcitable cells commonly demonstrate T-type channels. T-type channels are low-voltage activating channels, or channels that will activate for

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small depolarizations near the resting potential of the cell (Figure 4). These channels, therefore, provide an obvious interaction site for ELF-field-induced membrane potential perturbations.

T-type calcium channels have two other distinct qualities relevant to ELF field interactions. First, like all calcium channels, these are rectifying channels; in fact, they operate as a negative conductance within the plasma membrane. Decreasing the membrane potential acts to increase the calcium current, which rapidly results in a large change in the local calcium concentration within the cell. One to two milliseconds of a 0.5-pA calcium current flow through a single calcium channel will result in the intracellular calcium concentration rising to 10 μM within a 0.1-μm radius of the channel and then diffusing outward as a calcium wave. A membrane potential change of opposite polarity or a depolarization of the opposite side of the cell does not produce an opposing physical response. The second important aspect of these channels is that they open transiently. Following a 50–100-ms open time, the channels normally progress into an inactivated state. This intrinsic delay results in a cycle time on the order of 10–20 Hz, a range commonly associated with optimal ELF field responses.

The importance of identifying calcium channels in nonexcitable cells is accentuated by the work of Morris and Lecar (*36*), who have demonstrated that a membrane with only voltage-dependent calcium and potassium ion channels forms a system with the ability to undergo relatively complex oscillations. Be-

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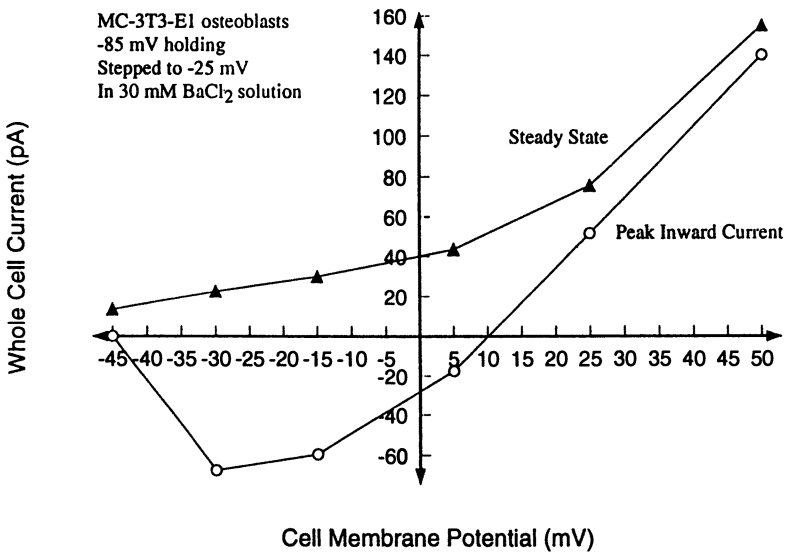

Figure 4. Current–voltage curves for whole-cell currents recorded from an osteoblast-like cell (MC–3T3E1) showing transient inward current associated with T-type calcium channel activity (O) and steady-state currents (▲). Step depolarizations are from a holding potential of –85 mV, using permeabilized patch-recording technique with 30 mM barium as the divalent current-carrying cation.

cause most nonexcitable cell membranes also support BK-type potassium channels (a calcium-activated potassium conductance), most nonexcitable cells would apparently be capable of undergoing nonlinear calcium oscillations through modulation of their calcium conductance *(37)*. Moreover, recent numerical investigations have shown (*38*) that relatively small changes in the maximal conductances underlie the bursting activity in the Morris–Lecar oscillator. These developments lend strong support to the contention that voltage-dependent calcium channels could play a critical role in the origination of the ELF-field-induced responses, as even subtle modulation of these conductances (e.g., by ELF electric fields) may profoundly influence calcium transient behavior.

Gap Junctional Connectivity in Cell Ensembles

Although calcium dynamics represent a class of cellular events that have the potential to be perturbed by ELF fields through the plasma membrane calcium channels, a single cell would need to have a sensitivity approaching the theoretical threshold to detect even the largest environmental ELF field levels described in the literature. One approach for improving upon this situation involves amplification of the transmembrane potential perturbation, yet the only variable in the equation for the induced membrane potential perturbation by ELF fields is the radius of the cell. With the exception of nerve and muscle cells, cell radii are consistently on the order of only a few micrometers, but the effective size of a cell can be increased if it is part of an ensemble of cells that are electrically coupled. Recent work has shown (*39*) that the electrical interconnection of adjacent cells through gap junctions is more the rule than the exception. These gap-junction interconnections have the potential to play an important role in enhancing the sensitivity of cells to extracellular electric fields.

The concept of a tissue using a gap-junction-interconnected array of cells to spatially amplify the influence of an extracellular field was first proposed by Cooper (*40*), and the implications as to the frequency dependence of such an ensemble was reconsidered by Pilla et al. (*41*). More recently, we (*42*) extended this concept to two and three dimensions specifically to address the issue of detection of ELF environmental fields. If a collection of N cells are grouped as a two-dimensional interconnected ensemble, then the effective radius of the ensemble will increase as $N^{1/2}$. Correspondingly, the magnitude of the induced membrane potential perturbation for the cells at the periphery of the ensemble will also increase as $N^{1/2}$. Although in theory the size of the cell ensemble can be increased sufficiently to permit the cell ensemble to detect any arbitrarily small extracellular field, in reality this theoretical model would result in an unrealistically large number of cells in the ensemble.

A more useful estimate of the role of cell ensembles can be obtained by considering the effect of three-dimensional ensembles. Extension to three dimensions does not increase the magnitude of the field-induced membrane potential perturbations, but it does result in a decreased cell ensemble noise compo-

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nent because ensemble noise will decrease as the square root of the number of cells in the ensemble. The benefits of extending the dimensions of a cell ensemble do not accumulate linearly because cell plasma membranes are leaky insulators and ensembles more than a few space constants (typically on the order of 1 mm) in length contribute no additional benefit (*43*). Nonetheless, incorporating three-dimensional considerations into a gap-junction-coupled tissue model of field–cell interactions demonstrates a significant increase in ensemble sensitivity. By arranging the cells in a cylindrical pattern, similar for example to that found in bone tissues, a collection of only 20,000 cells can be shown to form a signal detection system that is capable of consistently detecting an electric field intensity as low as 1 mV/m.

Calcium plays two critical roles within gap-junction-coupled networks of cells that could significantly influence cellular sensitivity to ELF electric fields. First, gap junctional conductivity and gating is sensitive to intracellular calcium levels. Although initial reports of gap junctional responses to calcium led (*44*) to suggestions that channel uncoupling in high calcium was a safety mechanism that allowed cells to protect themselves from injured neighboring cells, more recent work has demonstrated (*45*) that, at least in some cell types, significant alterations in gap junctional conductance can be observed when calcium concentrations are altered within the normal physiological range of 125–500 nM. The implication of this observation is that alterations in intracellular calcium levels could modulate cell network sensitivity to extracellular electric fields. If, as suggested, field exposure affects calcium permeability of the cell membrane, then the ability of calcium to alter coupling in an interconnected cell network would result in a closed-loop system in which the sensitivity of the network to extracellular electric fields could be readily titrated. This possibility holds further promise given the second demonstrated role of calcium in gap-junction-interconnected networks: that of an intercellular messenger. Research shows (*46*) calcium transients or oscillations that occur in single cells can pass through gap junctions and proceed as a calcium wave into adjacent cells. As a result, even though only the cells at the edge of an interconnected network have maximal sensitivity to extracellular fields, an effect of field exposure on these peripheral cells may well be rapidly transmitted into the interior cells through calcium waves mediated by gap junctions.

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Developments in Network Theory

The common existence of gap-junction-interconnected cell systems not only provides a means for amplifying and distributing the effects of ELF field exposure, it also provides a mechanism for temporal integration, or system memory, a feature that would also enhance the probability of ELF signal detection. The fundamental basis of memory, that is, how biological systems map data from the outside world into internal states, falls within the realm of network theory. Much of the current research in network theory is being applied to the problem of learning and memory in neural networks, the basis of which is a fundamental

principle of learning proposed by Hebb (*47*). The essence of Hebbian learning is that a network of spiking cells can store and retrieve spatial–temporal patterns with a time constant substantially less than the temporal integration time of any single cell. This capability arises from the synchronization of the local spiking patterns within individual cell processes. Studies have extended (*48–51*) these concepts by addressing the possibility of coherent activity among large ensembles of cells.

Analysis of realistic models of ensembles of cells with spiking capability shows that by simply changing the communication delay times between adjacent cells, the oscillatory state of the ensemble can be significantly altered (*52*). For a weak external signal, a short delay time between cells results in strongly locked oscillation in the ensemble that will be sustained only for the duration of the applied stimulus. Conversely, with long delay times between the cells, exposure to an external signal results in a weak locking state, and the ensemble oscillation will sustain itself even after the signal is removed. Interestingly, intermediate delay times can lead to unsynchronized spiking activity in the ensemble. In addition, such modeling studies show the effect of adding noise is to lower the amplitude of the oscillations and shift the system from global stationary oscillation to a weakly locked state. The relevance of understanding the origin of such coherent activity becomes clear when the ensemble activity of nonexcitable, calcium spiking cells is observed (Figure 5). Clearly, just as in neurons, ensembles of gap-junction-connected nonexcitable cells can undergo both local transient behavior as well as weakly coupled, low-level ensemble oscillations.

Theoretical work on collective oscillations suggests ensemble oscillatory

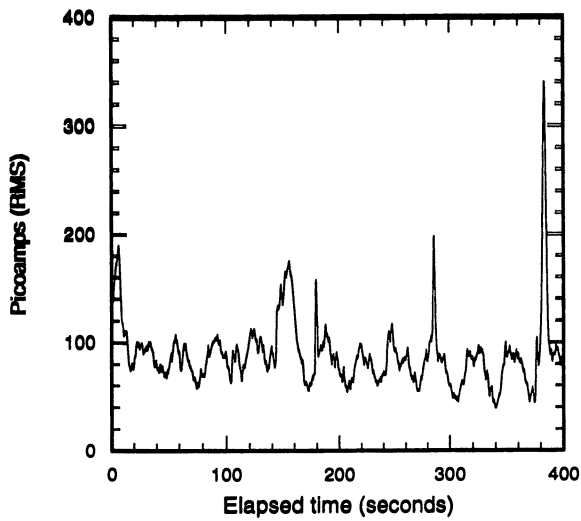

Figure 5. Low-frequency (0.03-Hz) collective oscillations and concomitant single cell spiking arising spontaneously in an ensemble of aequorin-loaded osteoblastic cells growing in monolayer.

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activity should be particularly susceptible to weak stimuli in the environment, and work on ELF-field-stimulated cellular responses appears to confirm this suggestion. Perhaps the first demonstration of the sensitivity of oscillating systems to ELF fields was the reported work on *Aplysia* pacemaker cells (*53*). Induced current densities as low as 2 $\mu A/cm^2$ were shown to alter the firing rate of the cells at a frequency of 0.5 Hz. Similarly, Blackwell (*54*) studied 15-, 30-, and 50-Hz field effects on spontaneously firing neurons in the brain of anesthetized rats in response to an externally imposed field of 100 V/m. For a typical coupling coefficient of 10^{-7}, the induced fields in the brain of the animal would be on the order of 10 μV/m. Although no effect on average firing rate was found, induced synchronicity of the firing rate with the applied fields at 15 and 30 Hz were significant. Undoubtedly the best example of the sensitivity of oscillating systems to weak ELF fields is the response of the electroreceptive organs, the ampulae of Lorenzini. Recently reported voltage-clamp studies of isolated ampullary epithelium (*55*) demonstrate membrane potential perturbations of only 1 μV are capable of modulating firing rates of the afferent nerve. Moreover, these investigations demonstrate a distinct negative conductance in the apical membrane surface that is believed to be a calcium conductance.

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Real-Time Measurements of ELF Effects

Numerous experimental reports support the theoretical prediction that, at least for excitable cells, ensembles of cells in an oscillatory state are exquisitely sensitive to weak ELF electric field stimuli. Also, nonexcitable cells can clearly sustain an oscillatory state, most likely calcium oscillations or spiking. If calcium indeed plays a critical, direct role in the field–cell transduction process, then demonstrating a direct effect of ELF field exposure on calcium dynamics should be possible. Consistent with this expectation, several laboratories are currently developing techniques to study the real-time response of intracellular calcium to ELF fields. One approach has been to utilize differential fluorescence spectroscopy to assay changes in both calcium levels and calcium influx during ELF field exposure (*56*). These studies have confirmed the existence of remarkably small (4%), although highly significant ($P < 0.01$), increases in calcium influx occurring within 120 s of the onset of a 60-Hz, 2-mV/m root mean square (rms) electric field exposure induced by a 2-mT magnetic flux.

We have initiated a similar series of investigations of alterations in calcium activity but have emphasized the dynamic changes in calcium spiking activity in response to ELF field exposure. Ensembles of aequorin-loaded, osteoblast-like cells ($100–200 \times 10^3$ cells) growing in monolayer culture at 37 °C and a pH of 7.4 consistently demonstrate calcium spiking patterns that arise within 8–24 h after plating (Figure 6A). Exposure of this preparation to a 60-Hz, 35-mV/m rms electric field can be shown to significantly suppress the spiking activity over a time course of minutes (Figure 6B). Moreover, spiking activity is reinitiated upon field removal and returns to baseline levels within tens of minutes.

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Advances in Chemistry; American Chemical Society: Washington, DC, 1995.

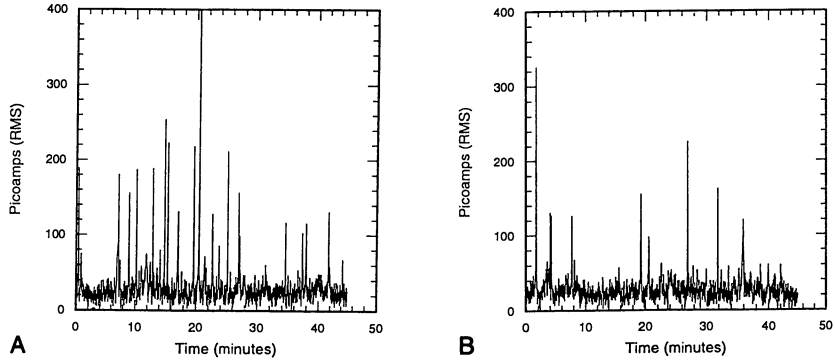

Figure 6. Effect of uniform ELF field exposure at 60-Hz, 35-mV/m rms, on the calcium transient activity in an ensemble of osteoblastic cells growing in monolayer for 48 h prior to exposure: (A) 45 min immediately preceding exposure and (B) 45 min immediately following onset of field exposure.

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Although such real-time studies are only beginning, increases in temporal resolution combined with studies on the influence of gap junction blockers, calcium channel blockers, and further single-cell exposure studies should be capable of definitively identifying the initial events associated with cellular detection of ELF electric fields. Our current understanding of the critical role of calcium in mediating numerous environmental signal detection processes suggests that calcium will likely play an immediate and critical role in ELF field detection as well.

Summary

In considering the conceptual requirements of a transduction process through which cells could detect a low-level ELF electric field induced within a tissue, four critical characteristics of the process were identified. These include (1) a nonlinear or rectification characteristic that would permit detection of symmetric fields, (2) the existence of memory within the process to permit temporal signal averaging, (3) temporal characteristics that could explain the reported frequency dependence of ELF field interactions, and (4) the ability to spatially integrate the field to provide amplification and enhance the tissue's ability to detect these low-level fields.

A review of the more recent developments in our understanding of calcium activity within cells leads to the suggestion that voltage-dependent modulation of calcium conductances within the cell plasma membrane may be sufficient to explain many of the phenomena observed in ELF exposure experiments. Calcium channels exist in both excitable and nonexcitable cells and serve as a highly nonlinear, rectifying process with distinct temporal characteristics. In addition, oscillating systems are highly susceptible to weak environmental per-

turbations, and calcium oscillators appear to exist in many, if not most, cells because of the activity of their voltage-dependent calcium channels. Finally, gap-junction-connected cell ensembles commonly occur in tissues, and calcium serves both as a mediator of these interconnections as well as an important carrier of intercellular information through these channels.

On the basis of these observations, the regulation of calcium conductivity at the cell plasma membrane is proposed as a potentially critical process through which ELF electric fields could significantly interact with cells. The definitive experiments testing this hypothesis remain to be done.

Acknowledgments

This work was supported by Electric Power Research Institute Grant 2965–21 and National Institutes of Health Grant ES–06287.

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Received for review April 25, 1994. Accepted revised manuscript March 29, 1995.

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Magnetoreception and Electromagnetic Field Effects: Sensory Perception of the Geomagnetic Field in Animals and Humans

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Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch021

This chapter reviews numerous discoveries that have been made during the past 15 years concerning the ability of living organisms to respond to the geomagnetic field. These include (1) the magnetotactic response of bacteria and protozoans, (2) magnetic effects on homing and navigational behavior by migrating animals, (3) the discovery of magnetically influenced signals in nerve fibers from the trigeminal system in birds and fish, and (4) the testing of two biophysical hypotheses for the magnetoreception mechanism (biogenic magnetite and optical pumping). A final discussion concerns possible biological effects of environmental electromagnetic fields on the basis of the energy required to rotate the small crystals of biogenic magnetite that have been discovered in various human tissues.

THE QUESTION OF WHETHER SOME LIVING ORGANISMS might be sensitive to the Earth's magnetic field has been one of the most controversial topics in the behavioral and neural sciences for more than a century. Earlier reports of magnetic effects on animals were criticized properly by biologists because of the difficulty of replication and by physicists who could not think of a plausible biophysical mechanism by which animals could detect the weak magnetic field of

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In Electromagnetic Fields; Blank, M.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1995.

the Earth (e.g., *1, 2*). However, this situation changed radically during the past 15 years by developments in three separate areas.

First came the discovery of many highly reproducible magnetic effects on behavior. These effects include the magnetotactic response of bacteria and protozoans, magnetic effects on homing and navigational behavior by migrating animals, and the development of some robust psychological magnetic conditioning techniques. Apparently, two separate magnetic sensory systems exist in higher animals: a directional compass and a magnetointensity sense.

Second, at least two plausible biophysical mechanisms are now known through which the Earth's magnetic field can be transduced to the nervous system. These mechanisms include use of the ferromagnetic mineral magnetite (Fe_3O_4), which is a biochemical precipitate in virtually all groups of higher organisms, including humans. In several groups of vertebrates it forms chainlike structures ideally suited for responding to magnetic fields (*3–6*). Optical pumping is another potential transduction mechanism (e.g., *7–9*) by which Earth-strength magnetic fields could influence charge-transfer reactions in organic molecules. Both mechanisms have received support from laboratory-based experiments, as discussed in this chapter. A third mechanism, electrical induction, is not a plausible transduction hypothesis for terrestrial organisms (e.g., *10*).

Finally, two separate groups obtained clear records of magnetically influenced signals in single nerve units connecting magnetite-bearing tissues with the brain, and these signals suggest strongly that the magnetite-bearing tissues of the ethmoid sinus contain a magnetoreceptor. The ophthalmic branch of the trigeminal nerve appears to be the main conduit of magnetic intensity information to the brain; the origin of the compass is as yet unknown.

A brief review of each of these three important developments will be presented, followed by a review of the past work on magnetoreception in humans.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch021

Experimental Evidence for Magnetoreception

Directional Compass Effects. ***Microorganisms.*** Magnetotactic bacteria and algae provide the clearest examples of a directional compass response in any living organism (*11, 12*). When observed under the microscope, living cells swim in straight lines parallel to a magnetic field, and their swimming directions can be changed instantly by using a small hand magnet. Each cell contains between 0.5 and 2% by weight magnetite or greigite (Fe_3S_4), and the arrangement of crystals into chains provides each cell with magnetic moments large enough to align the organism passively like a compass needle in the geomagnetic field.

Why is magnetotaxis useful for these microorganisms? In the Northern hemisphere, the magnetotactic bacteria swim to the North, or toward the South pole of a magnet, whereas in the Southern hemisphere they do precisely the opposite (*13*). In both cases, the inclination of the Earth's magnetic field leads them down, back to their natural environment at the mud–water interface. Most

of these bacteria do not like a high concentration of oxygen in the water, so using the geomagnetic field to stay near the surface of the mud, which has a low oxygen concentration, is convenient for them. Hence, the geomagnetic field is very important to their survival. On the geomagnetic equator (or in environments where the magnetic field is parallel to the mud–water interface), the population density of magnetotactic bacteria is reduced by several orders of magnitude (*14*). The magnetotactic behavior of the algae is more complex, as they respond to other environmental clues.

Invertebrate Magnetic Compass Effects. Of all terrestrial invertebrates that have been examined for magnetic field sensitivity, the honeybee (*Apis mellifera*) stands out as having the most extensive and reproducible behavior. Table I shows a summary of the known magnetic effects, as well as the independent attempts to replicate them. Of the nine basic effects, six have been replicated by independent groups, and three by two or more groups. We know of no attempts to replicate these effects that were not eventually successful (some apparently took practice). Effects 2, 3, and 8 from Table I all demonstrate that honeybees have a very good directional magnetic compass sensitivity. These data have been reviewed extensively by Towne and Gould (*15*), Kirschvink (*16*), and Kirschvink et al. (*17*).

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Table 1. Summary of Magnetic Effects on Honeybee Behavior

Effects	*Original Reports*	*Similar Reports*
1. Misdirection in the waggle dance influenced by weak magnetic fields	Lindauer and Martin (*90*) Lindauer and Martin (*91*) Martin and Lindauer (*92*)	Hepworth et al. (*95*) Towne and Gould (*15*) Kilbert (*96*)
2. Dances on horizontal comb align with points of magnetic compass	Lindauer and Martin (*91*) Martin and Lindauer (*92*)	Brines (*97*) Gould (*98*) *see also* Kirschvink (*99*)
3. Magnetic orientation of comb building	Lindauer and Martin (*91*) Martin and Lindauer (*93*)	De Jong (*100*) Towne and Gould (*15*)
4. Time sense of bees influenced by geomagnetic variations	Lindauer (*94*)	partially by Gould (*98*)
5. Extinction test conditioning experiment	Walker and Bitterman (*37*)	Kirschvink and Kobayashi-Kirschvink (*41*)
6. Two-choice threshold conditioning experiment	Walker and Bitterman (*38*)	Kirschvink et al. (*42*)
7. Small magnets on anterior dorsal abdomen interfere with conditioning experiments	Walker and Bitterman (*39*)	No attempts reported
8. Pulse remagnetization converts North-seeking into South-seeking bees	Kirschvink and Kobayashi-Kirschvink (*41*)	No attempts reported
9. Two-choice conditioning to ELF magnetic fields up through 60 Hz	Kirschvink et al. (*42*)	No attempts reported

NOTE: ELF is extremely low frequency.

Phillips and Sayeed (*18*) have reported recently that males of the fruit fly (*Drosophila melanogaster*) also display a magnetic compass response when released into an eight-arm radial maze. This finding is an exciting development, as numerous genetic mutants exist for *Drosophila* that should aid in the localization of the receptor cells, as well as aid in the elucidation of the transduction mechanism. The experimental protocol appears to be simple and straightforward. If the results prove to be reproducible, the fruit fly could replace the honeybee as the organism of choice in magnetic studies, as it is much easier to maintain and work with.

A directional magnetic compass response also appears to be present in some marine mollusks, particularly *Tritonia diomeda* (*19*). Using intracellular electrophysiological recording techniques, Lohmann et al. (*20*) found that the large cells of the left and right pedal (lPe5 and rPe5) alter their firing patterns consistently (although slowly) in response to a rotation of the magnetic field direction. The ability to identify these discrete, magnetically sensitive brain cells offers hope that neurological staining techniques may ultimately locate the actual receptor cells and help clarify the transduction mechanism.

Vertebrate Magnetic Compass Effects. Magnetic compass effects have been reported in all five classes of vertebrates, including fish (*21*), amphibians (*22*), reptiles (*23*), numerous birds (e.g., *24–26*), and mammals (*27*).

Wiltschko and Wiltschko (*26*) first recognized that the magnetic compass response in European robins was insensitive to the polarity of the field. Reversing the vector direction of the local magnetic field (by inverting both the horizontal and vertical components) yields no change in the directional preference. On the other hand, inversion of the vertical component alone yields a 180° shift, and tests in horizontal fields (vertical component canceled) yield random directional preferences. Apparently, the animals are responding to the axis of the field relative to horizontal, and hence this behavior is generally referred to as an inclination compass. Numerous studies have shown that this pattern of response is a general characteristic of birds, and it has been reported in amphibians and reptiles as well [newts (*22*) and loggerhead turtle hatchlings (*23*)]. However, migrating salmon fingerlings (*28*) display a polar compass, as do migrating newts (*22*) and mole rats (*29a*). Unfortunately, very few reports of magnetic compass responses in animals include tests to distinguish axial from polar compass mechanisms, so the phyletic consistency of these observations is unknown.

The inclination compass is also interesting because the behavioral response combines sensory information from two separate systems: gravity and magnetism. This behavior unfortunately complicates attempts to elucidate the biophysical mechanism(s) underlying magnetoreception, as some important features that might be present at the receptor level (e.g., polarity sensitivity) could be eliminated in the complex sensory processing leading to the behavioral response.

Magnetointensity Perception. Most of the pre-1980 literature on magnetic sensitivity dealt with tests of various sorts on the ability of animals to

obtain directional information from the geomagnetic field (e.g., "compass" information), and this type of response has been difficult sometimes to replicate in subsequent attempts (e.g., *29b*). However, numerous reports in the literature deal with the effects of weak fluctuations in the background intensity of the magnetic field, with little change in the vector direction of the field itself. Examples include the release of homing pigeons at magnetic anomalies (*30, 31*), the preferential stranding of cetaceans at local magnetic minimum (*32–34*), the tendency of cetaceans to avoid high fields and field gradients while at sea (*35*), and virtually all of the successful attempts to condition animals to magnetic fields (*36–42*). The data suggest that migrating and homing animals derive useful information from magnetic variations that can be as weak as only a few tenths of a percent of the background field. Although many magnetic features, such as the marine magnetic lineations, and regional geomagnetic variations could be rich sources of position information for animals, the change in the vector direction of the magnetic field associated with them is rather small. For example, a 50-nT magnetic anomaly in the ~50-μT geomagnetic field could at most produce a directional change of 0.06°. As moving animals are not known to keep track of their spatial orientation better than this, the animals are thus probably monitoring some scalar component of the magnetic vector field (e.g., *43*).

Walker and Bitterman (*38*) report a remarkable measurement of the threshold level of static magnetic intensity perception in the honeybee (effect 6 of Table I). They first developed a two-choice training paradigm using two sucrose–water feeder assemblies mounted on a vertical window frame. Each assembly had a pair of double-wrapped coils that could either produce a sharply focused magnetic anomaly or a matching null-field anomaly, but with the same thermal effects. Individual foraging bees were trained via a reward–punishment scheme to feed preferentially from the feeder paired with the magnetic anomaly, and usually within 10 or 20 repeat visits they would learn to land at the feeder with the magnetic anomaly and avoid the nonmagnetic one. By starting with a moderately strong anomaly (3 mT) and by reducing the amplitude of the anomaly in small exponential steps, the threshold sensitivity could be determined by the point at which the bees were no longer able to discriminate correctly. Of nine bees run through the procedure, the median threshold was 250 nT in the presence of the Earth's field, a relative sensitivity of 0.6%. As shown in Figure 1, the best bee lost the ability to discriminate in fields below 25 nT (0.06% of background). Similar, but less direct, estimates of the magnetic sensitivity of bees were obtained from both the misdirection and circadian rhythm experiments (effects 1 and 4 of Table I).

In a previous study, we reported (*42*) the replication of this basic conditioning technique for strong fields and used it to obtain a first-order look at the frequency response of the honeybee magnetoreceptor system. Bees are able to discriminate oscillating magnetic fields at frequencies at least up through 60 Hz. Subsequently, we ran a series of bees through the Walker and Bitterman threshold procedure at 60 and 10 Hz in an attempt to measure the weakest power-line-frequency magnetic field they can perceive (*44a*); preliminary results are shown

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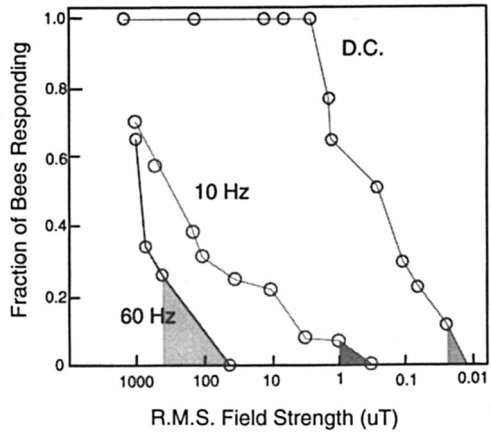

Figure 1. Measurement of the threshold sensitivity of honeybees to weak direct current (DC), 10-Hz, and 60-Hz magnetic anomalies. In each case, an individual bee is first conditioned to discriminate the presence or absence of a moderately strong magnetic anomaly using the two-choice paradigm of Walker and Bitterman (38). After reaching performance criterion, the field level is reduced to a lower value, and the bee is tested to determine if discrimination can be reestablished. This figure shows the proportion of bees able to discriminate the given field stimulus. Data for the nine bees exposed to the DC field are from Walker and Bitterman (38), and those from 15 bees tested at 10 Hz and 11 bees tested to a 60-Hz stimulus are from Kirschvink et al. (44a). All of the bees were initially able to detect the DC stimulus and were able to maintain discrimination through a reduction of 3 orders of magnitude. On the other hand, bees did far worse at detection of the 60-Hz stimulus, a result that is consistent with the viscous damping model of an elongate magnetosome chain (17, 42). RMS is root mean square.

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in Figure 1, compared with the results for static fields of Walker and Bitterman (*38*). Apparently the threshold sensitivity at 60 Hz is reduced by over 3 orders of magnitude compared with that at 0 Hz, with the 10 Hz value in between. As discussed below, this finding is consistent with the biophysical predictions of a magnetoreceptor that employs a linear magnetosome chain for detecting the magnetic field (*17, 42*).

Vertebrate Neurophysiology. This most important development involves the discovery of magnetically influenced signals in nerve fibers that ramify in the magnetite-bearing tissues of the ethmoidal region. The ophthalmic branch of the trigeminal nerve is the one that connects tissues in the ethmoid region to the brain, so this nerve is the most likely one with which to begin the electrophysiological search. Within the past 4 years, two groups have had spectacular success at making direct electrophysiological recordings of magnetically mediated signals in this nerve. The first report (*44b*) was recorded from cells in

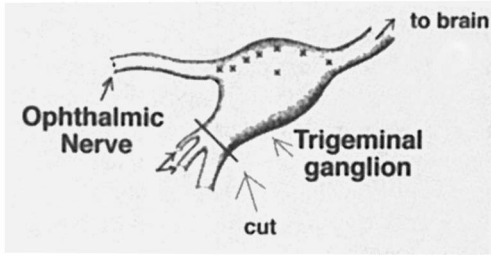

Figure 2. Schematic drawing of the bobolink trigeminal ganglion showing the nerves and recording locations (indicated by ×) of cells responding to magnetic stimulation. The line indicates where mandibulomaxillaris nerve was severed (Adapted from reference 44b, courtesy of R. Beason.)

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the trigeminal ganglion of a small migratory bird, the bobolink (Figure 2). In this report, about 21% of the fibers in the ophthalmic nerve and 9% of cells in the trigeminal ganglion responded to small changes in the intensity of the applied magnetic fields (Figure 3), including neurons that fired in response to changes in the field as weak as 200 nT (4% of the background geomagnetic field). The roughly logarithmic increase in firing frequency of the nerve with stimulus intensity is a pattern observed in many other sensory systems. Similar results were obtained by Walker et al. (*44c*) in New Zealand on the brown trout (a relative of the salmon). In both the bird and fish, no other sensory nerve fibers have yet been found to convey magnetic signals.

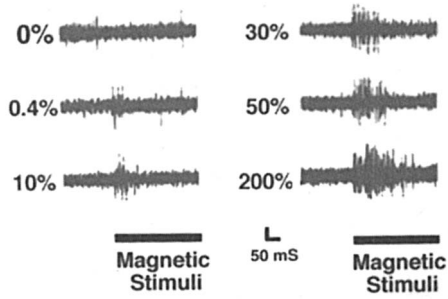

Figure 3. Electrophysiological recordings of magnetically mediated signals in a bobolink, showing the response of one ganglion cell to different vertical magnetic field intensity changes. On each of the six horizontal traces, the number with the % symbol indicates the size of the magnetic intensity increase compared with the background (static) field level, which was 50 μT. 0% is an example of the spontaneous activity of this neuron, 0.4% was with a stimulus of 200 nT, 10% was with 5 μT, 30% was with 15 μT, 50% was 25 μT, and 200% was 100 μT. The stimulus onset is indicated by the bar below each series. Horizontal scale is 50 ms; vertical scale is 2 mV. (Adapted from reference 44b; data courtesy of R. Beason.)

Human Magnetoreception. The question of whether humans also have a magnetic sensitivity is perhaps one of the most controversial areas in this entire field. Humans make magnetite in many tissues and have an ophthalmic nerve in their trigeminal system. However, most humans do not claim to perceive consciously the Earth's magnetic field, and magnetoreception is not listed among the five major senses (vision, hearing, smell, taste, and touch). Therefore, if magnetoreception exists in humans, it must either be buried deeply in our subconscious or masked in some other fashion. One research group in England has claimed for the past 13 years that humans do indeed have a subconscious magnetic compass sense like many other animals (*45–53*). However, no independent research group has yet claimed success at replicating any of these results, (e.g., *54–57*).

Two more recent developments indicate that now may be a good time to reinvestigate this human magnetoreception question. First, Bell et al. (*58*) obtained evidence from surface-based electroencephalogram recordings that environmental magnetic stimuli are somehow having an influence on neurological activity in the human brain. Second, Dobson et al. (*59, 60; see also* reference 61) report that a 1–2-mT static magnetic field applied through the head of epileptic patients was able to elicit epileptiform (epilepsy-like) activity, as recorded by electrodes implanted directly in the hippocampus. The ability to induce epileptiform activity on command led to the successful localization of the epileptic foci in several patients who otherwise had been difficult to treat. However, in one study (*60*) the induced activity developed into a full-blown epileptic seizure in one patient.

At present, the evidence suggests an intensity "window" for eliciting this response, as static fields below 0.9 mT do not work, and apparently no claims exist of epileptic seizures being triggered by exposures to the strong (1.5-T) magnetic fields of clinical magnetic resonance imaging (MRI) machines. This feature is as yet poorly understood. The only other report of a behavioral intensity window for a vertebrate is in birds (*26*), and it was centered on the geomagnetic field strength.

All of these more recent observations are consistent with the hypothesis that external magnetic fields are being transduced into neurological activity in humans. If so, some form of sensory transduction must be operating for this hypothesis to be true, as all known inputs to the nervous system arise ultimately in cells specialized to convert external stimuli into coded bursts of action potentials (e.g., *62*). Hence, the investigation of human magnetoreception is an area that needs much additional work.

Biophysics of Magnetoreception

The Ferromagnetic Transduction Hypothesis: Biogenic Magnetite. ***Theory and Occurrence.*** The simplest possible method for a living organism to respond to magnetic fields as weak as that of the Earth's is to use a

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permanent magnet, much as human navigators do today. However, most materials found in organisms are generally thought of as nonmagnetic, that is, either diamagnetic (repelled weakly from a magnetic field, like water and most fatty substances) or paramagnetic (weakly attracted to a magnetic field, like the deoxyhemoglobin in red blood cells). For materials of these types, the direct physical influence of the Earth's magnetic field is extraordinarily weak, with the energy of magnetic interaction being many orders of magnitude below that of the background thermal energy, kT (where k is the Boltzmann constant and T is the absolute temperature). However, another category of materials, termed ferromagnetic, interacts very strongly with the Earth's magnetic field. Unlike diamagnetic and paramagnetic substances, quantum mechanical interactions acting on unpaired electrons within ferromagnetic materials force the electron magnetic moments (Bohr magnetons) to form long-range alignments. The magnetic moments from each Bohr magneton within such a crystal are added vectorially, and in some materials a crystal of only a few tens of nanometers in size will have magnetic interaction energies with the geomagnetic field greater than the background thermal energy (kT). Motion of this material in response to external magnetic fields can in principle account for a variety of magnetic effects at the cellular level, such as the opening of mechanically sensitive transmembrane ion channels (Figure 4; references 17 and 63) or the biophysical transducer in a cell specialized to detect the magnetic field (e.g., a magnetoreceptor; *17, 43*).

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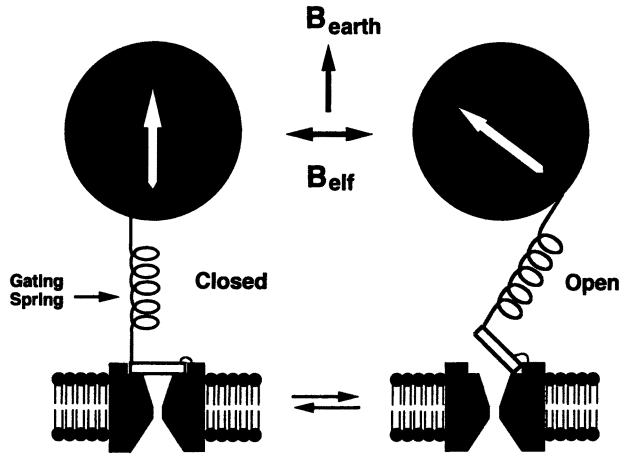

Figure 4. A schematic diagram for how a magnetosome might act to open or close a mechanically sensitive transmembrane ion channel. A magnetosome connected to an ion channel gate via a cytoskeletal filament (a "gating spring"), adapted from reference 88 but not drawn to scale as the magnetosome should be larger than shown. The geomagnetic field, B_{Earth}, is perpendicular to the plane of the membrane, whereas the extremely low frequency (ELF) magnetic field component, $B_{ELF} \cos(\omega t)$, is parallel to it (17).

At present, 12 iron minerals have been identified in organisms (*64*) although only three of these have been found so far as biochemical precipitates in vertebrates. These three are ferrihydrite ($5Fe_2O_3 \cdot 9H_2O$), which is the mineral in the core of the ferritin molecule, normally the chemical precursor of magnetite, and the substance often referred to in the medical literature as hemosiderin; goethite ($Fe_2O_3 \cdot nH_2O$); and magnetite (Fe_3O_4). Of these materials, ferrihydrite is paramagnetic above 240 K and goethite is antiferromagnetic, whereas magnetite has a variety of ferromagnetism termed "ferrimagnetism". These magnetic properties make magnetite interact over 10^6 times more strongly with external magnetic fields than does any other biological material. Magnetite crystals are permanently and spontaneously magnetized bar magnets, which cannot be demagnetized under physiological conditions.

In 1962 Heinz Lowenstam (*65*) first discovered that magnetite was the major mineral in the teeth of a primitive marine mollusk (the chitons, class Polyplacophora, Figure 5). Magnetite crystals in chiton teeth range from superparamagnetic to multidomain in size, implying that they have not been selected for on the basis of their magnetic properties. The chitons clearly use magnetite because it is much harder than any other biogenic mineral, not because it is magnetic. In contrast, all of the magnetite crystals that have been found subsequently in other living organisms are single domains, suggesting that the magnetic properties of these crystals have important biological functions. Numerous studies earlier in the 1980s revealed that virtually every group of vertebrates possessed anomalously high concentrations of magnetite in tissues in the vicinity of the ethmoid sinus (*4*).

An impressive variety of organisms are now known to biomineralize magnetite, including insects (*66*), birds (*67*), bacteria (*68*), fish (*69*), protozoans (*12*), and more recently humans (*70*). This list includes representatives of three of the five kingdoms of living organisms (bacteria, protists, and animals) and all five classes of vertebrates (fish, amphibians, reptiles, birds, and mammals). Only fungi and higher plants are not yet known to make it. Work by our group and others during the past decade has also shown (e.g., *14, 71*) that magnetite crystals formed by the magnetotactic bacteria are preserved in oceanic sediments as magnetofossils. By extracting magnetofossils from older and older sediments, we now know that the magnetotactic bacteria evolved at least by two billion years ago, about the same time as the appearance of the first eukaryotic cells. Hence, as shown on Figure 6, the biochemistry of magnetite formation quite possibly evolved only once in the magnetotactic bacteria, and all other groups inherited it from them. Magnetite biomineralization appears to be a common trait among most living organisms.

All of the magnetotactic bacteria when examined with the transmission electron microscope (TEM) contain chains of similarly sized magnetite or greigite crystals (around 30–50 nm) that span the bacterial cell (Figure 7). Under very high magnification (Figure 8), the crystal lattice can be imaged directly. The uniform fine stripes that cross the crystal are produced by successive layers of Fe atoms in the lattice. The high uniformity and structure of these fringe patterns are typical of biologically formed crystals. At present the biochemical

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pathways that form magnetite with such perfect, defect-free structures are unknown. Similar biogenic magnetites are also present in higher animals.

Largely due to the construction of a magnetically shielded clean laboratory at the California Institute of Technology, Kirschvink et al. (*4*) and Mann et al. (*5*) were able to demonstrate that the ethmoid tissues of the salmonoid fish

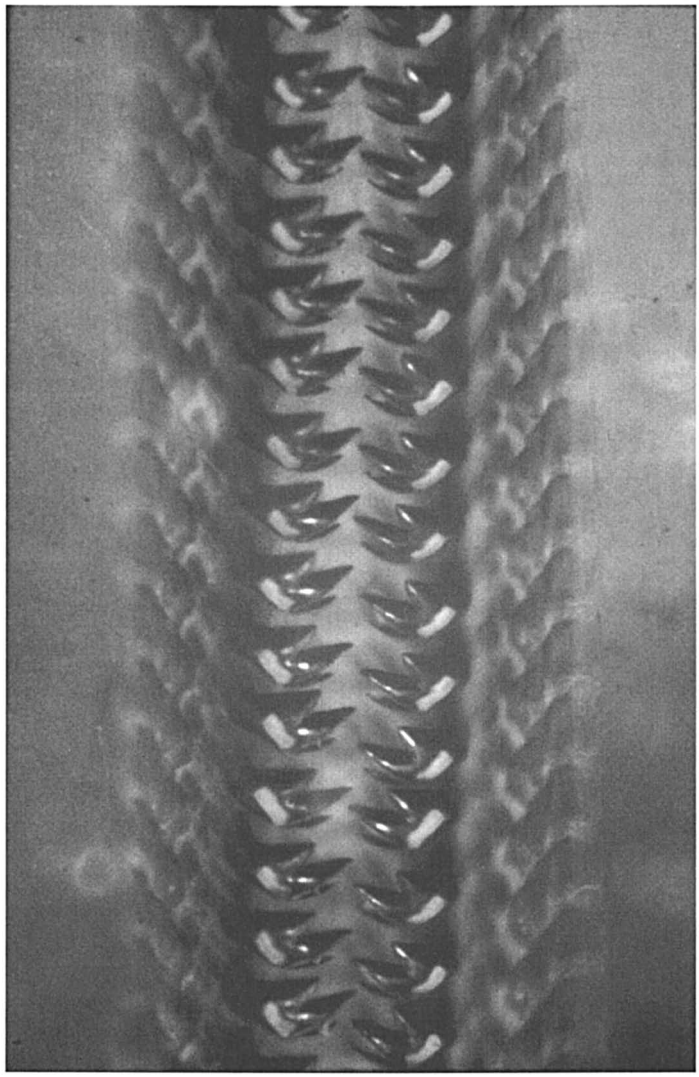

Figure 5. Chiton teeth. Length of one tooth is about 0.5 mm. Dark areas are covered with a thin (~10 μm) layer of magnetite. Red areas are made of other type of ferrihydrite, and their composition depends on the species. (Photo by H. Lowenstam. Reproduced with permission of California Institute of Technology.)

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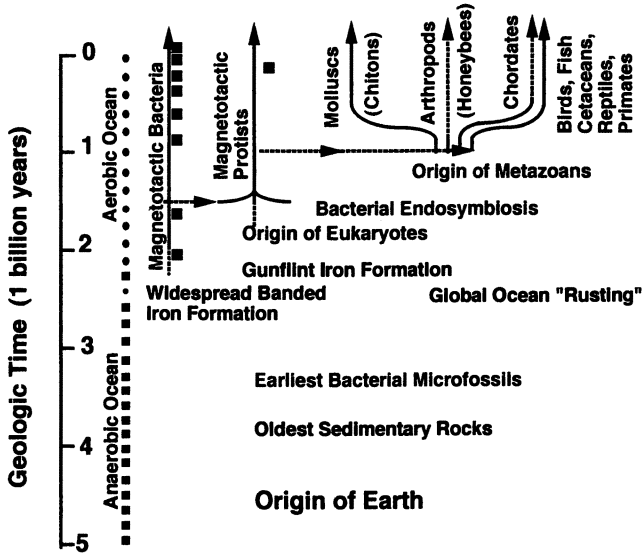

Figure 6. Evolution of magnetite biomineralization. Because biogenic magnetite has been discovered in three of the five kingdoms of living organisms, with a fossil record reaching back nearly 2 billion years, it probably shares a common descent in higher organisms. Presumably, the magnetotactic protists acquired this ability through an endosymbiotic relationship with a magnetotactic bacterium, and other groups of eukaryotes inherited it from them. (Adapted from reference 14.)

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contained chains of single-domain magnetite crystals (Figure 9), identical in morphology to those used for magnetotactic responses in bacteria and protists. These magnetites were discovered using a sensitive magnetometer, based on Superconducting Quantum Interference Devices (SQuIDs), which were designed originally to measure the direction and intensity of the magnetism of rock samples for geophysical studies. We modified these techniques for finding ferromagnetic minerals in frozen animal tissues. From these measurements, we located the largest concentrations of magnetite in the frontal regions of the head, particularly in the vicinity of the ethmoid–sphenoid sinuses (or their anatomic equivalents). By the fall of 1982, this pattern was demonstrated clearly in a variety of fish, birds, and mammals and was shown for amphibians shortly thereafter.

In a series of papers published between 1984 and 1988, Walker, Kirschvink, and others demonstrated (*4, 5, 69*) that extracts from the ethmoid tissues of pelagic fish (tuna and salmon) continued linear chains of single-domain magnet-

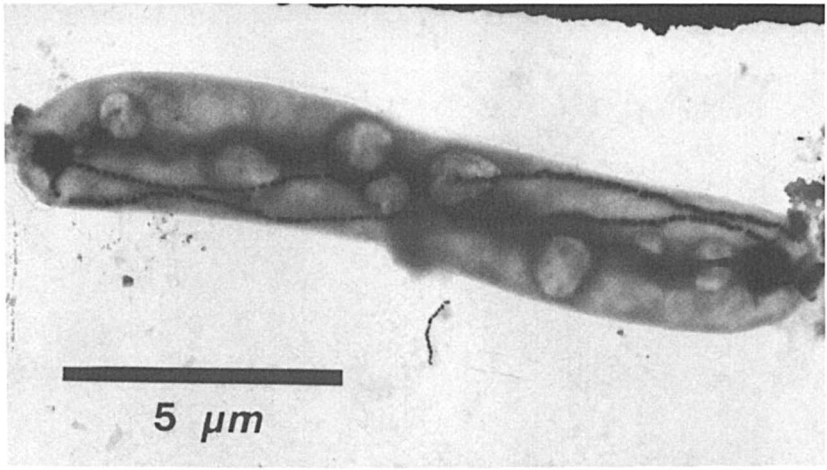

Figure 7. Transmission electron microscope photo of an unusually large magnetotactic organism from a depth of 44 m in the Ammersee, 50 km south of Munich. The 15-μm-long organism has two magnetosome chains and a total of about 180 crystals. These crystals are unusually large (170 × 80 × 80 nm), with magnetic-to-thermal energy ratios in the geomagnetic field of about 6 per particle, giving the entire cell a ratio of 1100 (assuming both chains are magnetized in the same direction). Crystals near the central portion of the chains are much smaller than average; this size is typical of immature grains near the growing end of magnetosome chains in other bacteria (e.g., reference 73). This oversized organism may be in the initial stage of division. Scale bar is 5 μm. (Reproduced with permission from reference 89. Copyright 1991 Plenum.)

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ite crystals, and the crystal shapes and structures are very similar to those found in magnetotactic bacteria. Furthermore, high-resolution TEM studies demonstrated that the {111} crystal axes of the magnetite were aligned along the chain length, as they are in most magnetotactic bacteria. This configuration yields a slightly higher magnetic moment per gram of magnetite (e.g., *72*) and is most easily explained as the result of natural selection for the magnetic properties, or as the result of new crystals growing in the strong magnetic fields at the end of a magnetosome chain (*73*). These are clearly "biological bar magnets". However, the ultrastructural localization of the magnetite in the tissues is not a trivial problem, as the maximum concentration in the ethmoidal tissues is still only a few parts per million, and the search for the magnetite-containing cells is not easy.

Even if we do not know the biological function of magnetite in humans, its presence in our tissues could have important implications for other biological effects of magnetic fields. Unfortunately, tissues associated with the human ethmoid sinus are not easy to study using the SQuID magnetometers, and the magnetite found in them in an earlier report (*74*) was contamination from a band-saw blade (*75*). For this reason, we focused instead on the soft tissues of the human

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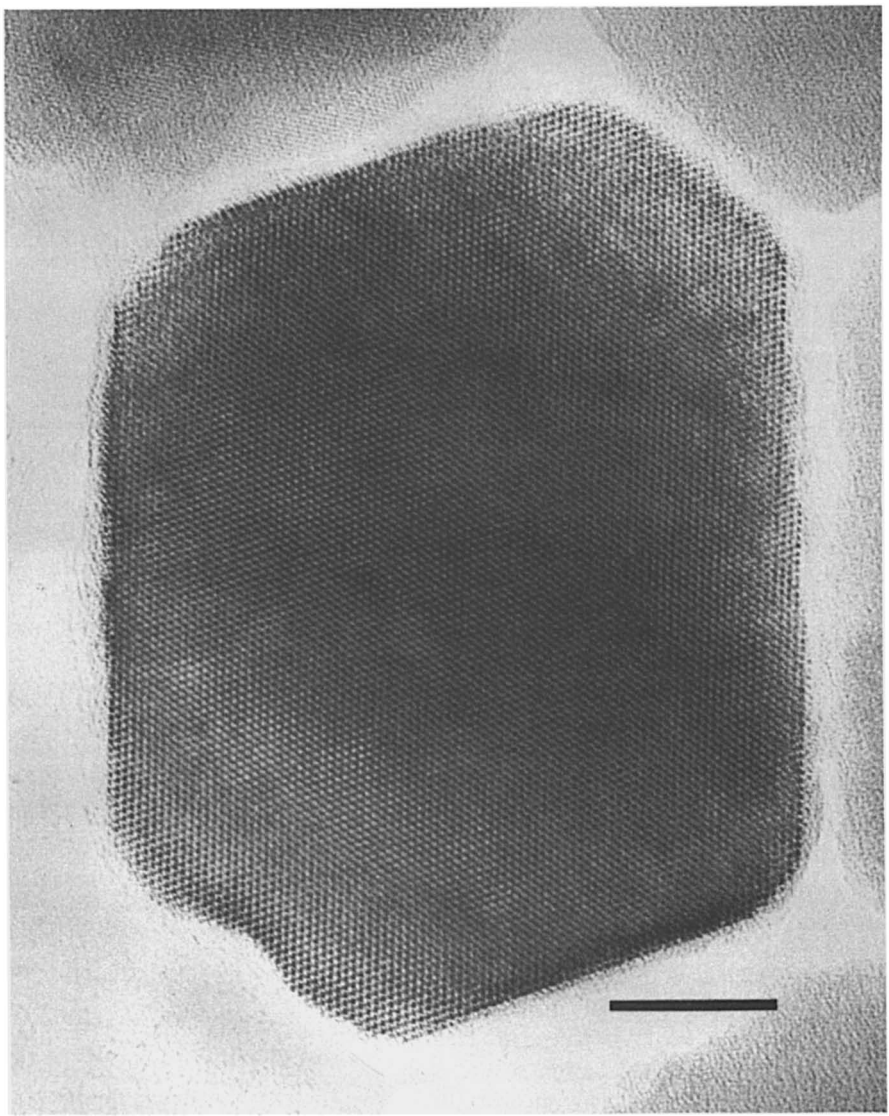

Figure 8. TEM images of single-domain magnetite extracted from the magnetotactic bacterium, Aquaspirillum magnetotacticum. *The scale bar is 10 nm. The high-resolution TEM (300-kV) image of the bacteria magnetite shows several sets of crystal lattice fringes (thin stripes) that correspond to three sets of {111} planes spaced a distance of 4.8 Å apart. (Photo by A. Kobayashi.)*

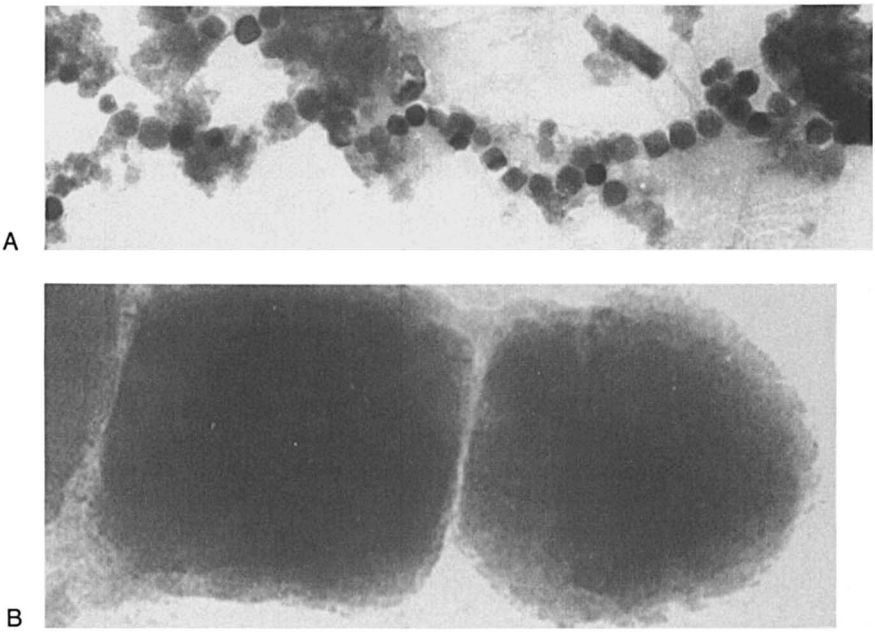

Figure 9. a: Magnetic extract from the ethmoid tissue of sockeye salmon showing a chain of ~50 nm electron-dense particles associated with organic material. (Reproduced with permission of S. Mann). b: Lattice images of two adjacent magnetite crystals showing the preferential crystallographic orientation of both crystals such that the {111} lattice planes (4.8-Å separation) lie perpendicular to the chain axis. (Reproduced with permission from reference 5. Copyright 1988 Company of Biologists Ltd.)

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brain, as this organ is the best studied tissue of any organism, both human and animal. Although in 1989 our initial SQuID measurements quickly demonstrated the presence of something ferromagnetic in the brain tissues, the absolute concentrations were rather minute: only a few parts per billion, much less than the concentrations of magnetite in the ethmoidal tissues mentioned already (Figure 10). After several more years of hard work extending our clean-lab extraction techniques, we were able to view the magnetic material high-resolution TEM (Figure 11a) using election diffraction (Figure 11b). Almost perfect lattice fringe patterns were present, with many of the crystal shapes very similar to the magnetosome of the bacteria and fish (*70*). These features imply that humans also have the process of magnetite biomineralization. Recently, Dunn et al. (*76*) reported an independent replication of the brain magnetometry work with similar results.

Experimental Evidence for Magnetite-Based Magnetoreception. Why is biogenic magnetite present in such a diverse set of living things? In all organisms except chitons, the particle sizes, shapes, orientation of crystal axes, and

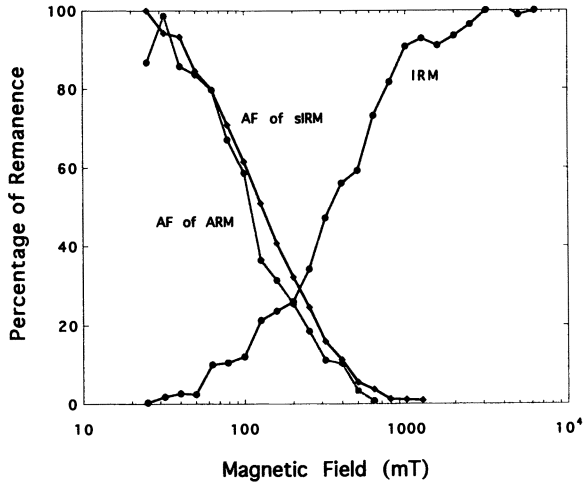

Figure 10. The curve labeled IRM (isothermal remanent magnetization) shows the relative magnetic moments remaining in the samples of human brain cortex after a brief exposure to a magnetic pulse of the indicated strength. The tendency of the curve to flatten at high field level is characteristic of the magnetite-maghemite solid solution series; most other ferromagnetic iron minerals saturate in fields >1 T. The curve labeled AF of sIRM shows the progressive alternating-field demagnetization of the saturation IRM, and that labeled AF of ARM shows the similar demagnetization of an anhysteretic remanent magnetization. The magnetic field value at which these two curves cross is the best measure of the average coercivity. The ordinate of the intersection point for noninteracting particles occurs at the 50% value; a depression or shift in this position is an indication of particle clumping effects.

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their alignment in chains act to produce single magnetic domains and to maximize their total magnetic moments. These observations suggest that the magnetic properties of the crystals are important biologically. Evidence that magnetite is used by organisms to respond to magnetic fields is very strong in bacteria, algae, honeybees, and in two groups of vertebrates, birds and fish.

A simple experiment first done by Kalmijn and Blakemore (*77*) demonstrates that this magnetotactic response is based on ferromagnetism. Single-domain ferromagnetic crystals, if slightly elongate or held in a chain, can be magnetized in one of only two stable orientations, either parallel or antiparallel to their length. A strong but brief magnetic pulse can cause the direction of the magnetic moment to jump from one stable state to the other, reversing the polarity of the crystal. A North-seeking compass would then point toward the South. (This principle is the same as that used to hold data on magnetic tapes and computer disks.) When applied to these magnetotactic microorganisms, a magnetic pulse actually causes them to reverse their swimming direction permanently. We

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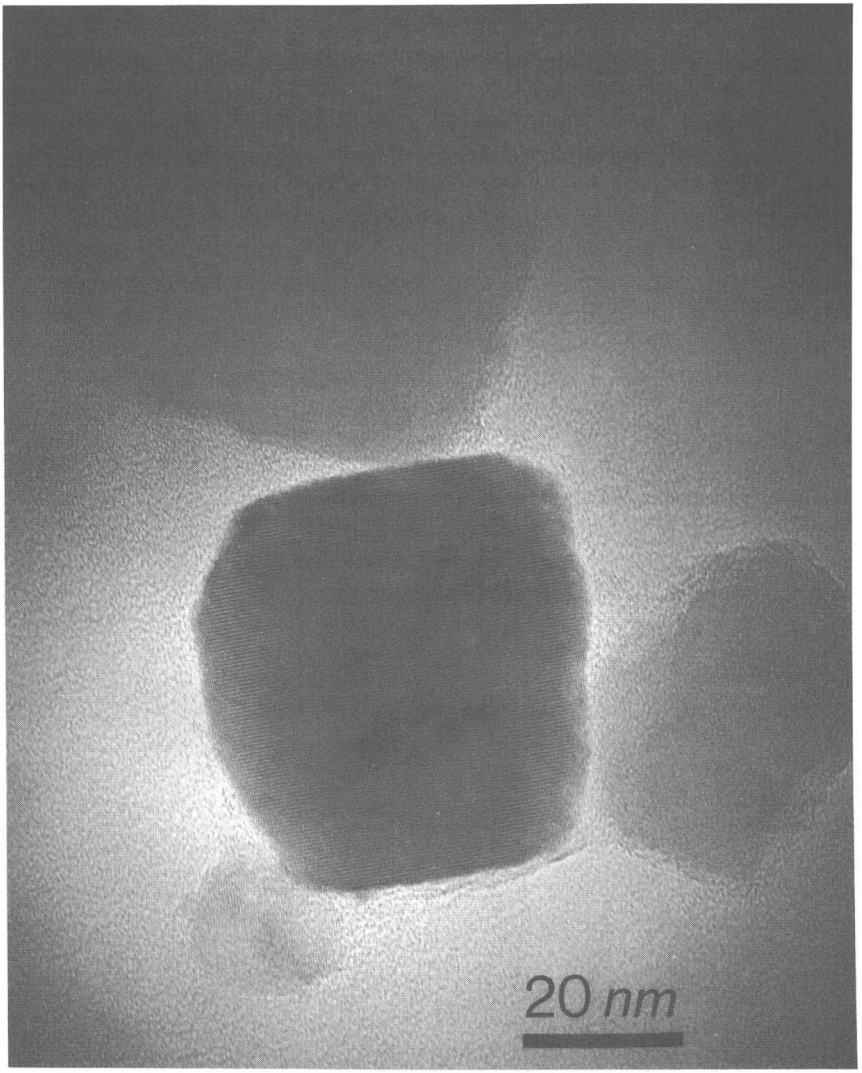

Figure 11a. TEM images of representative magnetite and maghemite crystals from the human cerebellum. High-resolution TEM image of the maghemite crystal shows the pattern of intersecting {111} and {022} fringes, with particle elongation in the {111} lattice direction. (Photo by A. Kobayashi).

(*78*) used this technique to isolate strains of magnetic bacteria with different particle sizes. The ability of a short magnetic pulse like this one to change behavior permanently is unique proof that a single-domain ferromagnetic material is responsible for the ability to detect the magnetic field. Similar pulse effects have been reported (*12*) on the magnetotactic algae.

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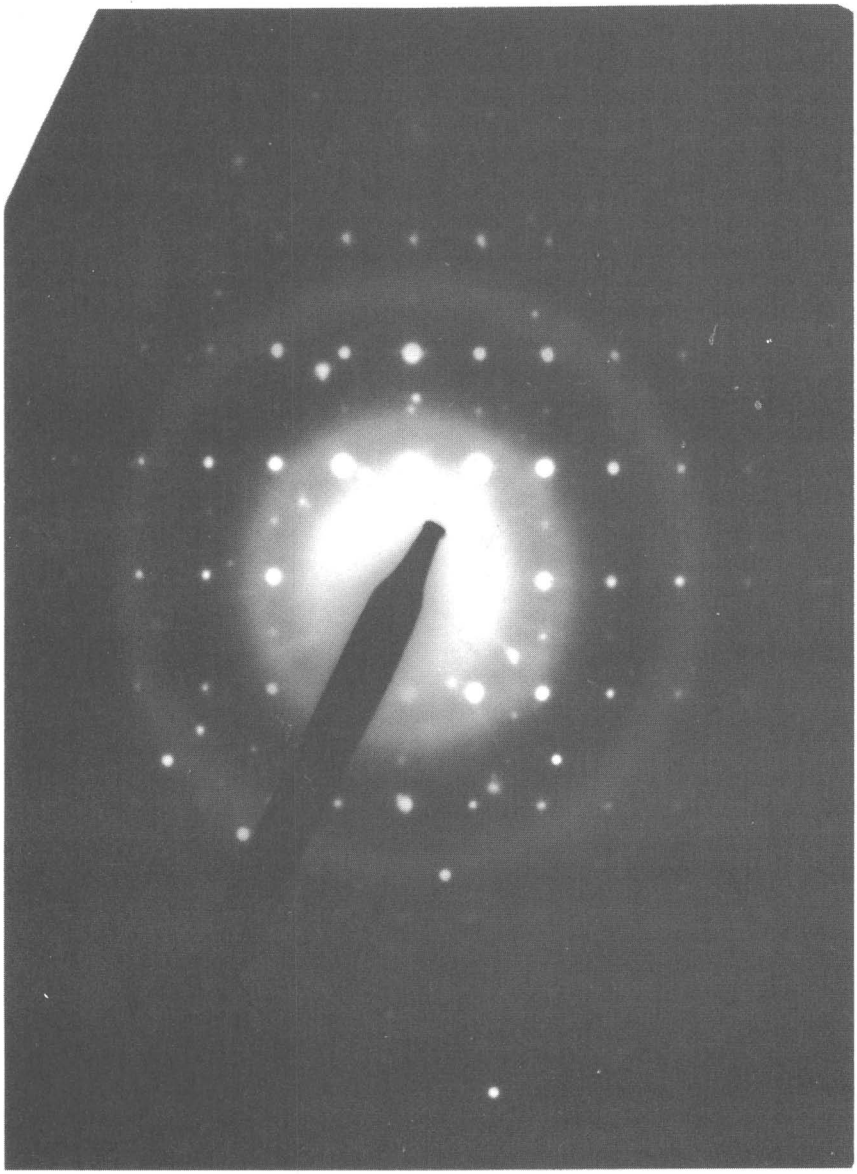

Figure 11b. The indexed selected-area electron diffraction pattern of this crystal, taken in the {211} zone. (A few miscellaneous spots are also present from the adjacent crystals seen in part a, and the faint row of spots midway between the bright rows are {011} and equivalent reflections that indicate the oxidation to maghemite) (Photo by A. Kobayashi). (Reproduced with permission from reference 70. Copyright 1992.)

Considerable support also exists for the hypothesis of magnetite-based magnetoreception in higher animals. Biogenic magnetite in honeybees is located primarily in the anterior dorsal abdomen (*66*), and small magnetized bits of wire glued over this location interfere with the bee's ability to learn to discriminate magnetic anomalies in conditioning experiments (effect 7 of Table I). Control bits of nonmagnetic wire, and magnetized wire placed elsewhere on the bees, do not interfere with the bee's ability to discriminate the magnetic cues. Hence, whatever the transduction mechanism, the magnetoreceptors must be in the anterior dorsal abdomen where the bulk of the magnetite is located. Furthermore, preliminary experiments with the pulse-remagnetization technique on bees trained to exit from a T-maze suggest that North-exiting animals can be converted to South-exiting ones, in a fashion similar to that for the magnetotactic bacteria (*41*).

In vertebrates, evidence for magnetite-based magnetoreception is slightly more circumstantial. As noted earlier, linear chains of magnetite crystals have been extracted from the vicinity of the ethmoid sinus in fish and birds, and nerves of the trigeminal system, parts of which ramify in the magnetite-bearing tissues, convey magnetic information to the brain. Unfortunately, the widespread existence of the axial magnetic compass ought to limit the ability to perform the pulse-remagnetization experiment on them. In theory, this type of experiment should be possible in mole rats (*29a*), salmon fry (*21, 28*), or newts (*22*), all of which display a polar compass response. However, despite the presence of the axial compass in birds, Wiltschko et al. (*79*) tried this experiment anyway and found a dramatic but temporary deflection in orientation. This finding implies that magnetite is indeed involved in the sensory system, but whether it is in the compass or intensity receptor is not clear.

Optical Pumping. ***Theory.*** Optical pumping is another potential biophysical mechanism that might enable organisms to transduce the geomagnetic field to the nervous system. This concept dates back to a suggestion by Leask (*7*), which was extended and developed further by Schulten (*8*) and Schulten and Windemeuth (*9*). The model in its more current form centers on the fact that chemical reactions can be influenced by two fundamentally different methods. The first method is to alter the energy levels of the reactants, intermediates, and products, and these steps are all governed by the well-known rules of statistical mechanics and thermodynamics. On the other hand, quantum mechanical constraints can also affect reactions, such as preventing two electrons with parallel spins from forming a bond. Such effects can easily block entire reaction pathways. As magnetic fields as weak as 0.1 mT can lead to significant hyperfine splits in orbital energy levels, the magnetic fields needed to produce this type of effect are much weaker (by factors of 1000) than those required to break the kT barrier with diamagnetic or paramagnetic effects. Numerous such reactions have been reported, some of which have even found commercial application, like increasing the molecular mass of polystyrene by photopolymerization in an applied magnetic field [reviewed by McLauchlin (*80*)].

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As a simple example, Schulten (*8*) discussed a situation in which the energy from a photon could lead to the breaking of a chemical bond, forming a pair of energetic electrons in a singlet state. As the spins in this electron pair would remain antiparallel immediately after the adsorption event, the back reaction (reforming the original covalent bond) is still permitted. However, because the singlet and triplet states of the pair are not in direct contact, the exchange interaction is negligible. Under these conditions, perturbation energies on the order of 10^{-8} kT can promote one of the spins to flip (or to evolve into one of the triplet states), thereby inhibiting the back reaction. This biochemical difference in subsequent reaction rates could then form the base of a magnetoreceptor function.

Experimental Evidence for Optical Pumping. Testing the optical pumping hypothesis is not such a simple matter, mainly because apparently no equivalent of the pulse remagnetization experiment, like that discussed earlier for the magnetite system, exists that could uniquely isolate optical pumping as the mechanism. However, the evidence presented so far is suggestive. First, an optically pumped system should yield an inclination compass, as has been observed in many groups of vertebrates. Second, using electrophysiological recording techniques, Semm and Demaine (*81*) found cells in the retina of pigeons that fired in response to magnetic stimuli. Third, behavioral studies in newts, migratory birds, and fruit flies (*18, 82, 83*) demonstrate that light of wavelengths that correspond to different receptor pigments has a strong influence on the compass orientation response, often shifting the mean orientation directions dramatically or abolishing the response completely.

On the other hand, the visual system exerts an extraordinary influence on many aspects of animal behavior, and all of the reports published to date could be explained by the interaction of the visual system with a separate magnetic sense. Similarly, the Semm and Demaine (*81*) preparations were done in vivo, and the vertebrate retina is known to receive inputs from elsewhere in the nervous system. Hence, the magnetic signals could have come from magnetoreceptors elsewhere in the bird, or, as magnetite has also been detected in vertebrate retinal tissue (*84*), the signals could arise from an occasional magnetite-based cell within the eye.

Two other problems further limit the viability of the optical pumping mechanism. First, it does not seem to be capable of accounting for the magneto-intensity sense described earlier. At best it could give an organism the axial direction of the field, and as such it cannot account for the polar compasses of honeybees, salmon fingerlings, newts, and mole rats. Second, it would have trouble functioning in the dark, and good evidence suggests that a broad spectrum of animals do not need light to perceive the magnetic field direction [e.g., bees (*41*), salmon (*21*), beetles (*85*), mollusks (*19*), turtles (*86*), and mole rats (*29a*)]. A chemically based "dark" reaction probably could not supply the energetic electrons necessary for this process to operate, as the chemical reactions needed for this process require access to molecular oxygen (*87*). Oxygen and highly toxic compounds are usually sequestered within the mitochondria, far

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away from the ordered membranes where the photopigments reside. Such a dark reaction should also produce an obvious side effect, as some of the electrons that were pumped up to higher energy levels would have the chance to decay spontaneously to their ground state, emitting visible photons in the process. Bioluminescence of this sort has not been reported in any vertebrate eye.

Discussion: Electromagnetic Field Effects, Biogenic Magnetite, and Magnetoreception

The preceding discussion indicates that the subject of magnetic field sensitivity (e.g., magnetoreception) in terrestrial organisms has evolved over the past two decades from a set of "fringe science" observations to one of serious scientific pursuit, and it now includes a body of highly reproducible effects. A variety of exciting and innovative experiments ranging from the behavioral, ultrastructural, and neurophysiological sciences have narrowed the range of potential transduction mechanisms, weeding them down to two major contenders: magnetite and optical pumping. In terms of the biological effects of environmental electromagnetic fields (EMFs), no other area of investigation appears to have such a solid foundation. For this reason, the biophysics of magnetoreception ought to be used as a starting point in any analysis of potential mechanisms for any reported biomagnetic effect, whether in whole animals or cells grown in tissue cultures. In particular, the discovery that magnetite biomineralization occurs in human tissues, even in structures that have no conceivable sensory role, argues that ferromagnetic bases for many deleterious EMF effects should be considered. This mechanism certainly raises far fewer objections about the thermal noise problem than virtually any other proposal.

Even though we as yet do not understand the function for all of the biogenic magnetite found in tissues, its mere presence has interesting implications for at least two areas of biomedical research. These include aspects of magnetic resonance imaging (MRI) and the possible health risks associated with low-frequency magnetic fields. MRI is one of the fastest growing fields in medical diagnosis. How and why the images show contrast between tissue types is a major field of investigation, but all MRI biophysical analyses so far have not considered the effect of biogenic magnetite. However, because a major component of the contrast is a function of the square of local magnetic field perturbation in a tissue, a simple calculation shows that the trace amounts of magnetite should play a significant role in providing this contrast.

Unfortunately, all past biophysical assessments of possible health effects from exposure to magnetic fields have assumed that human tissues did not contain ferromagnetic materials. In MRI, for example, the strong magnetic fields (which will soon reach up to ~5 T in clinical instruments) are often said to be too weak to break chemical bonds in diamagnetic or paramagnetic materials, and hence they cannot produce permanent physiological effects. This statement is completely untrue for a magnetosome, however, as its potential energy in an MRI instrument is over 1000 times the strength of the carbon–carbon bond en-

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In Electromagnetic Fields; Blank, M.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1995.

ergy and nearly 10,000 times more than hydrogen bonds. The million or so magnetosomes present per gram of brain tissue will rotate into alignment as a patient is inserted into the MRI instrument, and this rotation does have at least the potential to do cellular damage. (Translational forces due to the magnetic gradients are negligible.) On the other hand, most biological membranes act like flexible structures when they are deformed slowly, with time constants of about 10 Hz. Because strong magnetic fields of the sort used in MRI instruments act at very low frequencies, no permanent damage should result. Evidence suggests that they are certainly much safer than imaging techniques based on X-rays, which are known to initiate cancer.

At frequencies above about 10 Hz, however, biological membranes and other structures tend to behave in a more rigid, brittle fashion, and ultrastructural damage and other biological effects may be possible. Figure 4 shows a simple biophysical model of a magnetosome floating in the cytoplasm of a cell and connected to an ion channel; this model can be used to predict what frequencies and field strengths are necessary to produce an effect. The simplest biological effect that the motion of a magnetosome might produce is the opening of an ion channel in a membrane. These channels control the diffusion of ions and molecules into, out of, and within cells, and in turn these are fundamental regulators for many cellular processes. Many ion channels are also mechanically activated; applying enough tension to a cytoskeletal filament can cause them to open. They actually have little "gates" that open like trap doors to allow molecules to pass through. From detailed studies of these ion channels in hair-cell mechanoreceptors (*88*), we know that the little doors move through a gating distance of about 4 nM, and the opening force needs to be about 1 pN. The energy associated with opening the gates, a force times a distance, is $\sim 4 \times 10^{-21}$ J, which is $\sim kT$, the background thermal energy.

The problem is to determine which combinations of field strength and frequency will move a magnetosome enough to just barely open an ion channel. Two factors compete to influence this motion. On the one hand, the magnetic force on the magnetosome produced by an external oscillating magnetic field is trying to force the crystal to twist back and forth like a torsional pendulum. Resisting this torque are the viscous drag of the cytoplasm and the restoring torque of the static geomagnetic field. The resulting motion of the magnetosome and the attached ion channel is given by the solution of a fairly simple but nonlinear differential equation. These results show that we need 50- or 60-Hz oscillating magnetic fields of about 100 μT to contribute ~1 kT of energy to an ion channel from an individual magnetosome.

How does this result scale in biological systems, and how can we compare it to the alternating magnetic fields found in a modern, industrialized society? Two factors are important. First, the energy in a system responding to the magnetic field generally increases as the square of the field strength, that is, $\Delta E \sim B^2$, where ΔE is the energy change and B is the magnetic field strength. Second, the relative biological effect generally follows an Einstein–Boltzmann relationship, or $\sim \exp(\Delta E/kT)$. Hence, if we double the threshold field in our magnetosome

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model, the energy ΔE increases by a factor of 4 and the biological effect increases by e^4 or by a factor of ~54. Similarly, for fields below the threshold, the relative energy quickly falls below thermal noise, and magnetic effects will be difficult to detect (unless some special form of signal averaging occurs).

We can now compare this threshold level for biological effects with the measured extremely low frequency (ELF) EMFs produced by man-made objects in our environment, as summarized in Figure 12. Most of the magnetic fields produced by electrical power transmission and distribution lines are much weaker than the threshold levels discussed already, unless one approaches very close to them. On the other hand, electrical appliances like hair dryers, electric razors, and electric blankets do expose parts of our anatomy like our hands, fingers, and skin to alternating fields well above these thresholds. A 1-mT, 60-Hz magnetic field, for example, can give enough energy to a magnetosome to break covalent bonds (~100 kT). Because of the small size of most household appliances and the rapid decay of the field strength with distance, the actual volume of tissues that are exposed to these strong fields is usually quite small.

This discussion leads naturally to the important question of whether alternating magnetic fields actually produce harmful effects such as cancer in hu-

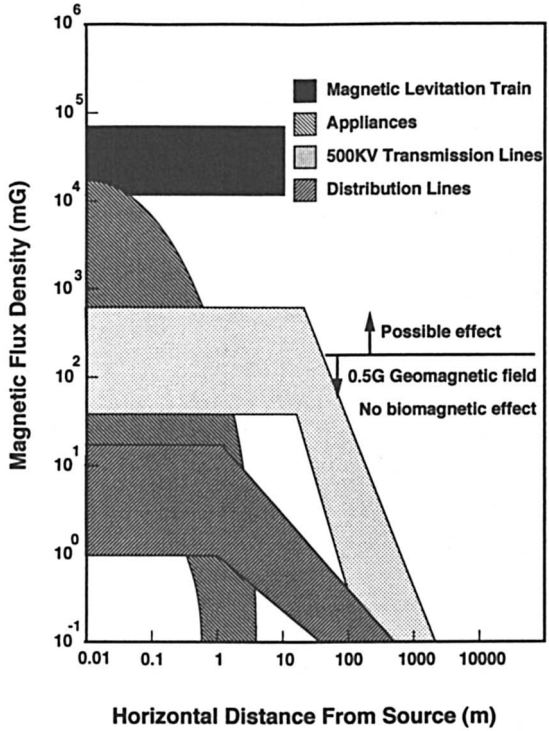

Figure 12. Magnetic field exposure depending on the distance and type of equipment.

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mans; this topic is now extremely controversial in the United States and Europe. The assessment of medical risk generally falls into two major categories of research, including animal experimentation and epidemiology. Although numerous studies of magnetic fields on mice and other laboratory animals have been done in the past 200 years, surprisingly little research has been done using continuous magnetic exposure systems on large numbers of animals to assess the effect on rare diseases. These studies are expensive, particularly when experimental controls are done properly. Several epidemiological studies have implicated slight increases of diseases such as childhood leukemia and brain tumors with electromagnetic exposure, but the underlying mechanisms are unknown, and the results need to be confirmed by replication. However, as a precautionary measure all electric blankets now sold in the United States have been designed to minimize external fields, and magnetic shielding materials are being incorporated into many electrical appliances. New power lines are being routed away from populated areas or buried in shielded cables. In 1992, the U.S. Congress authorized the National Institutes of Health and the Department of Energy to spend $65 million for research on magnetically related health effects over a 5-year period. We hope this new work will help us to understand the basic biophysical mechanisms through which magnetic fields interact with life at the cellular level.

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Acknowledgments

This study was supported by Electric Power Research Institute contract WO4307–03 and is contribution 5392 from the Division of Geological and Planetary Sciences at the California Institute of Technology.

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RECEIVED for review April 6, 1994. ACCEPTED revised manuscript January 3, 1995.

22

Magnetokinetic Effects on Radical Pairs: A Paradigm for Magnetic Field Interactions with Biological Systems at Lower Than Thermal Energy

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A common assumption is that any primary electromagnetic interaction with a biological system must at a minimum overcome the system's mean thermal energy, kT, *per degree of freedom to be biologically effective. Applied magnetic field (MF) modulation of coherent quantum mechanical processes such as electron-spin motion in "in-cage" radical pairs, however, represents a well-defined mechanism, termed the radical pair mechanism (RPM), that makes sub-*kT *biological MF effects (magnetic flux density* B ≈ *20–2000 μT) physically feasible. A two-stage model of RPM-mediated MF coupling to biological signal transduction–amplification pathways is presented. Special conditions are proposed that would allow for nonlinear, resonance-like intensity or extremely low frequency (ELF)-dependent MF bioeffects. This chapter further reviews (1) the physicochemical principles that underlie the RPM, (2) the evidence for RPM-mediated MF effects on biochemical in vitro reactions (biomagnetochemistry), (3) potential biomolecular targets (e.g., reactions involving NO•, $O_2^{\bullet-}$, •OH, or transition-metal ions) of the primary physical MF interaction in cells, and (4) secondary biological mechanisms such as Ca^{2+} and redox signaling.*

0065–2393/95/0250–0395$13.50/0

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch022

In Electromagnetic Fields; Blank, M.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1995.

WEAK MAGNETIC FIELDS (MFs) ranging from a few hundred microteslas to several milliteslas may modulate the rates of chemical reactions that involve transient free-radical states. This modulation has been demonstrated unambiguously in numerous experiments over the past 20 years (for reviews, *see* references 1–5). The application of static or time-varying MFs as a method for probing the existence as well as elucidating the reaction kinetics of radical intermediates has proven successful, for example, in bacterial and plant photosynthetic reaction centers, photo-induced electron-transfer processes, reaction pathways during the formation of organic radicals, and most recently in biochemical in vitro studies other than on photosynthesis. Importantly, mathematical models to describe magnetochemical effects on transient radical pairs (RPs) or biradicals have been developed (for reviews, *see* references 2–4). They predict the effectiveness of magnetic interactions with electron-spin dynamics in spin-correlated RPs; these interactions magnetically alter the chemical reactivity of the RP members with each other and involve magnetic interaction energies that are orders of magnitude lower than the energy of the reaction systems' background thermal noise, kT. For some organic chemical reactions, the quantitative predictions of the models have been experimentally confirmed (e.g., reference 6).

Nearly 20 years ago, the question of the possible significance of magnetochemical concepts in understanding the physical basis of MF interactions with living systems had already been raised (*2, 7, 8*). In 1976, Buchachenko (*7*) reviewed the then-known interaction mechanisms of MFs with chemical reactions and pointed out the possible importance of the radical pair mechanism (RPM) as an explanation of MF effects on "biochemical reactions involving species having unpaired electrons (e.g., processes involving the transfer of electrons along the chain of cytochromes and the associated reactions of oxidative phosphorylation, many enzymic reactions, the oxidation of nonheme iron by oxygen, [and] certain stages of photosynthesis)" (*7*). Recently, in light of the continued absence of a realistic biophysical model for describing MF effects, we (*9–12*) and others (e.g., *13–15*) again proposed a role for the RPM in biological MF interactions. For example, McLauchlan (*13*) pointed to the importance of the RPM in general terms, whereas we (*9*) suggested the possibility that the RPM, specifically in conjunction with nonlinear dynamic principles, might provide a biophysical basis for biological MF interactions. As one specific application we proposed a possible role for the RPM in the induction of MF effects on lymphocyte proliferation and cellular Ca^{2+} homeostasis via MF-dependent perturbations in the cellular redox state (*10, 12*).

The purpose of this chapter is to show that the possibility of direct magnetic interactions, in accord with the RPM, with cells, tissues, or the intact organism cannot be neglected a priori in the construction of any complete biophysical MF interaction model. This chapter specifically deals with (bio)magnetochemical effects from the application of static and extremely low frequency (ELF) time-varying MFs. Field effects from higher-frequency electromagnetic fields (EMFs) will not be considered. For clarification, the concept of "magnetochemical effects", as used in this text, refers to "magnetokinetic

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effects on radical pairs". The term "biomagnetochemistry" is introduced in analogy to the use of the term "bioelectrochemistry". The discipline of bioelectrochemistry is concerned with the application of electrochemical principles to biological problems, whereas the emerging field of biomagnetochemistry explores the possible role of magnetochemical concepts in biological function and regulation.

Thermodynamic Considerations

A major barrier toward acceptance by the general scientific community of the existence of biological EMF effects involving weak energy interactions is the apparent lack of a physically plausible mechanism (*16*). A number of theoretical studies estimated the minimum electric field intensities to influence a biological cell (*16–20*). Common to these calculations, based on purely electrical interactions, is that, at a minimum, mean thermal noise, *kT,* sets a fundamental physical constraint for processes in thermal equilibrium. It is therefore of interest to consider the possibility of direct magnetic effects on coherent electron-spin motion in transient RPs during biological free-radical generation, which can occur when the involved interaction energies are below *kT.* Furthermore, in weak, static, and ELF electric fields, the coupling site of the fields is limited to the outer surface of the cell membrane; the cell's interior is electrically isolated from externally applied electric fields. In contrast, the cell membrane does not present a barrier to MFs. Applied MFs pervade the whole cell and allow bioactive MF interactions within the cytoplasmic space and the cell nucleus, in addition to interactions at the plasma membrane level.

Several hypotheses have been put forward on the basis of direct MF interactions for explaining the results from bioelectromagnetics experiments. These hypotheses include models based on concepts such as cyclotron resonance (*21*), Zeeman interactions at ion-binding sites (*22, 23*), or what has been termed the "Larmor effect" (*24*). Another notable MF coupling mechanism evolved from the finding of biogenic magnetite in mammalian tissues (*25*). Kirschvink and coworkers (*see* chapter in this book and reference 25) suggested a model in which the torque induced by an MF on magnetite coupled to a membrane ion channel might alter channel activity. In summary, although some of the suggested mechanisms have been criticized on theoretical grounds, others, although theoretically promising, still lack convincing experimental confirmation at the cell biochemical level.

Magnetic-Field- vs. Electric-Field-Induced Cellular Field Effects

This chapter considers the possibility that the magnetic field vector may be the cause of some bioelectromagnetic effects. This proposition is not meant to imply that the electric field vector is unimportant in mediating bioelectromagnetic interactions. It remains an open question, however, whether electric fields induced

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch022

from the application of time-varying MFs to biological tissues (1) are fully sufficient to explain a field effect, (2) are biologically ineffective, or (3) might act in concert with the magnetic force to induce biological responses. The consideration of the MF as the active agent is of special significance for MF exposure experiments with single-cell suspensions and very weak induced electric fields ($E \leq 10$ mV/m). In such cases, the proposed electrical coupling over cell assemblies, through gap junctions to enhance the collective cellular electric field sensitivity (as discussed in reference 18), cannot be assumed. For example, my work (unpublished data) with suspension cultures of human leukemic Jurkat T-cells (radius of cell ≈ 5 μm) has demonstrated that cellular Ca^{2+} homeostasis can be significantly affected during a single, 120-s exposure to a 2-mT, 60-Hz sinusoidal MF with a maximum induced electric field of only 1.8 mV/m. None of the current approaches based on electrical interactions (*16–20*) could account for these effects for thermodynamic reasons. Still, the possibility cannot be excluded that future research will find that bioactive interactions of single T-cells, even with very weak electric fields (<2 mV/m), are feasible. Such interactions might theoretically be possible because of the potential nonlinear dynamic behavior of an as-yet-unknown interaction mechanism (e.g., *9, 10, 26*). Pending the availability of further information, it is reasonable to assume that the particular type of field interaction (electrical, magnetic, or possibly a joint electric and magnetic action) depends on the specific EMF-exposed biological system and the exposure circumstances.

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The Concept of Nonthermality in Bioelectromagnetic Experiments

The term "nonthermal" as used in the bioelectromagnetics literature has often been applied within different contexts. One frequently used definition of "nonthermality" is consistent with the absence of any detectable macroscopic heating. In other words, the global temperature rise in the biological sample due to the field influence is generally assumed to be <0.001 °C (i.e., such a small temperature rise is commonly assumed to be biologically insignificant). The second use is related to fundamental thermodynamic considerations. Here, the notion of nonthermality is that the energy deposition of the EMF in the exposed biological sample is $<kT$, the mean thermal energy per degree of freedom within the sample at the experimental temperature, where k is the Boltzmann constant and T is the absolute temperature. Nonthermality as used in this chapter is in accordance with the second, more stringent definition (i.e., energy deposition $< kT$).

Geomagnetic Flux Densities and the Radical Pair Mechanism

Can the RPM be relevant for describing chemical and biochemical effects from very weak MF intensities, such as the Earth's field (magnetic flux density $B \approx$

20–70 μT)? This question has already been addressed in a few theoretical studies that demonstrate that, in principle, such interactions are indeed physically possible. For example, Brocklehurst (*27*), on the basis of predictions from RP theory, concluded that "even very low fields, smaller than 1 G, such as the Earth's, can produce effects at ['in cage' RP life] times of 100 ns to 1 μs". As will be discussed, RP lifetimes on this order may occur under certain circumstances.

Schulten and co-workers (*8, 28, 29*) theoretically explored the RPM as a physical basis for a biomagnetic sensory mechanism that would allow organisms to detect the presence as well as the orientation of the Earth's MF. In this effort they calculated that effective interactions between geomagnetic flux densities and spin-correlated RPs (e.g., generated by biological electron transfer processes) are theoretically feasible. With regard to the results on the effects of MF orientation they predicted that "if a biradical reaction is spin-dependent and slow, i.e., the reaction rate is about 1 μs or slower, then external fields somewhat lower than 1 G can induce a significant orientational dependence" (*29*). With respect to lower MF detection limits of RPM-mediated (bio)chemical effects, they pointed out "that electron transfer processes which are ubiquitous in the metabolic pathways of biological cells can be influenced by magnetic fields of 10 G. However, there is no reason why fields as weak as the geomagnetic field of about 0.5 G should not also affect such transfer processes" (*8*). Recent experimental results showing presumably RPM-mediated effects on chemical or biochemical in vitro reactions from MF exposures as weak as $B \approx 1$–5 G (100–500 μT) will be discussed in later sections.

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A Two-Stage Model of Magnetic Field Coupling to Biological Systems

A simple scheme of the proposed temporal sequence of events during bioactive, direct MF coupling to cellular systems is shown in Figure 1. As illustrated in Figure 1, a distinction is made between primary physical interaction mechanisms and secondary biological mechanisms (*12*). The primary mechanism must explain how the effective field coupling is accomplished, at the microscopic level,

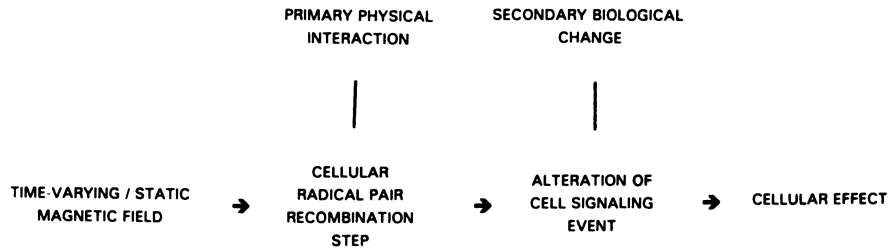

Figure 1. Proposed temporal sequence of events during magnetic field coupling to cellular systems via the radical pair mechanism. See text for details.

to an initial molecular "target" in the presence of thermodynamic noise. The secondary mechanism(s) must explain how the primary field coupling to the target is translated via biochemical steps (e.g., signal transduction–amplification pathways) to a response detectable at the macroscopic biological level.

In the model presented herein the primary physical interaction of a static or ELF time-varying MF is through its influence on magnetically sensitive cellular RPs or biradical recombination events. During this initial coupling step the external MF interferes with cell biochemical events, which specifically must involve free-radical intermediate states, for example, the formation of transient RPs or biradicals. As discussed later, thermodynamic laws are not violated by this type of field coupling, even though the involved interaction energies are $<kT$, because only the kinetics of a radical-mediated (bio)chemical reaction are affected, not its thermodynamics, hence the term "magnetokinetics" (e.g., reference 4). Consequently, the nature of the reaction product is not magnetically influenced; only the reaction rate is. If, however, the rate of a reaction was altered by an MF that was part of, or interacting with, a cellular signal transduction and amplification chain, even small rate changes might be translated into biologically significant secondary changes.

One candidate that we currently explore is the tight regulation of cellular Ca^{2+} homeostasis because evidence exists that free-radical-mediated changes in the cellular redox state may modulate Ca^{2+} fluxes (*12*). In particular, the recognition that intracellularly generated active oxygen species (*30*) and the nitric oxide radical (*31*) may act as messengers in cell signaling is highly significant in this context (for details and additional references, *see* later sections). Furthermore, multiple cellular targets might be affected simultaneously and thus give rise to complex interaction behaviors. Finally, RPM-mediated secondary biological effects may lead to cellular effects, such as on cell proliferation, differentiation, motility, secretion, carcinogenesis, and cytotoxic activity, which in turn may powerfully affect the physiology of an MF-exposed organism.

For this proposal key concepts of the physicochemistry of the MF interaction with spin-correlated RPs or biradicals are first outlined, and the available evidence for RPM-mediated MF effects on biochemical and related in vitro reactions is summarized. Then, selected cell functions that involve the generation of radical intermediates and therefore might potentially serve as cellular MF interaction targets are described. Finally, specific recommendations for future studies are given, the significance of the model for understanding potential adverse health effects is briefly discussed, and conclusions are drawn.

The Radical Pair Mechanism

Numerous reviews have been written on the RPM [for their listing up to the year 1988, *see* the review by Steiner and Ulrich (*4*)]. This literature demonstrates that (1) the existence of MF effects on chemical reactions is unquestionable on the basis of the experimental record, and (2) classical and quantum mechanical RPM models have been firmly established. In this chapter selected physicochemical

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch022

concepts that are critical to understanding the RPM are described and are illustrated by experimental examples when appropriate. This introduction to the RPM is written primarily for the reader with a biological background. The more physically oriented scientist, who is already familiar with the RPM, will probably find most informative the later sections, which suggest possible biomolecular targets of the primary field interaction and secondary biological mechanisms.

Radicals and Electron-Spin Effects on Chemical Reactivity

A radical is any atom or molecule with one or more unpaired electrons. Radicals can either be charged or neutral species, and they have, because of the magnetic moment of the unpaired electron spin, paramagnetic properties (i.e., radical species are slightly attracted to an MF). Radical species are often highly reactive, and an appreciation of the role of the electron spin in determining chemical reactivity is fundamental to understanding (bio)magnetochemical effects. Briefly, the respective reactivity of molecules is normally determined by the spin state of their outer-shell electrons, which means that chemical reactivity is spin-selective. Under certain conditions, electron-spin states can be modified by interactions with external MFs; these interactions change the molecule's subsequent reactivity. The concept of magnetic interactions with the electron spin is central to understanding the RPM. In the following sections, the specific physicochemical requirements will be summarized that make possible the interactions between applied weak MFs, static or time-varying, and electron spins that lead to MF effects on chemical and biochemical reaction kinetics.

Spin-Correlated Radical Pairs and Radical Pair Recombination

Radicals are formed as reaction intermediates in many (bio)chemical transformations. In general, radical molecules are created via homolytic cleavage of a precursor molecule. Diffusion, then, causes the cleavage products (i.e., the two members of the RP) to physically separate. Nevertheless, as a consequence of the principle of the conservation of spin angular momentum, the spins of the RP members initially remain correlated. To describe this special kind of reaction intermediate, the concept of the spin-correlated RP was first introduced by Kaptein and Osterhoff (*32, 33*) and Closs (*34*).

Immediately after its formation the spin-correlated RP is either singlet- or triplet-correlated depending on the ground state of the precursor molecule or the cleavage conditions. A spin-correlated RP at this stage is also called a geminate RP. The dissociation of a precursor molecule in the singlet ground state generates an RP whose spins initially are singlet-correlated (i.e., the electron spins have an antiparallel orientation relative to each other; $S = 0$). Correspondingly, for example, splitting of an excited triplet molecule leads to the formation of a pair of radicals with triplet-correlated spins immediately after pair formation (i.e., electron-spin orientation is parallel; $S = 1$). Whereas RP recombination is normally spin-forbidden in triplet-correlated RP members, in the singlet-

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch022

correlated RP a pair reencounter attributable to the antiparallel spin orientation of the RP's electrons can lead to the immediate re-formation of the precursor molecule. This step is known as the geminate reaction. An initially triplet-correlated RP, however, may still recombine if the relative orientation of the involved spins evolves to a singlet-correlated state before the reencounter probability is negligible. Conversely, spin evolution in an initially singlet-correlated RP may prevent RP recombination if the electron spins on the pair members evolve into the triplet configuration. The evolution of singlet (S) into triplet (T) states, and vice versa, both in the absence or presence of external MFs, depends on spin interactions with intramolecular magnetic fields, and this process is described as S–T intersystem crossing (ISC). The modulation by an external MF of S–T ISC rates is fundamental to understanding the physical basis of RP-mediated MF effects.

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Singlet–Triplet Intersystem Crossing

As stated, S–T ISC is the result of the interconversion of pure singlet and triplet spin states subsequent to RP formation. Spin conversion results from the fact that the magnetic moments of the spins of each of the odd electrons associated with the RP members experience different local MFs. The effective local MFs are defined primarily by interactions of the electron spins with (1) the MF associated with the orbital motion of the electron itself (spin–orbit coupling, SOC), (2) the magnetic moments associated with nuclear spins (hyperfine coupling, HFC), and (3) an external MF (Zeeman interaction). In principle, any of these interactions, or a combination of them, can lead to S–T interconversion among the members of a spin-correlated RP. Although SOC is a key mechanism by which S–T ISC may occur in organic molecules (especially those containing transition metal ions), this chapter emphasizes more external MF modulation of the HFC interaction. The reason is that the applied MF intensities required to influence HFC-induced ISC rates are typically much smaller than the lowest external MFs needed to modulate SOC-dependent ISC, such as in the triplet mechanism or the Δg mechanism (where Δg is the difference between g-values of radical-pair members), which will be briefly discussed later (for details, *see* references 2–4).

Hyperfine Coupling and Zeeman Interactions. Probably most relevant for understanding weak magnetic interactions in RPs is the HFC mechanism because for most reaction systems, the action of weak external MFs (millitesla intensities and less) on RP kinetics is primarily through modulation of the nuclear–electronic hyperfine interaction. Historically, the basic concepts describing the HFC mechanism come from the RP theory of chemically induced dynamic nuclear polarization (CIDNP; e.g. *32, 33*). HFC describes interactions of magnetic moments associated with the nuclear spins and the electron spin, individually. The magnitude of the hyperfine interaction is given by the HFC constant, a, commonly expressed in units of gauss (1 G = 10^{-4} T). The HFC

constant for free radicals in solution is available from electron-spin resonance measurements. For RPs for which the recombination kinetics are primarily controlled by hyperfine interactions, an external MF of a magnitude comparable to or even less than the intramolecular hyperfine fields may influence RP recombination kinetics.

HFC constants for small organic compounds are on the order of 1–100 G (*2*). Normally, however, hyperfine interactions are suppressed by the magnitude of the electron-exchange interaction, J_r. J_r depends on the overlap of the electron state functions of the RP. For example, close RP reaction partners have a comparatively high J_r value, for example, 1 eV at close distances. However, weak hyperfine interactions can lead to S–T ISC only if J_r is less than the hyperfine interaction energy, which is typically 10^{-6} to 10^{-7} eV. This requirement is met after pair creation, when the RP members diffuse away from each other and therefore J_r falls off rapidly. At this point hyperfine interactions become significant. While RP separation allows hyperfine interactions to occur, at the same time the RP members must remain close enough and not diffuse away too quickly to keep the RP reencounter probability significant. Thus, the applied MF effect on RP recombination arises from the competition between MF-dependent ISC and field-independent diffusion (*3*).

Generally, external MF modulation of the hyperfine interaction will decrease S–T ISC rates. This decrease is a consequence of the Zeeman splitting effect, in which the applied MF (B > HFC) removes the degeneracies between the triplet sublevels T_{-1}, T_{+1}, and T_0 (*see* Figure 2). At some MF strength, the triplet RP states $T_{\pm 1}$ become fully decoupled from T_0 and S; thus, only S–T_0 ISC remains probable, as illustrated in Figure 2. An overall decrease in the S–T ISC

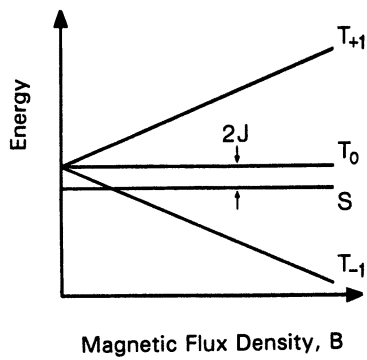

*Figure 2. Diagram illustrating the energy variation of the radical pair (RP) electron-spin states with increasing magnetic flux densities (*B*). Spatial separation of the radical pair members is shown to be constant as indicated by the constant value of the electron exchange energy (2J). At* B = *0, the energies of the three sublevels (T_0, T_{+1}, and T_{-1}) of the triplet RP are equal. Increases in* B *remove the sublevel degeneracies, which eventually shuts down the S–T_{+1} and S–T_{-1} interconversion channels.*

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rate is the result. For an RP generated in the singlet-correlated configuration, this result means that the RP recombination yield is increased upon application of an MF. Conversely, the respective recombination yield is decreased for a triplet-correlated RP because only T_0–S ISC remains a possibility, not, however, ISC via the T_{-1}–S or T_{+1}–S ISC channels. Another phenomenon associated with MF-induced triplet sublevel splitting is the crossing of T_{-1} and S energy levels; this crossing may occur only within a narrow MF intensity range; below or above that range, S and T_{-1} levels remain decoupled (*compare* with Figure 2). In this special case, the rate of S–T ISC increases at a certain external MF intensity as a result of T_{-1}–S level crossing, and field effects in the opposite direction may be observed (*2*). Biphasic MF effects may be observed by changing B from lower to higher intensities, most likely as a consequence of T_{-1}–S level crossing. An experimental confirmation of this predicted effect was reported, for example, by Hamilton et al. (*35, 36*).

One of the first experimental demonstrations combined with a quantitative theoretical description of the external MF modulation of RP kinetics in solution, in accord with the HFC mechanism, was given by Schulten et al. (*37*) and Werner et al. (*38*). These authors found that in the pyrene–3,5-dimethoxy-*N,N*-dimethylaniline (DMDMA) system in polar solvents, the triplet product yield could be decreased by a few percent as a result of an exposure to MF intensities as low as 1–2 mT. Since then the pyrene–DMDMA and related systems have been used in various studies that confirmed and greatly expanded the original results (*6, 35, 36, 39, 40*). Importantly, in a systematic study, Weller and coworkers (*6*) arrived at a mathematical model that correctly predicted the applied MF magnitude needed to cause half-saturation of the experimentally observed MF effect, termed $B_{1/2}$, on the basis of the calculated HF interaction energies of the respective RPs.

Besides the availability of a theoretical description of the MF effects on the model RP reaction, another issue of great interest within the context of this chapter is that of the lowest effective MF intensity. With a high-resolution detection technique, McLauchlan's group (*35, 36*) was able to resolve the direct effects on the pyrene–dimethylaniline system from magnetic flux densities that were, at least, as low as 100 μT. The field effect size at $B \approx 100$ μT, however, appeared to be on the order of less than a tenth of a percent. Nevertheless, these experiments show that MF intensities as weak as 100 μT, and possibly somewhat weaker, can measurably affect chemical reaction rates in vitro (*35*).

Δg Mechanism. For substantially higher MFs (ca. ≥0.5–1 T), magnetic interactions are mostly accounted for by the Δg mechanism. This mechanism describes S–T ISC in an RP because of modification of the Larmor precession frequencies of the two radical electron spins upon application of an external MF. In weak MFs (millitesla intensities), the Δg mechanism may be operative only in extreme cases, for example, when the difference between the g factors of the RP members is large, that is, Δg is 2 or more, such as is often found in enzyme active sites containing transition metal ions (e.g., Fe^{2+} and Co^{2+}). In typi-

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch022

cal, metal-free organic radicals, Δg is 100–1000 times smaller, that is, $\Delta g \approx 0.01–0.001$. Generally speaking, MF effects due to the Δg mechanism have an opposite sign compared to effects from hyperfine interactions; that is, $S–T_0$ ISC rates are always increased upon application of an external MF. Consequently, in the study of the MF strength dependence of RP recombination yields, biphasic MF responses can be observed when the recombination kinetics are dependent upon both the Δg mechanism (in stronger MFs) and the HFC mechanism (in weaker MFs). [For an overview of the Δg mechanism, *see* Steiner and Ulrich (*4*)].

The Cage Effect and Radical Pair Lifetimes

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch022

For the singlet-correlated members of an RP to recombine, they must encounter each other before they are irreversibly separated by diffusion and escape as (free) radicals into the surrounding medium. Radical encounters are a stochastic phenomenon, and several mathematical models provide estimates of the RP encounter probability (for reviews, *see,* e.g., references 2–4). The notion of the "cage effect", that is, the influence of the medium surrounding the RP members such as solvent molecules, is of critical importance. The idea is that the pair members are prevented from freely diffusing apart by restrictions imposed by the cagelike environment of the surrounding medium. In the cage the probability that the individual spin-correlated members of an RP will encounter the members from other RPs or nonradical molecules is low compared to the probability that they will encounter each other (*2*).

Pair recombination can either occur at first encounter of the RP members or at subsequent reencounters of the same pair members, provided, of course, that the respective electron spins are in an overall singlet configuration. Reencounter events will drastically enhance the RP recombination probability. The influence of the cage effect is thus to increase the recombination probability by increasing the reencounter probability before the pair members diffuse away. The reaction product resulting from recombination in the cage is known as the cage product or geminate product. The free radicals that escape the cage environment by diffusion before they can recombine (to form the cage product) encounter similarly escaping radicals or other molecules at random and form the escape product.

The lifetime of the spin-correlated RP is prolonged by the cage effect. One also speaks of the cage RP lifetime, in which the lifetime is the duration from RP creation to escape of the RP from the cage. Typical RP lifetimes are on the order of 0.1–10 ns. Much longer RP lifetimes, for example, several hundred nanoseconds to 1 μs, may occur in special circumstances that will be discussed later. Because external MF effects on spin-correlated RPs are induced by modulation of S–T ISC within the RP lifetime in the cage, the cage RP lifetime is thus a critical parameter in defining the MF sensitivity of RP recombination kinetics. The cage RP lifetimes, which make effective interactions of weak external MFs with RPs feasible, may range from the nanosecond to the microsecond time

scale. At RP lifetimes approaching the microsecond time scale, however, spin relaxation events may randomize electron-spin motion in RPs (*2–4*). Major factors that determine the magnitude of the cage influence are (1) the density of the medium (i.e., viscosity) and, for RP ions with opposing charges on each of the pair members, (2) the Coulomb attraction and consequently the dielectric constant of the reaction medium. For the purpose of illustrating these concepts, the qualitative effect of an externally applied MF on the recombination characteristics of an initially singlet-correlated model RP is shown in Figure 3. Also, special circumstances are described in which additional factors might further increase the cage RP lifetime and, hence, increase the susceptibility of the RP reaction to weak MF influences.

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Micellar Supercage Effects and Interface Phenomena

One circumstance by which the MF influence may be enhanced is exemplified by the so-called "micellar supercage effect" that occurs when RPs are enclosed in micelles (i.e., a secondary cage or "supercage"). The term "supercage" was coined by Turro and Weed (e.g., *41*), who observed a great enhancement of the MF effect on RP recombination in micellar media compared to homogeneous media. This influence on the recombination yield is thought to stem from the fact that "normal" cage RP lifetimes are even further extended by containing the RPs in micelles. In magnetochemical studies, micellar solutions are made, for example, by dissolving micelle-forming agents such as sodium dodecyl sulfate (SDS) in the reaction medium. For example, a 50-fold enhancement in cage RP lifetime in SDS micelles compared to aqueous solutions was observed (*42*). In biological MF exposure studies, micellelike structures containing RPs may be present in living cells, such as in intracellularly located lipid vesicles. Besides limiting diffusion in a micellar cage, there is also the possibility of magnifying the cage effect by restricting RP diffusion to a two-dimensional surface. Modeling has shown that the probability of RP reencounters is increased when diffusion is limited to a two-dimensional plane compared to unrestricted diffusion in three-dimensional space (*2*). Membrane interfaces that might act as planar diffusion limits are abundant in biological cells. In the next section another geometry that may enhance the cage effect is discussed, namely that represented by biradical molecules.

Biradical Recombination

A biradical molecule can be formed by the breaking of a single bond; the biradical formed has properties that can be described by the interactions of its two unpaired electrons (*43*). For many purposes the biradical can be considered a special case of a caged or geminate RP, and the basic physical manifestations describing the MF dependence of both the biradical and RP reactions are identical. However, the important difference between a biradical and an RP is that the spin interactions in the biradical (1) are dependent upon the geometry of its mo-

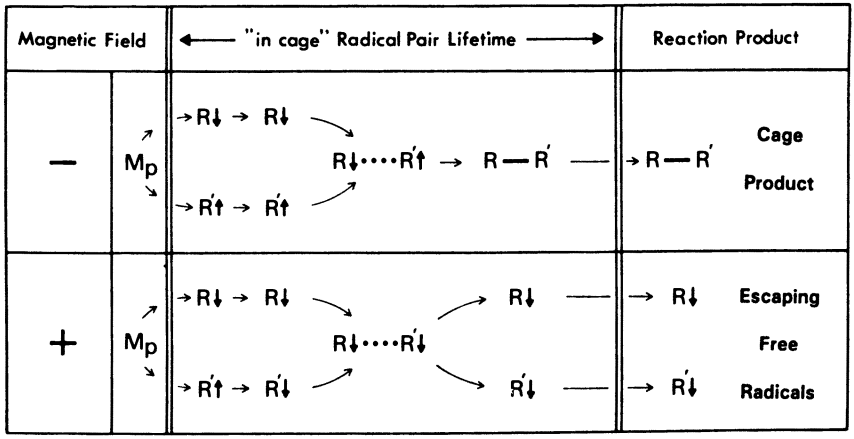

Figure 3. Illustration of the applied magnetic field (MF) effect on radical pair recombination. In the absence of the MF (–), the two members (R and R′) of the singlet-correlated RP, created by homolytic cleavage of a precursor molecule, M_p, reencounter each other within the cage RP lifetime. Upon reencounter of the RP members, because of the singlet overall spin state of the two odd electrons associated with each of the RP members, the RP recombines via covalent bond re-formation before the cage influence becomes negligible. The reformed molecule represents the cage (or geminate) product. In contrast, in the presence of the MF (+) the relative spin orientation evolves into an overall triplet configuration because of external MF modulation of the intrinsic S–T_0 ISC rate after pair creation. Upon reencounter of the two RP members, now in the overall triplet configuration, bond re-formation is spin-forbidden. As a result, the individual RP members diffuse apart again without undergoing a recombination event, and they escape the cage environment as "free" radicals. Subsequently, the free radicals react at random with each other or other molecules in the reaction environment. The resulting species represent the escape product. The chemical nature of the respective molecular species is not affected. The action of the applied MF is only to alter the ratio between cage and escape product yields.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch022

lecular configuration and (2) are also coupled to the motion of the biradical molecular chain (e.g., the hydrocarbon backbone of an organic molecule). Unlike paired radicals in solution, biradicals have the two unpaired electrons coupled to the same molecular chain, and therefore these cannot escape by diffusion. Because, as stated already, MF effects on RP recombination are diffusion-limited, the biradical configuration consequently may increase the magnitude of the MF effect. On the other hand, in short-chained biradicals in which the two radical centers are less distant from each other than in their long-chained counterparts, exchange interactions between the unpaired electrons may remain significant and consequently suppress MF-induced ISC.

The behavior, however, of long-chained biradicals often resembles that of a regular RP. An example that demonstrated large, biphasic MF effects on bi-

radical decay rates in strict dependence on molecular chain length was reported by Wang et al. (*44*). These authors investigated the MF dependence of the decay rate of 1,*n*-diphenyl 1,*n*-diyl biradicals (*n* is the number of carbons in the biradical chain) generated by photolysis of diphenylcycloalkanones. Whereas for $n = 4$ and 5 biradicals, no field effect was observed, for the $n = 9$, 11, and 14 molecules the ISC rate first increased and then decreased as the magnetic flux density was increased. The maximum stimulation of the ISC rates due to the MF was at 30 mT for $n = 9$, 12 mT for $n = 11$, and only 3 mT for $n = 14$ biradical molecules. Thus, this result was consistent with the prediction from theory that in shorter-chained molecules the action of weaker MFs would be suppressed more easily by electron exchanges. Work by Staerk and co-workers (*45*) with polymethylene-linked radical ion pairs that were generated by photoinduced intramolecular electron transfer in pyrene–$(CH_2)_n$-*N,N*-dimethylaniline compounds also showed a definite dependence of the magnitude of the MF effect on the biradical carbon chain length. For example, for one experimental condition the triplet yield of the $n = 9$ biradicals was maximum at $B = 21$ mT, whereas for $n = 11$ molecules a maximum enhancement effect was already reached at an eightfold lower flux density, namely, at 2.5 mT. Interestingly, in the latter case, when the flux density was raised to 4.3 mT, the field effect vanished. Further increases in the flux density even lead to significant reductions in the triplet yield (*45*).

Random Radical–Radical Reactions

Up to this point only MF effects on RP reactions of geminate origins, that is, on initially spin-correlated RPs, have been discussed. An applied MF may, however, also affect the reactivity of nongeminate RPs that are formed from random collisions. As pointed out by Cozens and Scaiano (*14*) and Scaiano et al. (*15*), MF effects on such random interradical reactions might also be of considerable importance for understanding biological MF effects because such reactions also determine the final concentration of bioactive free radicals. Radical–radical reactions continuously occur in all types of cells and tissues as part of their normal metabolism. As described before with geminate RPs, an applied MF may change bioactivity by altering the concentration of free-radical species with either positive or negative consequences for the organism. Recent experiments have confirmed that weak MFs ($B < 10$ mT) can indeed have dramatic effects on random free-radical encounters in vitro: For example, the self-reaction rate constant of benzyl radicals in micellar solution was reduced by 35% with $B_{1/2}$ at 12 mT. A minimum effective flux density was not reported, although their data still show effects at $B \approx 5$ mT (*14*). (For a detailed discussion of the special reaction conditions under which MFs may interfere with recombination events from random interradical encounters, *see* reference 14.)

Magnetic Field Effects on Biochemical and Related Reactions In Vitro

As described in the previous sections, the process of free-radical generation is under certain conditions susceptible to weak external MF influences in accor-

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch022

dance with the RPM. Singlet- and triplet-derived RPs of geminate origins, as well as random, nongeminate radical–radical reactions, are sensitive to the external MF influence. This sensitivity has been firmly established in magnetochemical experiments, and some of the results have been cited in this chapter. On the other hand, much of cellular biochemistry also involves the creation of singlet- or triplet-derived RPs and random interradical reactions. Therefore, their respective recombination rates may also be sensitive to the MF environment. For example, the activity of some cellular enzymes is thought to proceed via biradical recombination steps in the solid-state matrix of the enzyme active center involving transition metal ions, such as in cytochrome *P*-450s (*46*).

Importantly, the involvement of cage radical recombination steps has been proposed in cytochrome *P*-450 activity (*47*) and cobalamin enzymes (*48*). We had previously proposed that transition metal enzymes, like cytochrome *P*-450s, may act as primary MF coupling sites (*10*). Thus, we carried out a theoretical simulation study of magnetic field effects on *P*-450s on the basis of the RPM and found that flux densities on the order of 1–10 mT may indeed affect *P*-450 activity by a few percent (C. Eichwald, J. Walleczek, and F. Kaiser, unpublished data). With the exception of MF effects on photosynthetic reactions centers (for one example, *see* Table I; reference 49), only a few other experimental examples

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Table I. Static Magnetic Field Effects on Biochemical and Related In Vitro Reactions

Reaction System	B_{min}	$B_{½}$	B_{sat}	*Magnetic Field Effect*[a]	*Ref.*
Porphyrin–viologen dyad	~0.1	NR	5	50% reduction in recombination rate	55
Cytochrome *c*-oxidase	0.3	NR	NR	35% increase in redox activity	54
Potassium ferricyanide luminol oxidation	~0.5	1.5	10	increase in luminescence yield	57
Hemin-H_2O_2 oxidation of luminol	~0.5	3.0	12	increase in luminescence yield	5
Vitamin B_{12} cofactor methyl(Co^{III})cobalamin	~5	NR	50	25% reduction in rate of photolysis	51
Bacterial photosynthetic reaction center	~5	7.5	60	5.5% increase in fluorescence yield	49
Cytochrome *c*-oxidase	8–10[b]	NR	NR	40% increase in redox activity	54
Vitamin E phenoxyl–butyrophenone	~10	~40	500	2.6-fold increase in radical pair escape	56
Ethanolamine ammonia lyase	NR	NR	100	25% reduction in V_{max}/K_m enzyme kinetic parameter	52
Cytochrome *P*-450	800[c]	NR	NR	70% reduction in hydroxylation rate	53

NOTE: All values are in millitesla. B_{min} is reported minimum effective magnetic flux density (B); $B_{½}$ is B at which half-saturation of the effect is observed; B_{sat} is B at which effect saturates. NR is not reported; K_m is the Michaelis constant; V_{max} is the maximal reaction rate under saturation conditions.

[a]When given, the estimated effect size is for the highest listed flux density value.

[b]A 50-Hz sinusoidal magnetic field was applied.

[c]No lower B was tested.

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of MF effects on specific biochemical reactions have been observed. A review of MF effects on in vitro biochemical reactions that were reported prior to 1986 revealed no substantial evidence for such effects (*50*). A summary of results (*see* Table I) almost exclusively based on more recent reports, however, shows that such MF effects do indeed exist, including effects on the vitamin B_{12} cofactor methyl(Co^{III})cobalamin (*51*), coenzyme B_{12}-dependent ethanolamine ammonia lyase activity (*52*), cytochrome *P*-450 enzymatic activity (*53*), cytochrome-*c* oxidase activity (*54*), electron transfer in the porphyrin–viologen dyad (*55*), the vitamin E phenoxyl–butyrophenone reaction (*56*), and bioluminescent luminol oxidation by potassium ferricyanide (*57*) and the hemin–hydrogen peroxide system (*5*). (For a comparison, *see* Table I.)

Bioelectromagnetic Effects and a Possible Role for the Radical Pair Mechanism

Numerous effects from exposures to weak, time-varying MFs ($B \approx 0.1$–10 mT) in cell, tissue, or animal studies have been reported (for review, *see* references 9 and 58–60), and these include field effects on cells of the immune system (reviewed in references 61 and 62), the musculoskeletal system (*63*), the nervous and neuroendocrine systems (e.g., references 64 and 65), and a report of MF effects on in vitro tumor promotion (*66*). So far, however, only a few cellular MF studies have been independently replicated. Published successful replication attempts, for example, describe the effects of 3-Hz pulsed MFs on lymphocyte proliferation (*67, 68*) and lymphocyte $^{45}Ca^{2+}$ uptake (*10, 12, 67*).

The thesis of this chapter is that RPs or biradical species may be physically plausible, primary targets of MF interactions with cells, tissues, or the intact organism on the basis of the RPM. As summarized in Table I, MF exposure studies of biochemical in vitro reactions involving transient RP or biradical formation have already begun. These experiments have demonstrated that, under certain conditions, defined biochemical in vitro activity can be influenced by MFs ranging from a few hundred microteslas to several milliteslas (*see* Table I). Therefore, from the perspective of bioelectromagnetics, it is reasonable to begin to study the following questions. (1) What are potential biomolecular targets in cells that might serve as initial magnetic coupling sites, in accord with the RPM? (2) How could MF-modulated RP recombination reactions and, consequently, small product yield changes influence biological function and regulation? (3) Under what circumstances, if any, could RPM-mediated, resonancelike intensity-dependent or frequency-dependent MF effects be observed?

The Functional Role of Free Radicals and Radical Recombination in Biological Systems

The essential functional role of radical reactive intermediates is increasingly recognized in many different areas of biology and medicine (for an overview, *see* reference 69). In fact, many basic cellular functions have been shown to be

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partly regulated, or influenced, by radical species, such as in cell signaling (*30, 31, 70*), gene expression (*70, 71*), cell proliferation (*72, 73*), or macrophage activity (*31, 74*). Certain biologically generated radical species are tumor promoters and in some cases weak tumor initiators (*75–77*). Free-radical generation or reactions that proceed via radical intermediate stages are abundant in biological systems and may mediate many cellular functions. Thus, not only from a physicochemical perspective but also from a biological viewpoint, it is reasonable to explore the possible involvement of the RPM in the causation of nonthermal MF effects in living systems.

Of possible significance to understanding bioelectromagnetic effects are MF effects on cell biochemical reactions that can be grouped in the following three categories: (1) radical reactions that lead to the formation of unwanted free-radical by-products with adverse biological activity; a primary example of the damaging effects of radicals is lipid peroxidation; (2) radical reactions generating free radicals that have necessary functions in biological regulation such as potential cell signaling functions and immune cytotoxic activity; and (3) radical reactions involved in the reaction cycle of enzymes in which transient RPs or biradicals function as necessary reaction intermediates. Among the best-known biologically significant radicals are reactive oxygen intermediates (e.g., $O_2^{\bullet-}$ and •OH), the nitric oxide radical (NO•), and transition metal ions (e.g., Fe^{2+}, Co^{2+}, Cu^{2+}, Mo^{2+}, and Cr^{2+}).

However, instances of radical formation also involve other elements, for example, in so-called "protein-radical enzymes", which involve a free radical located on an amino acid residue, such as tyrosine, tryptophan, or glycine (for reviews, *see* references 78–80). Other examples are the oxidation of the thiol group (R–SH) or of NADH, which yields the sulfur-centered thiyl radical RS• or the NAD• radical, respectively (*69*). The following discussion deals only with reactive oxygen and nitrogen intermediates that have been shown to modulate cell biochemical activity. Any process that either generates or depends on these reactive species may serve as a primary MF coupling site for RPM-mediated cellular MF effects. This discussion is not meant to rule out the potential importance of the many other cellular radical reactions.

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Reactive Oxygen Intermediates

Reactive oxygen intermediates (ROIs), including the superoxide radical ($O_2^{\bullet-}$), the hydroxyl radical (•OH), and hydrogen peroxide (H_2O_2), are generated by electron reduction steps from molecular oxygen (O_2). Cellular locations that are known to generate ROIs include mitochondria, the endoplasmic reticulum, the nuclear membrane, the cytoplasm, and the cell membrane. Thus, because of the potential cell-damaging effects of ROIs, or conversely a potential regulatory role for intracellularly generated ROIs, the prooxidant state of the intracellular environment must be tightly controlled by the cell. This control is achieved, among other ways, by ROI-neutralizing enzymes like catalase, superoxide dismutase, or the glutathione–peroxidase system (*69*). Additionally, the pineal gland hormone

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melatonin was recently discovered to be a potent •OH scavenger (*81*). Thus, if an external MF interfered with the normal yield of ROI production or availability via RPM-mediated processes, this interference could upset the cellular redox state and lead to biological effects.

ROI Generation by Phagocytic Cells. One key element of the body's immune defense is phagocytosis, that is, the killing or digestion of pathogens by the phagocytic cells of the immune system. Phagocytosis involves the generation of $O_2^{\bullet-}$ and H_2O_2, mostly by neutrophils and macrophages. The increased consumption of oxygen that accompanies ROI generation in these cells is known as the "respiratory burst". The consumed oxygen subsequently is reduced by the NADPH-oxidase system, located at the plasma membrane, to $O_2^{\bullet-}$, and H_2O_2 is formed by dismutation of superoxide. $O_2^{\bullet-}$ and H_2O_2, together with enzymes (e.g., myeloperoxidase), are released into the phagocytic vacuole to kill and digest the pathogen (for review, *see* reference 69). An interference of an applied MF with these processes could lead to altered immune cytotoxicity.

ROI Generation by Nonphagocytic Cells. With the exception of ROI formation in the phagocytic cells of the immune system, until a few years ago it was thought that ROIs constituted only reaction by-products with negative biological activity. In the meantime new experimental evidence has emerged suggesting that cellularly generated ROIs may play a positive role in cell regulation and, indeed, possibly act as second messenger molecules (*30*). Evidence for this notion comes from experiments that detected ROI generation in nonphagocytic cells, as well as studies that suggest an important regulatory role for ROIs in cells. Evidence of the first kind was shown for various cell types, including human leukemic Jurkat T-lymphocytes (*82–84*), transformed human B-lymphocytes (*85*), and fibroblasts (*73, 86*). A regulatory role for cell-generated ROIs was suggested from studies that tested the involvement of ROIs in cell proliferation and during signal transduction events. For example, addition of $O_2^{\bullet-}$ concentrations, similar to the concentrations that are released by fibroblasts, were found to stimulate fibroblast proliferation, although at higher doses $O_2^{\bullet-}$ was toxic to the cells (*73*). Other examples of the effects of ROIs or antioxidants, that is, agents that either scavenge ROIs or inhibit ROI generation, on mammalian cell proliferation, including on HeLa and BHK-21 cells, have been reported (*72, 87*). Furthermore, in cells of the immune system, like T-lymphocytes, ROI generation may be critical for inducing proliferative events. For example, mitogen-induced murine T-cell proliferation was observed to be inhibited, in a dose-dependent manner, by antioxidants such as ROI scavengers or lipoxygenase inhibitors (*88*).

ROIs as Modulators of Signal Transduction Events and Gene Expression. A possible pathway by which MF effects on cellular proliferation could be mediated is through ROI modulation of cellular signal transduction

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and amplification steps, such as during Ca^{2+} signaling. Indeed, evidence exists showing that the lectin-triggered rise in intracellular free Ca^{2+} concentration, $[Ca^{2+}]_i$, in mouse and rat thymic lymphocytes is suppressed in the presence of the lipoxygenase inhibitor nordihydroguaiaretic acid (NDGA; references 88 and 89). In addition, our work has shown (reference 12 and unpublished data) that Ca^{2+} transmembrane influx and $[Ca^{2+}]_i$ in Jurkat T-cells is also affected by ROIs and antioxidants. Consistent with these findings is also the observation of NDGA-dependent inhibition of inositol phosphate hydrolysis in mitogen-stimulated human T-cells (*90*) and the inhibition of ornithine decarboxylase activity in concanavalin A -stimulated murine T-cells by NDGA and other antioxidants (*91*).

ROIs have also become known as inducers of gene expression. For example, Schreck et al. (*92*) found that the nuclear factor (NF) κB is an oxidative stress-responsive transcription factor in mammalian cells. The activation by ROIs of NF-κB upregulates the synthesis of new mRNA. In particular, NF-κB activation by tumor necrosis factor-α, interleukin-1, viruses, phorbol esters, UV light, or ionizing radiation, which leads to gene induction, was found to be mediated by intracellular ROIs (for review, *see* reference 70). On the basis of their results with Jurkat T-cells and other findings, Schreck and Baeuerle (*30*) proposed a role for ROIs in intracellular signaling and suggested that ROIs may constitute a new class of second messenger molecules. Evidence also suggests that ROIs may be directly involved in the activation of K^+ channels in various cell types (*93, 94*). Furthermore, Ca^{2+} adenosinetriphosphatase (ATPase) activity was also reported to be affected by ROIs (*95*). In line with these findings, Cerutti (*77*) proposed that ROIs "may act as a mediator and activate signal transduction pathways that ultimately modulate the expression of immediate early genes such as *fos* and *myc* as do the phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (TPA), serum, and certain polypeptide growth factors". In summary, the preceding evidence suggests that ROIs are intimately involved in the mediation of cellular signal transduction steps. Therefore, MF-induced changes in cellular ROI homeostasis via the RPM may lead to cellular field effects, including effects on ion transmembrane transport and gene expression.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch022

Reactive Nitrogen Intermediates

Reactive nitrogen intermediates are biologically highly active species (for reviews, *see* references 31 and 74). Specifically, the gaseous free radical nitric oxide (NO•) is now recognized as an important physiological regulator. To date, two separate functions of NO• have been characterized: first, its importance in the bactericidal and tumoricidal activity of macrophages (*74*), and second, as a regulator of vascular tone and as a neuronal signaling molecule (*31*). New evidence also suggests (*96–98*) that reactive nitrogen intermediates have inhibitory as well as stimulatory effects on lymphocytes, particularly on lymphocyte proliferation. In neurons and endothelial cells, nitric oxide is generated by the enzyme nitric oxide synthase (NOS), which is constitutively expressed in these cell

types. In macrophages, however, NOS activity must be induced by activation of the macrophage by cytokines or pathogens.

In 1992 spectroscopic and biochemical evidence appeared establishing NOS as a new member of the cytochrome *P*-450 heme protein family (*99, 100*). Evidence was also presented indicating that NOS contains a flavin semiquinone radical that might magnetically interact with the NOS heme iron during the NOS catalytic cycle (*100*). As mentioned before, we found theoretical evidence that NOS/*P*-450 activity may be altered by MFs on the order of 1–10 mT (Eichwald, Walleczek, and Kaiser, unpublished data). In summary, MF modulation of the yield of NO• generation, or of NO• interactions with ROIs or other reactive nitrogen intermediates, could interfere with neuronal signaling events, lymphocyte activity, and macrophage antitumor activity.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch022

The Question of Resonancelike Intensity or Frequency Dependencies

Nonlinear magnetic flux density dependencies of the resonance type (intensity "windows") for well-defined chemical systems have been experimentally demonstrated and, importantly, have been theoretically explained on the basis of the RPM (for reference citations, *see* earlier sections). The observation of intensity windows in biological MF exposure experiments is, thus, in line with the RPM. In contrast to nonlinear intensity-dependent effects, resonancelike frequency dependencies in the ELF range cannot be accounted for by the RPM alone. In general, the physicochemical principles that underly the RPM exclude this possibility. In principle, however, ELF-dependent, RPM-mediated MF effects in biological system might still occur. The possibility proposed here does not stem from the physics of the RPM but from the functional organization of biomolecular or biological systems themselves. That is, any biological activity that depends on the periodic generation of reactive radical intermediates may not only be susceptible to the specific intensity but also the temporal characteristics of an oscillating MF. In this scenario, the existence of a mechanism for the periodic generation of radicals must be postulated, whereby the (parametric) coupling of an appropriately oscillating MF via the RPM would allow the induction of resonance like frequency-dependent MF effects on a particular biological endpoint.

One such option is the frequency-dependent coupling of oscillating MFs to cyclical radical steps that occur during the reaction cycle of certain enzymes, such as *P*-450s (Walleczek, in preparation). Another possibility would appear if a functional role for oscillating concentrations of bioactive species at the cellular level could be demonstrated. A functional role for oscillating bioactive species is not a new concept. Repetitive or oscillatory increases in the concentration of second messenger molecules such as Ca^{2+} during cellular signal transduction have been documented in a variety of cell types (reviewed in references 101 and 102). For example, oscillations in the cytoplasmic free Ca^{2+} concentration, $[Ca^{2+}]_i$, have been proposed to be part of a digitally encoded cell signaling mechanism. In this scheme, cellular signals are not only transmitted by a simple

increase in $[Ca^{2+}]_i$, but by the repetitive nature of the Ca^{2+} signal. The frequency of the $[Ca^{2+}]_i$ oscillations ($f \approx 0.001$–3.0 Hz) is believed to transfer crucial information across cytoplasmic regions (*101, 102*). Similarly, repetitive or oscillatory concentration changes in radical second messenger species (e.g., ROIs and reactive nitrogen intermediates) may be part of the inter- or intracellular signaling network. A model based on the theory of nonlinear self-sustained oscillators, which describes the influence of externally applied ELF EMFs on signal transduction pathways involving oscillating concentrations of second messenger species, has been worked out by Eichwald and Kaiser (*26*) for Ca^{2+}. A similar approach could be taken to model the case of free-radical messengers. At present, it is not known, however, whether oscillating concentrations of free-radical intermediates do exist in cells and, if they do, whether they could play any role in modulating cellular functions. Only new experiments will be able to clarify whether oscillating cytoplasmic concentrations of free radicals have a biological function. In summary, a resonancelike MF amplitude dependence might be due to physical characteristics of the RPM alone, whereas an RPM-mediated, nonlinear MF frequency dependence in the low-frequency range would only occur because of the intrinsic biological characteristics, for example, periodic free-radical processes, of an MF-exposed system.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch022

Recommendations

As outlined in the previous sections, efforts to study the potential role of the RPM in the induction of nonthermal MF effects on biological systems seem justified, both from a physicochemical as well as a cell biological perspective. More experimental and theoretical work, however, is needed to be able to conclusively evaluate the importance of biomagnetochemical concepts in cellular bioelectromagnetics. Future steps should involve (1) the development of new methods for the detection of real-time MF effects with 0.1% or better resolution, which should enable the systematic study of the MF action on biochemical in vitro activity even at very low intensities ($B < 0.1$ mT); (2) the construction of mathematical models that describe RPM-mediated effects from weak MFs, including the effects from the combined action of static and time-varying MFs on RP recombination events; and (3) the determination of physicochemical information with regard to relevant biomolecular structures (e.g., transition metal enzymes). This information is needed for modeling biomagnetochemical effects, which are of direct relevance to the current questions in bioelectromagnetics. Data concerning, for example, g values, HFC constants, or RP lifetimes for most biologically relevant RPs or biradicals are still lacking. As mentioned before, modeling approaches for describing MF effects in comparatively simple chemical systems, in which the appropriate parameters are known, have proven successful (e.g., *6, 38*). For this reason similar successes may be achievable for biomolecular RP-forming systems, pending the availability of the needed physicochemical information.

In Electromagnetic Fields; Blank, M.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1995.

A Rationale for Understanding Possible Adverse Health Effects from Magnetic Field Exposures?

With respect to the epidemiological findings that suggest a statistical link between environmental EMFs and increased cancer risk in humans (*see* other chapters in this book), it is unclear whether the proposed model could provide a mechanistic rationale. For example, it is unknown whether long-term exposures (i.e., many years) of an organism to much weaker field intensities than have been discussed in this chapter, for example, as weak as ~1 μT, 50–60-Hz MFs, may affect a health outcome on the basis of primary MF coupling to RP recombination steps. One reason is that the lowest effective magnetic flux densities that are still capable of altering biologically relevant chemical reaction rates have not been determined, either theoretically or experimentally. Another reason is that no information is yet available on the capacity of a living organism to amplify subtle biomagnetochemical primary effects from oscillating MF exposures via highly nonlinear secondary biological interactions. More research is needed to determine whether the MF interaction mechanisms discussed herein can provide a rationale for explaining potential adverse health effects from MF exposures ≤1 μT. However, for large magnetic flux densities associated with clinical magnetic resonance imaging techniques ($B_{AC} \approx 0.1$–1 mT; $B_{DC} \approx 1$–2 T), it seems reasonable to consider the possible safety implications on the basis of the already available information (*see* Table I for comparison of flux density values).

Conclusions

Many of the EMF effects reported in the bioelectromagnetics research literature may potentially be a direct consequence of the interaction of the MF vector with biological biradical or RP recombination processes. Like for all other proposed magnetic interaction mechanisms (*21–25*), as yet no direct proof exists for the importance of the RPM in the bioelectromagnetic interactions discussed in this chapter. Nevertheless, at present, what sets the RPM apart from the other proposals is that (1) the RPM is an already well-understood and proven mechanism for describing nonthermal (sub-kT) interactions of relatively weak MFs ($B \approx 20$–20,000 μT) with chemical reaction systems and (2) experimental evidence exists for RPM-mediated MF effects on biochemical in vitro activity at intensities that are comparable to the intensities applied in many cellular bioelectromagnetics experiments (*see* Table I).

The usefulness of external MFs as a probe for identifying selected radical recombination steps in biological systems seems limited, however, because of the possibility that many different biradical or RP-dependent processes may simultaneously occur during biological activity. In contrast, the use of MFs as a probe in well-defined reaction systems (e.g., on the activity of transition metal enzymes) may yield important insights into the role of radical intermediates in biochemical reactions and thus help identify primary physical MF coupling sites in cells. First examples for this approach have been given in Table I. In any case,

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch022

the theoretical and experimental demonstration of sub-*kT* energy EMF effects on RP recombination processes, in conjunction with the finding that free radical and transient RP or biradical generation is critically involved in bioregulation, suggests that magnetokinetic effects on radical pairs represent a physically plausible research paradigm for bioelectromagnetics (*12*).

Acknowledgments

Funding for the Bioelectromagnetics Laboratory at Stanford University is provided by the Fetzer Institute. Comments on the manuscript by C. Eichwald and the anonymous reviewers are gratefully acknowledged.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch022

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Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch022

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RECEIVED for review March 8, 1994. ACCEPTED revised manuscript December 15, 1994.

23

Biosynthetic Stress Response in Cells Exposed to Electromagnetic Fields

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Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch023

*The biosynthetic response of cells exposed to electromagnetic (EM) fields has been studied at both transcriptional and translational levels. In addition to changes in the steady-state levels of several genes, including the protooncogenes c-*myc *and c-*fos*, increased transcript levels for the heat shock (stress) gene, HSP70, have been measured in samples from cells exposed to EM fields in the absence of elevated temperatures. Furthermore, the distribution patterns of proteins synthesized in response to EM fields resemble those following heat shock (HS). The cell responds to both EM and HS stimuli in a similar way, and the same sequence of changes with increasing energy occurs, even though the energies of the two stimuli differ by many orders of magnitude. When EM and HS stimuli are given simultaneously or sequentially, the protein biosynthetic response is indistinguishable from that of either HS or EM field alone. Results suggest that EM fields stimulate a pathway that is similar to the one used by cells in response to thermal shock and other stresses. EM fields appear to induce the normal biological response to stressful conditions, and this apparent interaction has implications for possible health effects.*

EXTREMELY LOW FREQUENCY (ELF) electromagnetic (EM) fields, present in all environments, may pose health risks. Epidemiological studies have shown (*1–3*) a low but significant risk of cancers in populations exposed to

0065–2393/95/0250–0423$12.00/0

In Electromagnetic Fields; Blank, M.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1995.

power-frequency (50- and 60-Hz) EM fields. Although several interpretations of the epidemiological data are possible, studies have shown that risk ratios cluster around 2, and in some occupational settings ratios of 6 and higher have been reported (*4, 5*). A recent study from Sweden (*6*) reported that the frequency of childhood leukemia increased approximately 2.7 times in homes in which the calculated average magnetic field exceeded 2 mG (0.2 μT) and that exposures above 3 mG increased the risk ratio to 3.8 (*6*). Occupational studies on seamstresses who use electric sewing machines for many years show a 4–6-fold increase in the incidence of Alzheimer's disease among this population (*7*). The persistence and weight of the epidemiological findings demand the discovery of a biologically plausible mechanism.

The likelihood that EM fields may affect humans is strengthened by evidence that time-varying EM fields have beneficial effects in accelerating the healing of bone fracture nonunions (*8*). To date, questions of EM field interactions with cells and their possible relation to cancer, as well as their clinical effectiveness, have motivated scientists to seek explanations for how these fields cause changes in cells.

ELF EM fields (<300 Hz) have wavelengths that are much larger than the dimensions of the structures with which they interact (*9*). It is unlikely that the fields can perturb transmembrane potentials because their energy level is too low to act through physical mechanisms (e.g., dielectric breakdown and electrophoresis) (*10*). These characteristics of ELF EM fields raise questions about how they could cause changes in biological processes (*11*).

The lack of a consensus on the mechanism of action of EM fields on cells is apparent in the diversity of scientific opinion concerning the cellular response to such low field strengths. At the same time, growing evidence suggests effects of EM fields on many biological properties. These include increases in the growth-related enzyme ornithine decarboxylase (*12*), changes in the ion-pump enzyme Na^+/K^+-adenosinetriphosphatase (Na^+/K^+-ATPase) activity (*13*), changes in protein synthetic patterns (*14*), altered cell-surface properties (*15*), altered endocrine and hormone expression (*16*), modulation of calcium flux (*17*), and altered quantities of steady-state levels of RNA transcripts (*18–20*). Inability to replicate reported findings for some studies (e.g., increases in c-*myc* steady-state levels in HL-60 cells) is undoubtedly due to failure of the replication studies to faithfully follow the original protocol (*21a–21c*).

Heat Shock as a Model for the Biosynthetic Response to Stress: Transcription

Recent data show (*21d*) that protein biosynthetic responses to EM field exposure are similar to those reported for sudden elevated temperature (heat shock). Activation of the stress response results in elevated and preferential synthesis of a family of stress-induced or heat shock proteins and inhibition of the synthesis of other proteins. Activation of stress gene expression and the resulting synthesis of heat shock proteins ensures survival under suboptimal stressful physiological

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch023

conditions. The stress-induced proteins (some of which are also known as "molecular chaperones") have essential roles in transport, translocation, and folding of proteins. The induction of eukaryotic heat shock genes is mediated by the heat shock factor, a transcriptional activator that binds to the heat shock elements in the promoters of the heat shock genes in a sequence-specific manner.

The stress response can be initiated by many stimuli, among them oxidative injury, viral infections, heavy metals, and free radicals (*22*). The genes encoding heat shock proteins are among the most highly conserved and exquisitely regulated. The molecular sizes of the classical heat shock proteins are mainly 104, 90, 70, 60, and 20–30 kDa. The majority of studies have concentrated on the HSP70 family of genes. The list of conditions known to induce HSP70 gene expression have been subdivided into three broad categories (*22, 23*). They are environmental stresses (including heat shock, amino acid analogs, and heavy metals); nonstress conditions (including normal cell growth and development, differentiation, and activation by certain oncogenes); and pathophysiology and disease states (e.g., antineoplastic chemicals and viral and bacterial infection). On the basis of experimental data discussed in this chapter, EM fields appear to be a form of environmental stress (*24, 25*).

It is important to determine the mechanism(s) used by the cell to detect and quantify physiological stress, as well as to determine how this information is transduced to the transcriptional and/or translational apparatus. The responses to increasing temperature and increasing EM field follow a similar sequence with increasing stimulus energy (*21d, 25*). Differences noted between EM field and heat shock protein patterns appear to be due to the acceleration of certain cellular reactions at the abnormally high temperatures of heat shock (*25*). Because only a very low energy is needed to elicit a cellular response to EM stimuli, the use of low-energy magnetic fields is an ideal and relatively nondisruptive tool for unraveling the stress response without the detrimental effects to the cell caused by changes in temperature, heavy metals, inhibitors of energy metabolism, or amino acid analogs.

Our initial hypothesis was that measurable changes in cells exposed to ELF EM fields would be reflected in changes in gene expression; splicing, transcription, processing, translation, and/or posttranslation might also be affected. Such changes could provide a unifying factor for the diverse effects that have been reported in cells and tissues exposed to ELF EM fields. Changes in steady-state levels of specific transcripts have been measured following exposure of *Escherichia coli* (*19*), *Drosophila,* and *Sciara* salivary gland cells (*26–28*); *S. cereviseae* cells (*29*); and a variety of human cells (*18, 30-33*) to ELF electric and magnetic fields.

Evidence that ELF EM fields act directly at the transcriptional level, rather than in the processing or storage of mRNAs, is shown by analyses of transcription autoradiograms of *D. melanogaster* and *Sciara coprophila* salivary gland cells (*26–28*). Salivary glands exposed to 60-Hz continuous sinusoidal and time-varying EM signals (20 min) showed increased transcription over specific chromosomal regions that were identified using published maps. Increased transcription was seen at the chromosomal locus for the HSP70 gene (locus 87AD),

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch023

as well as for other heat shock genes. The change in the HSP70 locus is particularly dramatic because the chromosomal region forms a "puff" in response to the stress; this response is an easily identified morphological expansion of the chromosome (Figure 1). The changes observed on the chromosomes from cells exposed to EM fields differ from the transcriptional patterns in the unexposed control chromosomes. Thus, EM fields appear to definitely initiate transcription per se. They may also increase mRNA stability or release mRNA storage forms, but this effect has not been reported.

EM fields have been shown to interact with the regulatory regions of both the *myc* gene and the HSP70 gene. In the c-*myc* gene a specific 900-base-pair region of the c-*myc* promoter is responsive to EM fields (*34*). We also have preliminary evidence that the *myc* binding sites on the HSP70 promoter are required for transcriptional response to EM fields (unpublished observation). Therefore, a

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch023

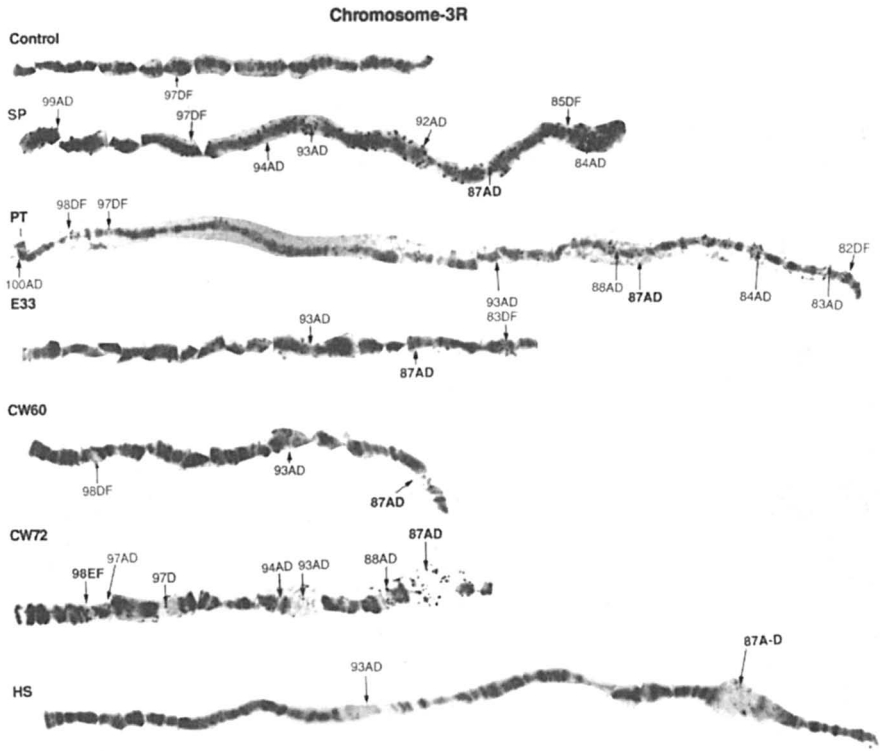

Figure 1. Autoradiogram of chromosome 3R from Drosophila *showing transcriptional activation of heat shock (HS) locus 87AD, which codes for hsp70 protein. Salivary gland cells were exposed to each of five different electromagnetic (EM) fields for 20 min. SP is single pulse; PT is pulse train; E is equine; CW is continuous sine wave. (Reproduced with permission from reference 28. Copyright 1992 Wiley-Liss.)*

coordinate increase in c-*myc* and HSP70 transcript levels by EM fields would be expected. The c-*myc* gene, as well as c-*fos* and c-*jun,* is regulated by heat shock in human lymphoid cells (*35*); c-*myc* protein binds to the HSP70 promoter regions and is involved in the regulation of this gene (*36*). Furthermore, overexpression of c-*myc* induces translocation of the endogenous hsp70 protein from the cytoplasm to the nucleus, where it becomes sequestered (*37*).

Further evidence that cells respond to EM fields by overexpressing heat shock genes comes from experiments in both *S. cerevisiae* and *E. coli.* In yeast, transcript levels for the SSA1 gene (a member of the HSP70 multigene family) were significantly increased within 10 min in cells exposed to an 8-mG, 60-Hz EM field (*29*). An increase in groEL/ES proteins (homologous to proteins for hsp60) was observed when *E. coli* were exposed to magnetic fields (*38*).

The stress–response system is a useful model for the mechanism by which cells exposed to EM fields overexpress some genes. Calcium, second messengers, and kinases, all of which may be implicated in the EM signal-transduction pathway, are known to be involved in activation of transcription of stress genes. Identifying the receptor for physiological stress would provide an important clue in determining the initiation of EM field interaction. From the foregoing evidence it appears likely that the cellular response to EM fields is part of a general physiological response to stress.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch023

Similarities and Differences Between EM Fields and Heat Shock: Translation

As expected, the synthesis of the stress protein hsp70 was increased by EM field exposure in both *Drosophila* and *Sciara* salivary gland cells (*14, 21d*). Furthermore, the overall protein pattern produced under EM field exposures was similar to the pattern seen following heat shock, including increased synthesis of major stress proteins in the 30-, 70-, and 90-kDa ranges.

Examining the effects of EM fields on protein synthesis enables one to scan the gene products of virtually all the genes that are being expressed. Thus, the response of the entire population of proteins, as well as changes in individual proteins, can be viewed. The use of two-dimensional gel electrophoresis allows the separation and tracking of proteins according to their molecular weight (MW; size) and isoelectric point (pI; charge). The amount of radioactive label ([^{35}S]methionine in this case) incorporated is a measure of the amount of each protein synthesized in a given period.

These data are converted into protein distribution curves in terms of either the molecular weight of polypeptides synthesized (%mass vs. MW) or their isoelectric points (%mass vs. pI). The results of repeated experiments are averaged, and the effects of different exposure conditions are compared. Analyses of protein distribution patterns show the level of synthesis of the proteins being expressed during EM field exposure (as compared with those in the unexposed controls) and provide evidence of changes in biosynthesis. Changes in the synthetic activity of individual proteins have also been followed.

In Electromagnetic Fields; Blank, M.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1995.

The effects of EM fields on protein synthesis have been studied using the larval salivary glands of the dipteran *S. coprophila* as a model system. Among the many advantages offered by dipteran salivary gland cells is that these cells are in synchrony and always in metabolically active interphase. We have used this well-described system to examine the effects of exposure to different field strengths and different levels of thermal stress as a way of characterizing the responses of the cells to varying energy levels. The MW and pI distributions of newly synthesized ^{35}S-labeled polypeptides were analyzed. Distribution curves for polypeptide synthesis in cells exposed to EM fields were compared with polypeptides in cells subjected to thermal shock (heat shock) (Figures 2 and 3). The use of either stressor showed new peaks in the distributions of proteins at MW of 30 kDa and pI of 7.1 compared with unstimulated controls. Similar changes of %mass were also found at approximately 20, 35, and 70 kDa. These molecular weights are within known ranges of major stress-response proteins. A shift of the maximum in pI distribution from 5.8 to 6.3 was part of the response to both EM and heat shock stimuli.

The distribution patterns of proteins synthesized by salivary gland cells of *Sciara* in response to EM fields strongly resemble those following heat shock (*25*). Also, a similar sequence of changes occurs with increasing stimulus intensity of both 60-Hz EM fields and heat shock temperatures. Two of the features in the MW distribution, the %mass in the 30- and 70-kDa ranges (regions with well-defined heat shock proteins), show a similar pattern in %mass with increasing stimulus intensity. Figure 4 shows the effect on %mass of both the

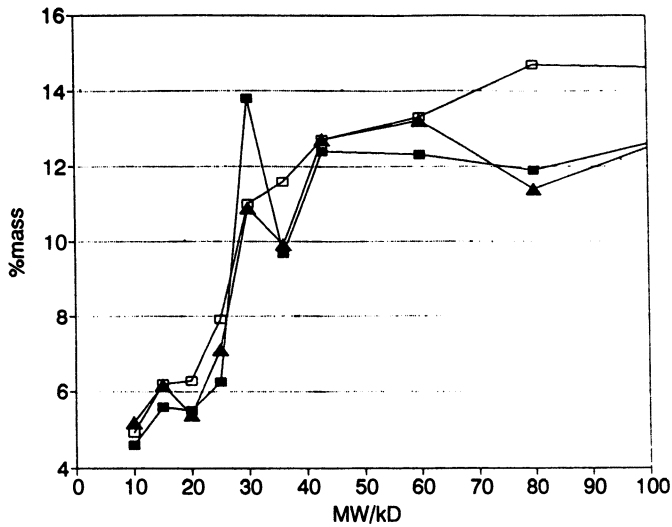

Figure 2. Distribution of mass as a function of molecular weight (MW) for EM-field-stimulated (0.8 μT; ■), heat shock (HS)-stimulated (▲), and control (□) protein synthesis. (Reproduced with permission from reference 21d. Copyright 1993 Elsevier.)

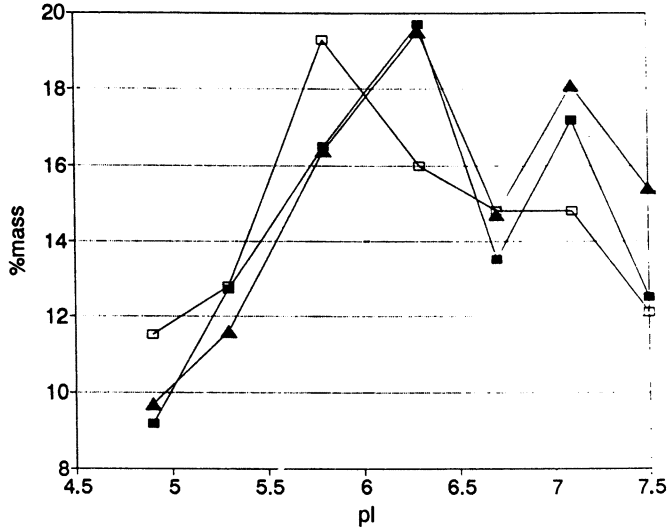

Figure 3. Distribution of mass as a function of isoelectric point (pI) for EM-field-stimulated (0.8 μT; ■), HS-stimulated (▲), and control (□) protein synthesis. (Reproduced with permission from reference 21d. Copyright 1993 Elsevier.)

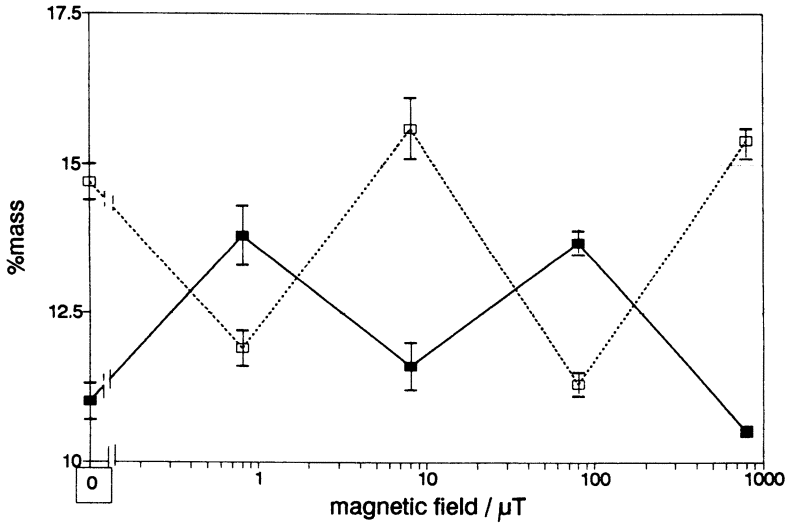

Figure 4. The variation of the %mass of 30-kDa (■) and 70-kDa (□) MW ranges vs. an increasing intensity of the magnetic field on a logarithmic scale. (The 30-kDa range is 29–32 kDa; the 70-kDa range is 66–84 kDa.) The points are averages of three experiments with standard errors indicated. (Reproduced with permission from reference 25. Copyright 1994 Elsevier.)

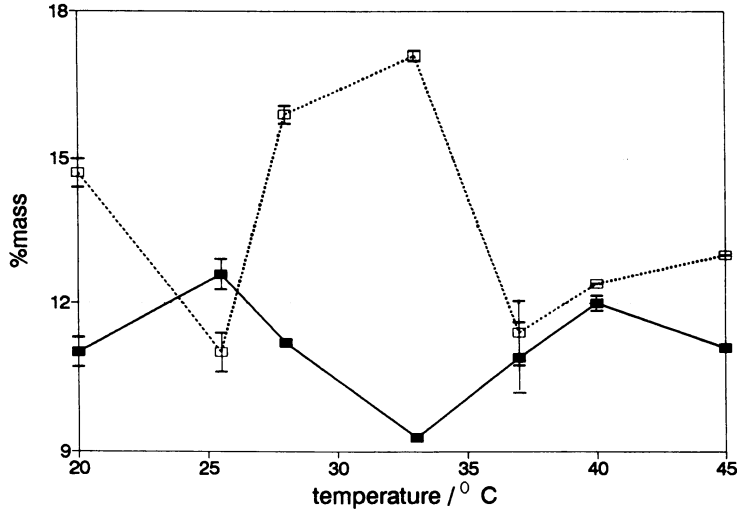

Figure 5. The variation of the %mass of 30-kDa (■) and 70-kDa (□) MW ranges vs. the temperature at which the HS experiment is carried out. (The 30-kDa range is 29–32 kDa; the 70-kDa range is 66–84 kDa.) The points are averages of three experiments with standard errors indicated. (Reproduced with permission from reference 25. Copyright 1994 Elsevier.)

30- and 70-kDa MW ranges from increasing magnetic field (0.8–800 μT). The zigzag pattern represents an alternation in the synthesis of these two major protein groups. Figure 5 shows a similar effect on the %mass of 30- and 70-kDa MW ranges due to increasing temperature (25.5–45 °C), with some distortion due to accelerated reactions at high temperatures. The induction of the heat shock response in dipteran cells is normally studied at 33–35 °C.

Even though the energy ranges are very different for EM field and heat shock, a similar pattern for 30 and 70 kDa occurs with increasing temperature and increasing magnetic field strength. The small differences between the responses to EM field and heat shock are probably due to the detrimental effects of the elevated temperatures in heat shock. The proteins that are more readily degraded at elevated temperatures have lower %mass in the heat shock experiments than in EM fields (*21d, 25*).

Changes in the pI distribution also show similarities between EM field and heat shock patterns with increasing stimulus intensity, and the differences are probably due to the changes in the heat shock experiments as a result of elevated temperatures (*21d, 25*). The changes of %mass due to both EM and heat shock stimuli show a relatively flat response in the middle of the range studied, with a return to control levels at the extremes. The ranges studied appear comparable and include the full spectrum of response for both thermal and magnetic field energy scales.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch023

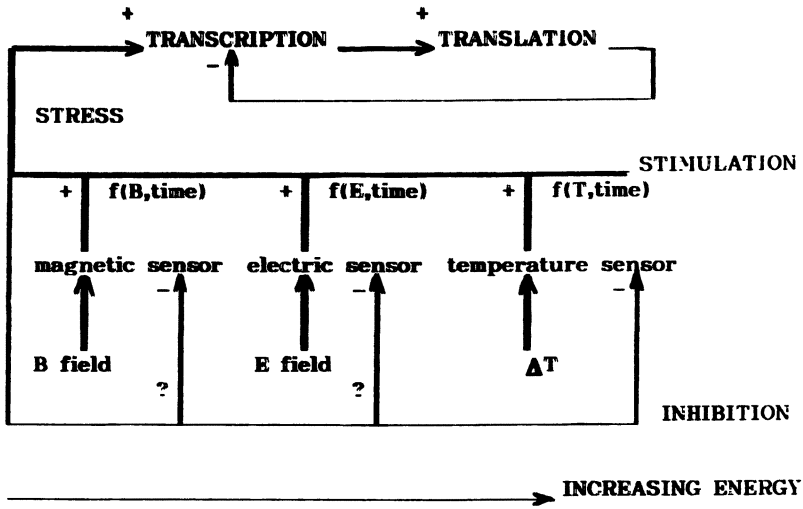

Figure 6. Flow chart showing model for the stimulation of transcription and translation via excitatory (+, heavy line) and inhibitory (–, thin line) stress pathways. E *is electric;* B *is magnetic;* T *is temperature. (Reproduced with permission from reference 25. Copyright 1994 Elsevier.)*

Additional evidence that the same response system is being stimulated by both EM field and heat shock has come from experiments exposing cells to both stimuli simultaneously or sequentially (*21d, 25*). The stimuli were deliberately chosen to exaggerate differences in the 30- and 70-kDa regions by using an EM stimulus of 80 μT (high synthesis of 30 kDa and low synthesis of 70 kDa) and a heat shock temperature of 33 °C (low synthesis of 30 kDa and high synthesis of 70 kDa) (*see* Figures 4 and 5). The responses to the two stimuli appear indistinguishable from the response to a single stimulus and are independent of the order of application.

The cellular responses are the same even though the energies of the two stimuli differ by many orders of magnitude. The overall pattern of data suggests that the cellular response to either EM field stress or thermal stress is via a common final pathway. A hypothetical control circuit (Figure 6) diagrams the properties we have described. These results suggest that EM fields stimulate a pathway that is similar to the one used by cells in response to thermal shock (and other physical stresses) and that the changes in protein distribution observed in our experiments may be characteristic of a general cellular response to stress.

The Cellular Stress Sensors

When cellular responses to EM and thermal stimulation are compared, the MW and pI distribution patterns are markedly similar, and similar sequences are ob-

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served with increasing stimulus intensities. However, the energy of the EM scale is much lower, the EM threshold is very much lower, and the two scales also differ in linearity. With reasonable assumptions about physical properties, we can calculate and compare the energy densities at the lowest stimulus intensities that elicit a response: increases of 0.8 μT for EM fields and 5.5 °C for heat shock. If a magnetic permeability comparable to air is assumed, then the energy density of the magnetic field is 2.6×10^{-7} J/m^3. If a heat capacity and density comparable to those of water are assumed, then the energy density for a 5.5 °C rise in temperature is 2.3×10^{7} J/m^3. The two values differ by 14 orders of magnitude!

An examination of electric and magnetic field stimulation of transcription in HL-60 cells allows us to compare the energy densities at the stimulus intensities that elicit similar responses (*39*). A maximum response occurred at 8 μT for magnetic fields and 5 mV/m for electric fields. If an electric permittivity at 60 Hz comparable to water is assumed, then the energy density due to the electric field is 9.0×10^{-3} J/m^3. The energy density due to the magnetic field is 2.6×10^{-5} J/m^3, or 300 times lower. Both fields stimulate biosynthesis, and one of the transcripts measured in the HL-60 cells is for the heat shock protein hsp70, so the experiments are comparable. These results suggest that the electrical energy needed to stimulate the sensor is at an intermediate level between the magnetic and thermal sensors. Table I incorporates the value of the electric field needed to stimulate transcription in HL-60 cells, along with the EM and heat shock values calculated from the *Sciara* experiments, to give a quantitative estimate of how the energies that activate the response vary with the modality.

The very low energy threshold of the "magnetic sensor" may indicate that the response of the "thermal sensor" could be due to a nonspecific reaction of the magnetic sensor. This mechanism is unlikely because the slightest increase in temperature would trigger a massive response from the magnetic field sensor, and this response is not seen. Also, the response to each stimulus varies with the energy of the stimulus, and the similarity of the responses suggests that the different sensors feed into a final common path. Furthermore, because of the great difference in sensitivity, no cross talk can exist between the sensors for different modalities. It is more reasonable to assume a separate sensor with its own sensitivity for each modality. These ideas are incorporated in the circuit diagram (Figure 6), together with the known inhibitory feedback of the heat shock system

Table I. Energy Densities of Different Stimuli of Biosynthesis

Modality	*Input*	*Energy Density (J/m^3)*
Magnetic	0.8 μT	$B^2/2\mu = 2.6 \times 10^{-7}$
Electric	5 mV/m	$\varepsilon E^2/2 = 9.0 \times 10^{-3}$
Thermal	5.5 °C	$\Delta T c \rho = 2.3 \times 10^{7}$

NOTE: B is magnetic flux density; E is electric field strength; T is temperature; μ is permeability, ε is permittivity; c is specific heat (in calories per gram per degree); ρ is density (in grams per cubic centimeter).

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Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch023

after stimulation. Preliminary experiments suggest that a common inhibitory feedback pathway for EM stimulation may exist, but such a pathway remains to be determined.

Imagining a cell sensory apparatus that can respond similarly to such widely different energy ranges is difficult, but the sensor may not be like a traditional receptor. Physical stimuli, including EM fields, may affect biological processes via a membrane-mediated mechanism (*40a*), and this mechanism may help explain how similar effects might be obtained with EM and thermal stimulation. Each physical stimulus could lead to the same end point by a different route. The real stimulus may be a decreased ionic gradient across the membrane. Alternating current from electric or magnetic fields results in a decrease of the ionic gradients across the membrane. Heat tends to permeabilize the membrane structure and to decrease ion gradients across the membrane. Such a mechanism would allow the different physical stimuli to operate through the same membrane-mediated mechanism and lead to similar effects. The sensor would be the membrane, and each stimulus would affect a different aspect at a particular energy level. A recent report shows (*40b*) that lipid bilayers in vitro are disturbed by electric fields, although at very much higher energy levels. However, in vitro transcription (the stimulation of transcription by EM fields in a cell-free medium) has been reported (*41*), and membranes may not be essential. Recent reports of electronic conduction within the DNA may indicate (*42a*) another possible mode of action of magnetic fields.

The activation of a common signal-transduction pathway by different kinds of stimuli was recently reported (*42b*) in another system. Nuclear responses to growth factors and cytokines appear to go through a common pathway even though they are activated by different cell-surface receptors. The diagram we propose may therefore be indicative of a more general property of cells. The diagram not only summarizes the observations but also indicates experimental approaches that could elucidate such ideas as the possible commonality of the inhibitory feedback pathway. If this pathway exists, then tempering the response of cells to a stress should be possible by prestimulation with a different stress or, for example, with one of lower energy. Responses in these experiments could be measured by the presence of the heat shock protein hsp70, which is increased in all of the stressed systems we have studied.

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Intersection of Stress Response and Oncogenic Pathways

With respect to the possible role of EM field exposure in cancer, 60-Hz fields may be more likely to promote tumor development than to initiate it. This view is supported by studies showing that exposure to EM fields accelerates tumorigenesis in experimental animals exposed to carcinogens (*44*). The failure to demonstrate chromosomal disturbances, DNA cross-links, changes in DNA repair, or sister-chromatid exchanges in cells exposed to 60-Hz EM fields is more consistent with a role in tumor promotion than in tumor initiation (*45, 46*).

The activation of stress gene expression resulting from exposure of cells to EM fields offers one possible explanation that could be related to neoplasia. The stress-induced proteins have essential roles in transport, translocation, and folding of other cellular proteins. The overexpression of stress genes in a number of human tumors has prompted Morimoto (*23*) to suggest that the presence of stress proteins, characteristic of a variety of neoplasias, may even be used as markers for the disease.

The molecular biology of the stress response provides one of the best-characterized paradigms for inducible gene expression. Many different stresses stimulate this response (*23, 47, 48*). Both electric and magnetic fields act as stimuli. Our results suggest a possible link between EM field exposure and malignancy through the overexpression of stress genes and increases in stress proteins. The hsp70 proteins are known to interact with oncoproteins. In addition, protein substrates that form stable complexes with hsp70 proteins include viral oncoproteins, SV40 large T antigen, polyoma virus middle T antigen and adenovirus E1A, as well as the cellular oncoproteins c-*myc* and mutant p53. The human hsp70 promoter has been shown to be regulated by p53 (*49*), and mutant p53 has been identified in at least 50% of all human cancers.

The possible deleterious effects of EM fields are public health matters and require attention. The critical gaps in our knowledge call for further research to develop a clear and unified explanation that would link all the reported effects of EM fields through a definition of the mechanisms involved. Clearly one of the mechanisms to be considered is the biosynthetic stress response that has been conserved through evolution to cope effectively with a variety of environmental stimuli.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch023

Acknowledgments

We thank the Electric Power Research Institute, the National Institute of Environmental Health Sciences, and the Department of Energy for their support.

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RECEIVED for review March 8, 1994. ACCEPTED revised manuscript January 12, 1995.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch024

24

Membrane Signal-Transduction Mechanisms and Biological Effects of Low-Energy Electromagnetic Fields

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The cell membrane represents a significant barrier between the extracellular environment and the interior of the cell, both in terms of electrical resistance and in terms of information transfer. Any attempt to explain the effects of low-energy electromagnetic fields (EMFs) on cells requires explanation of how the very low energy produced by environmental EMFs can overcome the barrier of the cell membrane. One plausible postulate is that low-energy fields may interact with already existing membrane signal-transduction mechanisms, which possess extremely high sensitivity and specificity for detecting and transducing low levels of signal in the extracellular environment. In this chapter, basic mechanisms of membrane signal transduction are reviewed, and specific examples of low-energy EMF effects on these processes are cited. A biochemical model is developed for possible interactions between EMFs and intracellular signal-transduction processes.

ENVIRONMENTAL ELECTROMAGNETIC FIELDS (EMFs) have been implicated as a possible causal factor in observed correlations between development of childhood cancers and proximity to high-current power lines (*1*). A major difficulty in accepting such fields as the cause of these correlations has been the lack of convincing data to indicate that fields of such low strengths can interact with

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cells in any way that might lead to development of cancer or other disorders (*2*). For example, the electric potential produced in tissues by exposure to power lines in the average household are in the range of 1 V/m (*3*), whereas the membrane potential of most mammalian cells is about 10^7 V/m. Nevertheless, a number of biological activities such as predation and navigation by animals have been shown to respond to changes in electric potential as small as (or even smaller than) the value of 1 V/m (*4*). This laboratory has studied the interaction of low-energy nonionizing EMFs with cells and tissues in a system in which low-energy EMFs are generally acknowledged to have biological effects: the stimulation of bone formation and fracture healing. Bone healing stimulated by electromagnetic energy has been a widely used and very successful therapeutic model for a number of years (*5*). In addition to fracture healing, a number of reports demonstrate that bone turnover rates can be modulated by EMFs, and this response suggests that EMFs may be a possible therapy for osteoporosis (*6*). A number of laboratories have proposed (*7*) mechanisms by which EMFs can alter bone metabolism; most have focused on the interaction between osteoblasts and regulatory agents such as hormones and growth factors. Our laboratory seeks to examine the mechanisms by which EMFs interact with cells, especially those that may be involved in production of changes in cell growth or differentiation. Our results indicate that EMFs can have effects on bone cells by modifying the sensitivity of membrane signal-transduction systems.

In this chapter the focus is on possible biochemical mechanisms that may be influenced by EMF exposure and not on the direct biophysical interaction between the field and membrane molecules themselves. The subject of biophysical models of interaction has been reviewed elsewhere in this book and in several recent reviews (*8*). In this discussion it is assumed that exposure of cells to a field existing in the external cell medium (e.g., the extracellular fluid in an animal) changes the properties of a susceptible type (or types) of molecule on the external surface of the cell membrane and that this change in properties is reflected by a change in function of one or more signal-transduction systems existing in the membrane (*4*). Other hypothetical biophysical mechanisms such as direct magnetic interaction with intracellular metals (*9*) may also operate either in parallel with or alternatively to the models discussed here.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch024

Receptor-Mediated Signal Transduction

The cell membrane presents a significant barrier to charged molecules (e.g., peptide hormones and neurotransmitters) that may carry a regulatory signal from one cell type to another. Similarly, the membrane, because of its high resistance, presents a major barrier to electric currents that are flowing in the medium outside the cell. The mechanisms developed by living cells to sense the presence of signaling molecules outside the cell have many of the properties that are required to receive an electrical signal as well: for example, sensitivity, amplification, rectification, and transduction can be accomplished by the enzyme systems residing in cell membranes. The receptors for hormones, neurotransmitters, and

growth factors thus are logical targets for examination as the sites of EMF effects. It is necessary to understand the properties of such signal-transduction systems before we examine the possible ways in which EMFs may interact with the systems.

One key feature of membrane signal-transduction systems is that the activity of the overall pathway is largely under the control of a protein molecule known as a receptor. Receptors bind the signaling agent and pass on the signal to other components of the cell. Receptors may or may not have enzymatic activities of their own. Agents that bind to a receptor are called ligands. In general, specific ligands have a high affinity for their receptor, and the target tissue has a limited number of receptors; thus the specific binding sites tend to become saturated at low concentrations of ligand. Only specific ligand binding generally results in a metabolically relevant response to the signal. The low concentrations of receptors in membranes is made up for by an extremely high affinity and specificity for the corresponding ligands. The signal-transducing activity of receptors is biochemically distinct from their ligand-binding properties; often the two activities reside in structurally distinct domains of the molecule. Receptors are susceptible in most cases to a variety of modulating agents, both intracellular and extracellular, such as antagonists, drugs, toxins, ionic composition of the medium, and, in the present case, electromagnetic energy.

In many systems target cells are much less sensitive to a second dose of signaling ligand than to the first dose. This phenomenon is known as "desensitization" or "downregulation". These two terms are often employed to refer to two different mechanisms of decreasing tissue receptor responses. Downregulation usually refers to a process that produces a decreased number of binding sites for ligand in the tissue. The best established mechanism for this phenomenon is internalization and degradation of ligand–receptor complexes. Desensitization, on the other hand, refers to a diminished amplification of signal by membrane enzymes or other intracellular transduction processes. In several well-known systems (e.g., adrenergic receptors), this decrease in transduction capacity is mediated by phosphorylation of the receptor.

Many different biochemical types of receptors exist, each of which utilizes a different mechanism for transmitting the extracellular signal into the cell for further effects on biochemical pathways. Each receptor has, at a minimum, a portion of the molecule that binds ligands, a portion of the molecule that pierces the cell membrane, and a portion of the molecule that interacts with other intracellular or membrane proteins to modulate their functions. Many receptors also have their own enzymatic activities that begin the cascade of events generating changes in cell function. Examples of different families of receptors are the tyrosine kinase family [e.g., the insulin receptor (*10*) and the epidermal growth factor receptor (*11*)]; the G-protein-linked receptor family (*12*), including receptors for epinephrine and many other ligands; the ion-channel receptors (*13*), many of which interact with neurotransmitters; the nerve growth factor receptor family (*14*); the guanylate cyclase family of receptors (*15*); the tyrosine phosphatase family (*16*); and the T cell receptor and homologous receptors for lymphokines and cytokines (*17*). One benefit to the organism that is derived from

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having such complex mechanisms for membrane signal transduction is that the pathways can interact synergistically, a phenomenon known as "cross talk" (*18*). This cross talk increases the degree of amplification of signal and also allows for sophisticated control at a multitude of steps. Another benefit is that the multiple pathways can interact in feedback loops to help regulate each other. Thus the regulation of target tissue response can be at least partly handled by the tissue itself, without having to invoke systemwide feedback systems that may produce (possibly unwanted) side effects in a number of different tissues. This type of regulation is beneficial from both a heuristic and an energetic point of view.

These receptor families present a significant but still limited variety of choices of possible mechanisms for transduction of the signal represented by the activated receptor into further intracellular actions. The detailed mechanisms of action of the various receptor types are well covered in other reviews (*19*). In this chapter we will focus on the ways in which bone cells in our laboratory have been found to respond to EMFs and the ways that these findings may illuminate the possible mechanisms of EMF effects on other cell types. The primary cell in which regulatory agents exert their effects on bone is the osteoblast; the osteoblast is in turn controlled primarily by the membrane receptors for parathyroid hormone (PTH). These receptors are members of the G-protein-linked family and carry out their actions by means of intracellular pathways involving adenosine 3′,5′-cyclic monophosphate (cAMP), phospholipase C (PLC), protein kinase C (PKC), and Ca^{2+} (*20*). Evidence suggests that each of these pathways can be influenced by EMFs either directly or indirectly (*7*). Figure 1 depicts key points of osteoblast signal-transduction pathways. The key point in PTH action is activation of the receptor, which in turn causes activation of at least two GTP-binding membrane proteins (G proteins). These in turn modulate the activities of enzymes that produce modifications in cell activities. The following paragraphs describe the G-protein-linked activity of the PTH receptor in osteoblasts.

Hormone binding induces a change in conformation of the PTH receptor. This change in conformation in turn increases the affinity of the receptor for membrane-resident G proteins. Many different types of G proteins exist, but they all share considerable sequence homology, and their functions are mediated in similar ways (*21*). The stimulatory G protein for adenylyl cyclase is designated G_s, and the inhibitory one is designated G_i. Most evidence suggests that PTH primarily modulates the G_s protein in osteoblasts. The receptor-activated G_s protein binds GTP and activates adenylyl cyclase. The cAMP produced by adenylyl cyclase diffuses into the cytoplasm, where it binds to the cAMP-dependent protein kinase (PKA). cAMP binds to the regulatory subunits of A kinase and causes dissociation of the protein and release of free catalytic subunits. These phosphorylate specific substrate proteins (their identities largely unknown in osteoblasts) and thereby lead to changes in their activities.

In addition to activating G_s and adenylyl cyclase, the PTH receptor can also activate (by means of a different G protein having different specificities) PLC, which hydrolyzes the membrane phospholipid phosphatidylinositol bisphosphate (PIP_2) to yield 1,4,5-trisphosphoinositol (IP_3) and diacylglycerol

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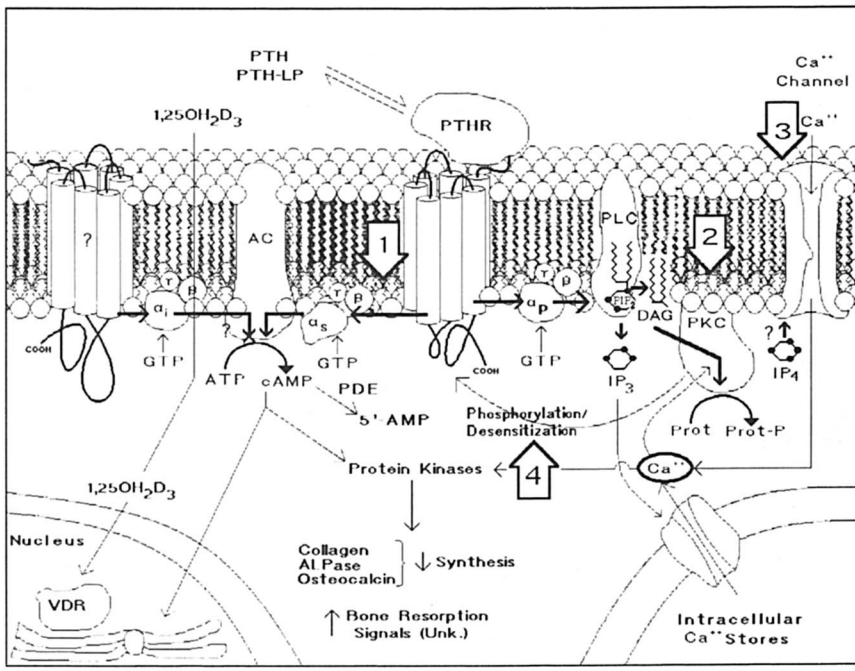

*Figure 1. Major signal-transduction pathways in osteoblasts showing sites of action of extremely low frequency (ELF) fields (*see *text for description). PTH is parathyroid hormone; PTHR is PTH receptor; PTH-LP is PTH-like peptide; 1,25OH_2D_3 is 1,25 dihydroxyvitamin D_3; PLC is phospholipase C; PKC is protein kinase C; DAG is diacylglycerol; PIP_2 is phosphatidylinositol bisphosphate; IP_3 is 1,4,5-trisphosphoinositol; IP_4 is 1,3,4,5-tetrakisphosphoinositol; AC is adenylyl cyclase; ALPase is alkaline phosphatase; VDR is vitamin D receptor; PDE is phosphodiesterase. (Reproduced with permission from reference 47. Copyright 1991.)*

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(DAG). Both the IP_3 and the DAG from this reaction can serve as second messengers (*22*). DAG activates a cellular protein kinase known as PKC (*23*). PKC phosphorylates a different set of proteins from protein kinase A (PKA), and this phosphorylation leads to a separate pattern of activities in the cell. The phorbol ester family of tumor promoter molecules, which are structural analogs of DAG, activates PKC independently of ligand–receptor signal transduction (*24*) and thereby produces inappropriate growth regulation in cells. Phorbol esters have been shown (*25*) to mimic some of the actions of PTH in bone cells by activating the PKC enzyme pathway. IP_3 also acts as a second messenger in bone cells (*19*) by interacting with receptors on intracellular membranes to release free Ca^{2+} from intracellular stores (probably endoplasmic reticulum or a related vesicular organelle). This release can raise intracellular free Ca^{2+} by as much as tenfold

(i.e., from around 10^{-7} M to about 10^{-6} M). This increase in turn can activate a variety of Ca^{2+}-dependent processes such as calmodulin-dependent protein kinases and/or phosphodiesterases. IP_3 also can be both phosphorylated and dephosphorylated to form other active metabolites, for example, 1,3,4,5-tetrakisphosphoinositol (IP_4), which seems to stimulate Ca^{2+} influx and efflux across the plasma membrane by opening ligand-gated and possibly also voltage-gated ion channels.

Importance of Phosphorylation in Signal Transduction

Regulation of the phosphorylation status of intracellular proteins is a key feature of the vast majority of signal-transduction pathways (*26*). The structures and functions of receptors, enzymes, structural proteins (e.g., microtubules), and intracellular modifiers (e.g., transcription factors) can be altered dramatically by phosphorylation. Changes in activity also can be brought about by removal of phosphate from previously phosphorylated proteins (phosphatase activity). Proteins may possess multiple possible phosphorylation sites, each of which may be phosphorylated or dephosphorylated by a different panel of enzymes. This property leads to a multiplicity of different possible phosphorylation states for most proteins regulated by phosphorylation. A variety of different activities may in turn be associated with the different possible phosphorylation states of each protein. Thus different signal-transduction pathways can modulate the same proteins in different ways, by activating different patterns of protein kinases and protein phosphatases. Because of the multiplicity of possible regulation levels, signal-transduction pathways have great flexibility in responding to diverse needs of the organism.

Protein kinases regulated by signal-transduction pathways are ubiquitous both in their number and in their substrate specificity; moreover, protein phosphatases may be nearly as universal (*27*). Signal-transduction pathways can modulate these kinases and/or phosphatases directly, as a result of processes set in motion by ligand binding to receptors, or indirectly, for example by stimulating the transcription of genes for kinase or phosphatase enzymes. Moreover, the response to signals may be determined by the preexisting phosphorylation state of receptors or other molecules participating in the pathway. Many of the interactions between pathways referred to already are commonly manifested at the level of phosphorylation or dephosphorylation of intermediate modifiers common to the interacting pathways (e.g., the kinase and phosphatase enzymes themselves). Thus, phosphorylation and dephosphorylation may be said to be central regulatory mechanisms by which many if not all signal-transduction pathways function and interact in the cell. Actual identities, however, are known for only a few of the multiple intracellular proteins phosphorylated or dephosphorylated in response to regulatory ligands. This area of research is active and expanding.

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In Electromagnetic Fields; Blank, M.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1995.

Regulation of Cell Growth

The relationships between signal-transduction processes, cell growth, differentiation, and neoplastic transformation of cells are far too complex for thorough discussion here. However, in the current context, two points should be made: first, many genes known to be oncogenes are clearly analogous to either hormones/growth factors or hormone/growth factor receptors. Second, intracellular regulatory pathways such as the cell division cycle discussed already and the promotion of differentiation and gene expression are very likely to be modulated by a multitude of signal-transduction pathways both in normal cells and in neoplastically transformed cells. For example, the well-known "tumor suppressor" RB gene product (*28*) not only acts as a cell cycle regulatory element but also undergoes a cell-cycle-dependent phosphorylation reminiscent of the cdc2 kinase (*29*). Moreover, the RB gene product is phosphorylated (inactive) in cells in which proliferation is induced by tumor promoters or differentiation agents that activate signal-transduction pathways, including PKC (*30, 31*). The RB gene product in turn appears to regulate the expression of c-*fos,* a transcriptional regulatory factor whose expression is tumorigenic, whose activity is regulated by phosphorylation, and which is the cellular homolog of one of the most potent viral oncogenes (*32*). These and many other examples suggest an intimate connection between signal-transduction pathways and the induction of tumorigenesis (*33*). Clearly, any environmental influence (e.g., EMF) that modifies signal-transduction pathways in normal cells could also influence the potentially tumorigenic pathways in susceptible cells, either by enhancing the likelihood of transformation by other tumorigenic stimuli or by acting in a directly tumorigenic manner.

Potential Interactions of EMFs with Signal-Transduction Pathways

Figure 2 summarizes several hypothetical ways in which low-energy EMFs could interact with membrane molecules to modulate or disrupt signal-transduction processes, by using the activation of adenylyl cyclase as a model (*34*). Each of the potential interactions with this signaling system could also be involved in interactions of extremely low frequency (ELF) EMFs with similar steps in other mechanisms of signal transduction. The model is based on the assumption that low-energy EMF effects are initially localized to the cell membrane. Possible interactions include the following.

1. Ligand binding could be modified because of interactions of the field either with free ligand or with the receptor. Possible charge flows due to field oscillations or cyclotron resonance might produce changes in the conformations of protein domains critical for binding. Alternatively, changes in the membrane–extracellular fluid interface could produce less specific alterations in the binding constants of the reaction.

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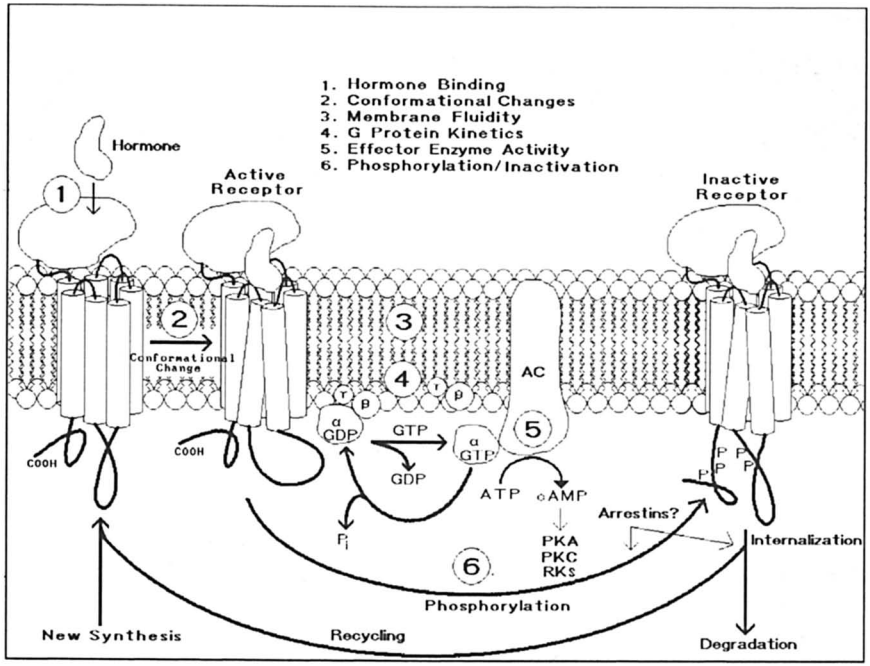

Figure 2. Possible interactions between ELF fields and signal transduction by the G-protein-linked adenylyl cyclase activation system. See *text for description. (Reproduced with permission from reference 47. Copyright 1991.)*

2. The conformational changes associated with activation of the receptor might be disrupted by ELF fields due to changes in membrane charge or phospholipid composition. Alternatively, the field might induce a specific change in receptor conformation, which would prevent both binding and activation by ligand. In some cases the receptor might be induced to undergo premature desensitization (or desensitization might be postponed and thus lead to enhanced activity of the receptor).
3. Membrane fluidity might be changed either directly (e.g., by field-induced changes in charge distribution of phospholipid groups) or indirectly (e.g., by changes in intracellular phospholipid synthesis). This fluidity change in turn would lead to changes in the association kinetics of the separate protein components needed to form the ternary complexes required for activation of adenylyl cyclase.
4. The properties, kinetics, and/or phosphorylation state of the G protein or its subunits could be altered. For example, even small changes in the GTPase activity of the α_s GTP complex could produce significant changes in cellular levels of cAMP. Changes in protein configuration or membrane charge or fluidity induced by the field could alter the reasso-

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch024

ciation kinetics of the G protein subunits or their interaction with other proteins. Similarly, the actions of the adenylyl cyclase enzyme itself could be modulated by the field in ways similar to the modulation of receptor or G proteins.

5. The activity of adenylyl cyclase, PLC, PKC, or other membrane-associated "amplifier" enzymes could be changed by direct interaction of the field with the membrane portions of the enzymes. Alternatively, synthesis of these enzymes could be modulated indirectly by interaction of ELF fields with the regulatory pathways controlling their transcription or translation.

6. The rate or pattern of phosphorylation of the receptor, and thus its activity, desensitization, internalization, recycling, or resynthesis, could be altered. These changes again either could be direct effects of ELF fields or could be mediated by the interaction of ELF fields with other signal-transduction pathways required for receptor phosphorylation.

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Effects of EMFs on Signal Transduction in Bone

Although the effects of ELF fields at the tissue level have been clarified somewhat by research over the past two decades, the primary biochemical and biophysical effects at the molecular or ionic level remain obscure. One clear likelihood for the effects of ELF fields on bone is that the plasma membrane of osteoblasts is likely to be the major site of action. Our laboratory has studied the effects of EMFs on bone cell function in vitro for a number of years. Our work has shown (*7*) that the pulsed EMF (PEMF) used in the most widespread clinical fracture treatment devices (*35*) produces activation of mouse osteoblasts in vitro by means of a strong inhibition of PTH responsiveness in the cells (*36*), leading to increased synthesis of collagen (*37*), decreased levels of bone resorption, and accelerated differentiation of osteoblasts from stem cells (*38*). The effects of EMFs on PTH responses were shown to be consistent with decreased coupling of receptors to adenylyl cyclase via the stimulatory G protein (*39*). Forskolin and cholera toxin had no effects on the changes induced by EMFs; this finding indicated that the hormone receptor, rather than some other component of the membrane signaling pathway, was the probable locus of the effects. Associated studies (*34*) using flow cytometry and immunohistochemistry indicated that exposure to EMFs produced a change in the accessibility of some PTH receptor epitopes to monoclonal antibodies; this behavior suggested that the fields induce a relatively persistent conformational change in the medium-accessible domains of the receptor and a corresponding decrease in signal-transduction capacity (i.e., desensitization). We proposed (*7*) that some or all of the fracture-healing effects of these devices in vivo may be due to localized desensitization of osteoblasts to endogenous PTH at the site of application of the electromagnetic energy.

The probability that the effect of EMFs on PTH responsiveness is specific to the membrane is increased by our previous observations (*37*) that the effects

of 1,25-dihydroxyvitamin D_3 and dexamethasone on collagen synthesis by bone cells were not modified by EMF exposure; both of these agents act primarily by means of cytosolic nuclear receptors rather than membrane receptors. Moreover, the effect appears to be receptor-specific because EMF exposure did not change the responses of the cells to insulin or prostaglandin E_2 (*37, 38*). However, the exposure of bone cells to EMF did modify the responsiveness of the β-adrenergic receptor (*40*). Thus these data are consistent with the hypothesis that EMFs may act at the membrane surface to cause a change in conformation of G-protein-linked receptors.

In more recent experiments we have begun (*40*) to extend the findings obtained with clinical field generators, both to lower field strengths and to the 60-Hz sine wave alternating field most commonly encountered in environmental and occupational settings. We have done a series of experiments similar to those reported in reference 36 using a 60-Hz, 1.0-G sine wave field. This field caused a significant inhibition of cAMP accumulation in osteoblasts in response to PTH at 10^{-9} M (*40*). A similar effect was observed on isoproterenol responses, that is, a desensitization of the β-adrenergic receptor at low drug doses but not so strong an inhibition at higher doses. These findings suggest that a similar process occurs with the 60-Hz, 1-G field as we have already observed in cells exposed to pulsed fields at ~10 G.

We have also begun to investigate the effects of the 1-G, 60-Hz sinusoidally varying magnetic field on activities of the signal-transducing enzymes PKC and PKA. Interestingly, exposure to the 1-G field had a very rapid and profound effect on PKC activity in these cells, indeed a much stronger effect than on the cAMP responses. Our initial findings (*41*) were that exposure to the field for times of 1 min to 24 h caused a transient activation, followed by progressive downregulation of PKC activity associated with the particulate fraction of the cell (usually interpreted as the fraction containing membrane-associated, activated PKC). When combined with our previous observations of the effects of EMFs on PTH receptor function, the current results support the hypothesis that EMFs may produce cellular responses by specifically modulating the conformation of G-protein-linked membrane receptors and thus perturbing associated signal-transduction processes. These findings have led us to hypothesize (*7*) that G-protein-linked receptors may be a particular target for the interaction of EMFs with cell membranes in responsive cells.

Changes in the functions of G-protein-linked receptors can be a factor in cell transformation as well as immediate cellular activities. Overexpression of the serotonin receptor, for example, has been shown (*42*) to cause neoplastic transformation of cells. G-protein-linked receptors also can be influential in controlling cell growth and development (*43*), for example, by controlling gene expression via transcriptional regulators (e.g., *fos* and CREB) that are phosphorylated by PKC, PKA, or both. Very small changes caused by EMFs in the surface charge distribution of cells bearing G-protein-linked receptors (*44*) could possibly lead to significant changes in the signal-transduction properties of those receptors. If substantiated, this hypothesis would ameliorate objections that low-

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energy fields are too weak to have any biological effects because the energy needed to produce small (but functionally significant) conformational changes in surface domains of membrane proteins may be very low indeed.

Possible Mechanisms for EMF Effects on Bone Signal Transduction

On the basis of observations of the biochemical effects of electromagnetic fields on bone in vitro, this laboratory has developed a hypothetical model for the ways in which bone may be induced to heal in vivo (Figure 3). One key observation is that osteoblasts exposed to PEMFs for as little as 10 min exhibit a persistent desensitization to the effects of PTH on adenylyl cyclase. This desensitization occurs in the absence of decreases in the total amount of adenylyl cyclase enzyme, in the number of hormone receptor sites, in the affinity of the hormone for the receptor, or in the fractional occupancy of the PTH receptor by hormone. Studies using biochemical probes of G protein coupling indicate that the ability of bound hormone–receptor complex to activate G protein α-subunits is impaired by treatment of the osteoblast with PEMFs. This desensitization of the

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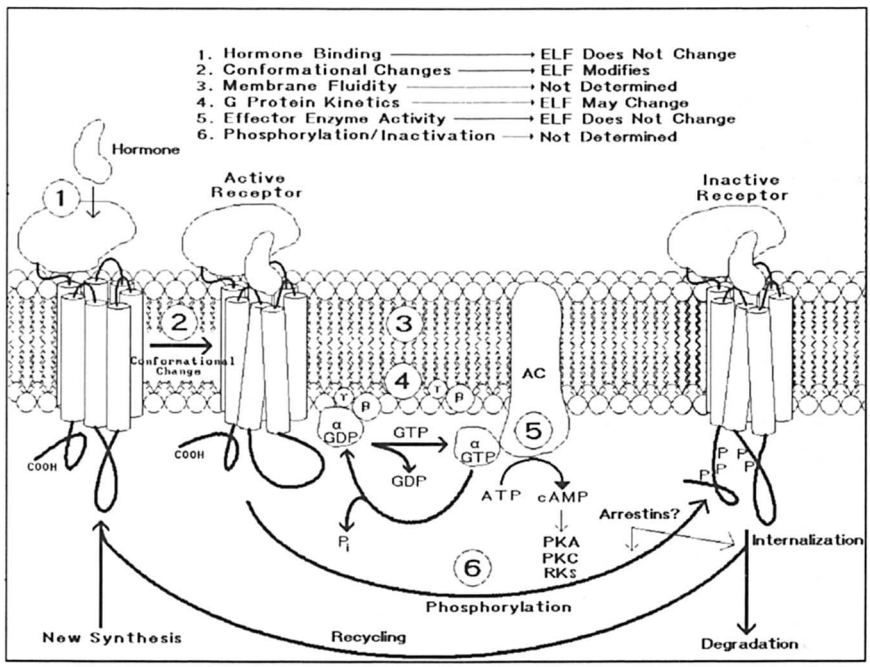

Figure 3. Summary of observed effects of low-energy EMFs on osteoblast signal-transduction pathways showing effects on PTH responses. (Reproduced with permission from reference 48. Copyright 1994.)

PTH receptor results in an elevated rate of synthesis of collagen by the osteoblast and a decreased rate of bone resorption by osteoclasts. Both of these effects would tend to increase the amount of bone in a localized area exposed to PEMFs in vivo, and these effects are in fact observed in healing bone under clinical circumstances.

Some potential clues exist to the possible molecular mechanism of PTH receptor desensitization by ELF fields. For example, in studies using monoclonal antibodies specific for the PTH receptor, we have observed that treatment with PEMFs produces changes in the expression of certain receptor determinants that are similar to changes observed in cells desensitized by treatment with hormone analogs. The receptor determinants whose expression is modified by ELF EMFs appear to be homologous to the signal-transduction domains of other G-protein-linked receptors. Desensitization of other G-protein-linked receptors is known to be associated with changes in the configuration of the transmembrane domains adjacent to intracellular phosphorylation sites; these configurational changes lead to phosphorylation of the receptor by intracellular enzymes. These findings suggest that ELF field treatment may change the conditions at the cell membrane surface in some as-yet-unknown way, such that the configuration of key residues of the PTH receptor is changed, leading to desensitization (possibly by phosphorylation) of the receptor and thus a shift in the balance of osteoblast activities toward increased bone formation.

Recent studies by this laboratory (*41*) suggest that a key site of action of ELF EMFs may be the PKC enzyme. We have shown that 60-Hz sine wave EMFs of 1.0 G can produce the same levels of PTH receptor desensitization as the clinical bone-healing devices and, most interestingly, that this desensitization is accompanied by a rapid decrease in PKC activity. Because PKC is believed to be the key enzyme involved in desensitization of the PTH receptor in bone cells (*45, 46*), these data suggest that effects of EMFs on PKC could be an important step in the signal-transduction cascade that results in increased bone formation. The possibility that PKC may be modulated by electromagnetic energy in other tissues is also under study. More broadly, it will be of interest to examine whether G-protein-linked receptors in general are particularly sensitive to the effects of ELF EMFs. If so, then a wide range of ELF field effects on hormonal, neural, and neurohormonal processes may become more amenable to direct molecular investigations.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch024

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RECEIVED for review April 25, 1994. ACCEPTED revised manuscript May 11, 1995.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch024

25

Melatonin Suppression by Time-Varying and Time-Invariant Electromagnetic Fields

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Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch025

This chapter reviews the experimental evidence that shows that both time-varying and time-invariant electromagnetic field exposures suppress the production and secretion of the pineal hormone melatonin in experimental animals. In general, the evidence that magnetic fields alter the circadian production of melatonin is more substantial than the data illustrating the ability of electric field exposure to suppress melatonin. The chapter also discusses potential mechanisms by which the fields couple to the organism, and it considers the significance of the melatonin changes with regard to the reported increased cancer risk in individuals exposed to an elevated electromagnetic environment.

THE PINEAL GLAND, a neuroendocrine organ located near the center of the human brain, produces and secretes an important hormone, melatonin. This gland is also present as a neuroectodermal outgrowth of the central nervous system of other mammals. The ability of the pineal gland to produce melatonin is directly related to the amount of visible electromagnetic radiation (i.e., light) detected by the eyes. Light exposure is always associated with minimal synthesis of melatonin in the pineal gland, whereas during darkness the production and secretion of the hormone increases. Because during the night blood melatonin

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levels are higher than they are during the day, melatonin has been referred to as the chemical expression of darkness (*1*).

More than a decade ago both power-frequency electric (*E*) fields (*2*) and static magnetic (*B*) fields (*3*) were, like light, reported to inhibit the production of melatonin by the pineal gland. Subsequently, time-varying *B* fields were also shown (*4*) to be effective in suppressing the synthesis and secretion of the pineal hormone melatonin. These initial reports set the stage for a flurry of studies; some confirmed the original observations, but others did not.

In general, the melatonin rhythm is considered to be highly stable, and therefore the fact that it was reportedly altered by very low energy electromagnetic fields was surprising. Likewise, the findings were important because the hormone melatonin has been functionally linked to a large number of important physiological events (*5*) including the suppression of cancer (*6*). Thus, very early a potential connection between the reported increased cancer risk (*7*) associated with electric residential wire codes in epidemiology studies and the suppression of melatonin was implied. This possibility has attracted even more attention in recent years (*8*).

The purpose of this brief review is to examine the published data related to the inhibition of melatonin production in the pineal gland of animals acutely or chronically exposed to *E* or *B* fields and to consider the mechanisms by which the fields interact with the system in question. Additionally, the potential consequences of a modification of the melatonin cycle will be summarized.

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Pineal Melatonin Synthesis and Secretion

Because the pineal gland responds to the prevailing light–dark cycle, and inasmuch as the gland in mammals is not directly light-sensitive, it was not surprising when an anatomic (and functional) connection was found between the eyes and the pineal gland. Thus, light detected by the retinas is transferred through the central and peripheral nervous systems to eventually end in the pineal gland. This circuitous pathway includes neuronal synapses in the suprachiasmatic nuclei (SCN or biological clock) of the hypothalamus and also involves fibers in the peripheral sympathetic nervous system (*9*). As noted already, retinal activation by light results in minimal pineal production of melatonin, whereas at night synthesis and secretion of the hormone increases (*1*). These changes in the metabolic activity of the pineal gland are reflected in blood levels of melatonin (Figure 1). Acute light exposure at night, like daytime light, quickly inhibits the production of melatonin in the pineal gland followed by a drop in circulating melatonin levels (*10*).

In the pineal gland, melatonin is a product of the metabolism of the amino acid tryptophan. After its uptake by the endocrine cells of the pineal gland, the pinealocytes, tryptophan is quickly converted to serotonin (5-hydroxytryptamine), a monoamine that is in very high concentrations in the pineal (*11*). Serotonin is the common precursor for several potential secretory products of the gland, but the only documented hormone produced by this organ is melatonin.

In Electromagnetic Fields; Blank, M.;
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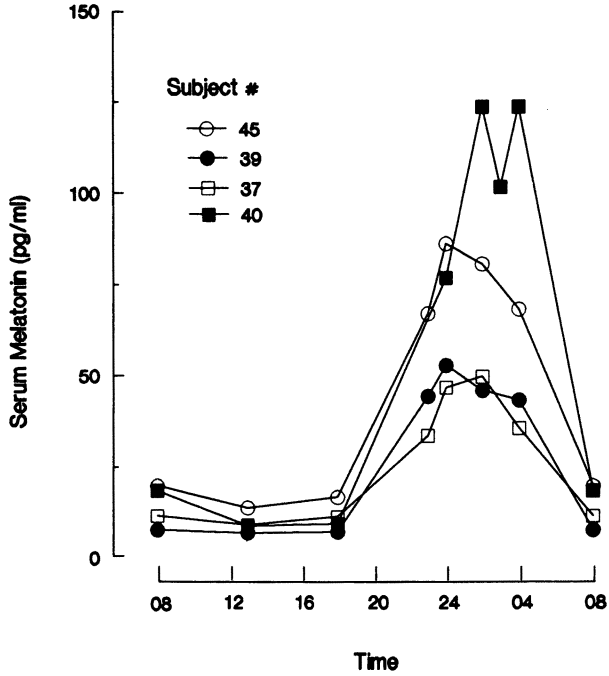

Figure 1. Twenty-four-hour rhythms in melatonin in the blood of four adult human males. Melatonin levels are always higher at night than during the day; however, the amplitude of the nighttime melatonin rise, as shown in this figure, varies between individuals. Within an individual, the melatonin rhythm is highly reproducible.

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Serotonin is converted to melatonin by a two-step process initially involving the *N*-acetylation of serotonin by the enzyme *N*-acetyltransferase (NAT) (Figure 2). The product of the action of NAT is *N*-acetylserotonin, which is *O*-methylated by the enzyme hydroxyindole-*O*-methyltransferase (HIOMT) to form melatonin (*12*). At night, as the production of melatonin increases, the concentration of its precursor (i.e., serotonin) drops. Thus, the pineal concentrations of serotonin and melatonin are characteristically inversely related. Once melatonin is formed, it is quickly released from the pinealocytes into the blood presumably by simple diffusion, because it is a highly lipophilic molecule. As a result, blood levels of melatonin are generally considered to be a good indicator of ongoing melatonin synthesis in the gland (*1, 12*).

Besides its metabolic conversion to melatonin, serotonin in the pineal gland can be oxidatively deaminated via a pathway that is usually evaluated by measuring pineal levels of 5-hydroxyindole acetic acid (5-HIAA). Also, serotonin can be acted upon directly by HIOMT with the resultant formation of 5-methoxytryptamine, a compound that has been proposed as a pineal hormone.

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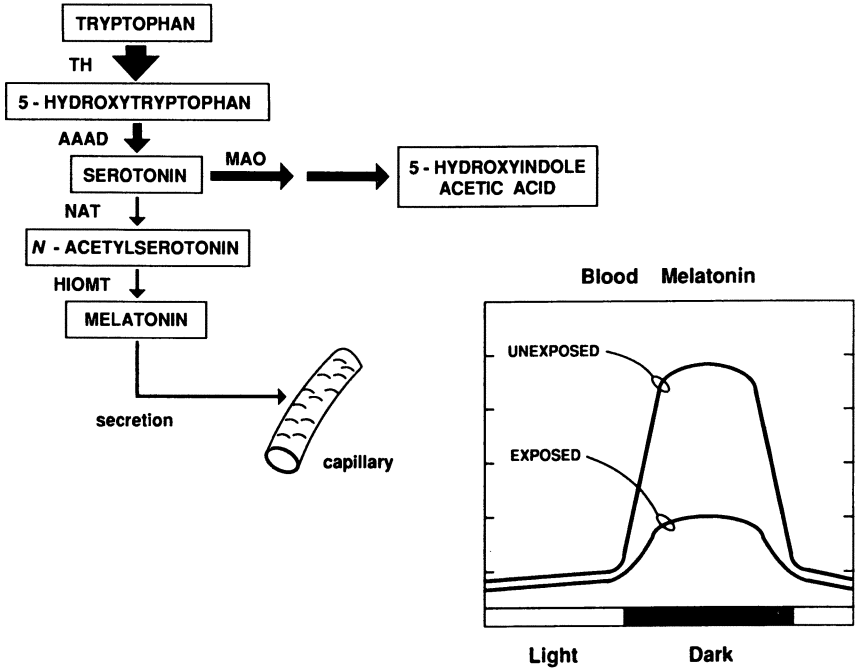

Figure 2. Diagrammatic representation of tryptophan metabolism in the pineal gland. The intermediate, serotonin, is metabolized via two pathways; that is., by monoamine oxidase (MAO) to 5-hydoxyindole acetic acid and by N-*acetyltransferase (NAT) and hydroxylindole*-O-*methyltransferase (HIOMT) to melatonin. The most important pathway, and certainly the one that is most applicable to the current report, is the conversion of serotonin to melatonin. Also represented is the reduction of melatonin in animals exposed to electromagnetic fields (see text). AAAD is aromatic amino acid decarboxylase; TH is tryptophan hydroxylase.*

Time-Varying E *Field Effects on Melatonin*

When Wilson and colleagues (*2*) described the inhibition of pineal melatonin concentrations in rats exposed to 60-Hz *E* fields, their evidence was the first to indicate that electromagnetic field wavelengths outside the visible range affected pineal metabolic activity. In this study, rats were exposed to a 39-kV (effective strength) field in a well-grounded exposure system in which the animals never experienced electrical shocks. The field was on 20 of each 24 h, and the exposure was chronic (30 days). After this period of exposure, nighttime pineal levels of melatonin, as well as the activity of the rate-limiting enzyme in melatonin synthesis, NAT, were significantly depressed. The changes were not totally uniform, and to achieve statistically significant differences the data from two nighttime points had to be combined. The control animals were indeed sham-exposed, having been placed in an exposure facility that was not energized. Al-

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though the study initially claimed an effective field strength of 39 kV/m, an erratum appeared two years later that corrected this value to 1.7–1.9 kV/m (*13*).

Wilson et al. (*2*) surmised that the responses of the pineal gland to sinusoidal *E* fields involved mechanisms similar to those related to the light suppression of melatonin. This conclusion implied that the retinas were the likely sites of coupling of the fields to the organism although the authors never actually made this claim.

The original publication was followed by another report by the same group in which they studied the time course of the change in pineal melatonin after rats were exposed to a 39-kV/m, 60-Hz *E* field (*14*). Rather than waiting 30 days to collect pineals, in this study the authors measured melatonin levels in the pineals of rats exposed for either 1, 2, 3, or 4 weeks. In this study they observed a gradual reduction in nocturnal melatonin over time with the suppression being significant after both three and four weeks of exposure. Whereas this study served as a confirmation of their original report, it also strongly suggested that the mechanism of the induced suppression by *E* fields was different from that by which light reduces the ability of the gland to convert serotonin to melatonin. In light-exposed animals, light exposure at night is followed by a rapid shutdown of pineal synthetic mechanisms with a similar quick reduction in melatonin (*15*). In the study of Wilson et al. (*14*) the animals had to experience 3 weeks of exposure to *E* fields (20 h/day) before a significant drop in melatonin was recorded.

With these two studies as a backdrop, another experiment was done on newborn animals exposed to sinusoidal *E* fields to determine whether exposing pregnant rats and, following their delivery, the young for 23 days, would compromise the development of the pineal melatonin cycle (*16*). Usually the circadian melatonin rhythm in 23-day-old rats is similar to that seen in adult animals (*12*). In this case three different field strengths were employed: 10, 65, or 130 kV/m. Control animals were sham-exposed as in the previous reports. This experiment showed that whereas all three experimental *E* fields (10, 65, and 130 kV/m) reduced nighttime pineal melatonin levels, the suppressions were very slight and occurred at only one time point during the night. This observation, although not directly contradictory to the original observations, raised some questions about the earlier findings.

This doubt was further strengthened when the same group that had published the 1981 (*2*) and 1986 (*14*) papers failed to confirm their own findings (*17*). In the presentation of the new data, the authors were at a loss to explain the different outcomes of the experiments. What was particularly perplexing was that all the studies seem to have been carefully done and the same exposure facility was used for all experiments.

More recently, Grota et al. (*18*) completed a comprehensive study related to the interaction of 60-Hz *E* fields with pineal melatonin production in rats. This study was designed specifically to duplicate the experiments of Wilson and co-workers (*2, 14*) at another site. The duration of exposure was 30 days, and the field strength was 65 kV/m. Control animals were appropriately sham-exposed. At the conclusion of the exposure, pineal and plasma melatonin levels were

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measured as were the two pineal enzymes, NAT and HIOMT, involved in the conversion of serotonin to melatonin. In this carefully conducted study, no effects of the sinusoidal field exposure in terms of nocturnal pineal melatonin, NAT activity, or HIOMT activity were apparent (*18*); a small drop in blood melatonin concentrations was recorded. On the basis of these results the authors concluded that no compelling evidence suggests that the time-varying *E* fields alter the circadian production of melatonin.

Enthusiasm for the notion that purely sinusoidal *E* field exposure interferes with the ability of the mammalian pineal gland to produce melatonin has waned. As a consequence, few if any follow-up experiments are currently being conducted.

Time-Invariant (Static) B *Field Effects on Melatonin*

Perturbed static magnetic fields have been frequently used (*19, 20*) as an experimental paradigm to alter the melatonin-forming ability of the pineal gland. The first of these studies dates back to the early 1980s when Semm (*21*) and Welker and co-workers (*3*) found that the mere inversion of the geomagnetic field induced a reduction of nocturnal melatonin in both the pineal and blood as well as a drop in the activity of the enzyme that *N*-acetylates serotonin. These were acute studies with the exposure to the inverted field usually lasting for less than 120 min. At least in the Welker et al. (*3*) study the pineal seemed to become refractory to the inverted field within about 120 min even when the geomagnetic field remained inverted.

The circumstances under which perturbed geomagnetic fields impede the melatonin-forming ability of the pineal gland were further defined in a series of studies by Olcese and colleagues (*22, 23*). Nighttime pineal NAT activity and melatonin levels in rats were found to be suppressed after the exposure of the animals to a 50° rotation of the horizontal component of the geomagnetic field for 30 min, but this suppression did not occur in rats that had been optically enucleated. The inference of this finding, and certainly one espoused by the authors (*22, 23*), was that the eyes serve as magnetoreceptors that subsequently signal the reduction in melatonin production. In support of this concept, Reuss and Olcese (*24*) found that weak background red illumination was also a factor required for the perturbed geomagnetic fields to change pineal melatonin synthesis. Although the importance of the eyes as a coupling organ for *B* fields with the organisms remains unproven, we have proposed that, if true, the isomerization of 11-*cis*-retinal to all-*trans*-retinal in the rod photoreceptor could be the chemical event that initiates the processes that eventually reduce nocturnal pineal melatonin formation (*25*). The isomerization of 11-*cis*-retinal requires very low energy input and leads to the activation of the photopigment rhodopsin; this activation is presumed to be the initial event in light-induced melatonin inhibition. Implicit in this proposal is that perturbed *B* fields change the conversion of serotonin to melatonin by the same means that acute light exposure does at night. This assumption, however, remains unproven.

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Systemic factors seem to interfere with the ability of a changed geomagnetic field to alter pineal metabolic activity in rodents. Thus, in the only study in which the Syrian hamster was used (*26*) in lieu of rats to test *B* field effects on melatonin production, no change was reported; this result implies species specificity. Melatonin production in the pineal gland of the Syrian hamster is, like that in the rat, very sensitive to light inhibition (*15*).

Cutaneous pigmentation has also been reported (*26*) to be a significant factor in determining the sensitivity of the Mongolian gerbil (*Meriones unguiculatus*) to time-invariant *B* fields (*26*). When both albino and pigmented gerbils were exposed to a 60° rotation of the horizontal component of the Earth's magnetic field for 30 min, only in the albino animals was a drop in melatonin measured. Further confounding the findings in this report was the observation that only in the males, but not in the females, were pineal melatonin levels suppressed (*26*); yet in both males and females pineal NAT activity was lowered as a consequence of the exposure. Such variable findings without seemingly a sound physiological explanation make generalizations concerning interactions of the static *B* field with the pineal gland difficult.

Using pineal metabolic parameters in addition to melatonin and NAT, Lerchl and co-workers (*27*) showed that the exposure of both rats and mice to intermittent changes in the Earth's static *B* field caused changes consistent with a drop in melatonin synthesis. Thus, when the animals were exposed, at night, to a repeatedly inverted (at 5-min intervals) horizontal component of the geomagnetic field (0.4 G or 40 μT) for 1 h, serotonin and 5-HIAA levels accumulated in the pineal gland. These findings signal a reduction in the conversion of serotonin to melatonin and thus lead to a buildup of serotonin and its subsequent metabolism via an alternate pathway to 5-HIAA. This study provided additional support for the notion that time-invariant *B* fields are effective, at least under some conditions, in curtailing the ability of the pineal gland to synthesize its most important hormone, melatonin.

As a follow-up to this report, Lerchl et al. (*28*) examined the possible parameters of the *B* fields that may be important in signaling the organisms of a change in the electromagnetic environment. They repeated their earlier studies with a couple of variations in the exposure parameters. In their initial studies and in half of the animals used in the second set of experiments, the geomagnetic *B* field was "rapidly" inverted ($dB/dt \cong 7.5$ ms) intermittently for 1 h at night. The remaining half were also exposed to a repeatedly inverted *B* field, but the inversion occurred over a 1-s interval; these were referred to as "slowly" inverted fields. The significance of these different type inversions is as follows. When the *B* field is rapidly inverted, weak electrical currents (eddy currents) are produced in the animals. Conversely, when the fields are slowly inverted, little or no eddy current is induced. The question being asked in this study was whether the induced electric currents were operative in causing the pineal melatonin changes.

The induced electric currents seemed to be operative in causing these changes in that the rapid dB/dt, which produced eddy currents, induced a reduction in pineal NAT and melatonin (Figure 3) and an elevation in pineal serotonin

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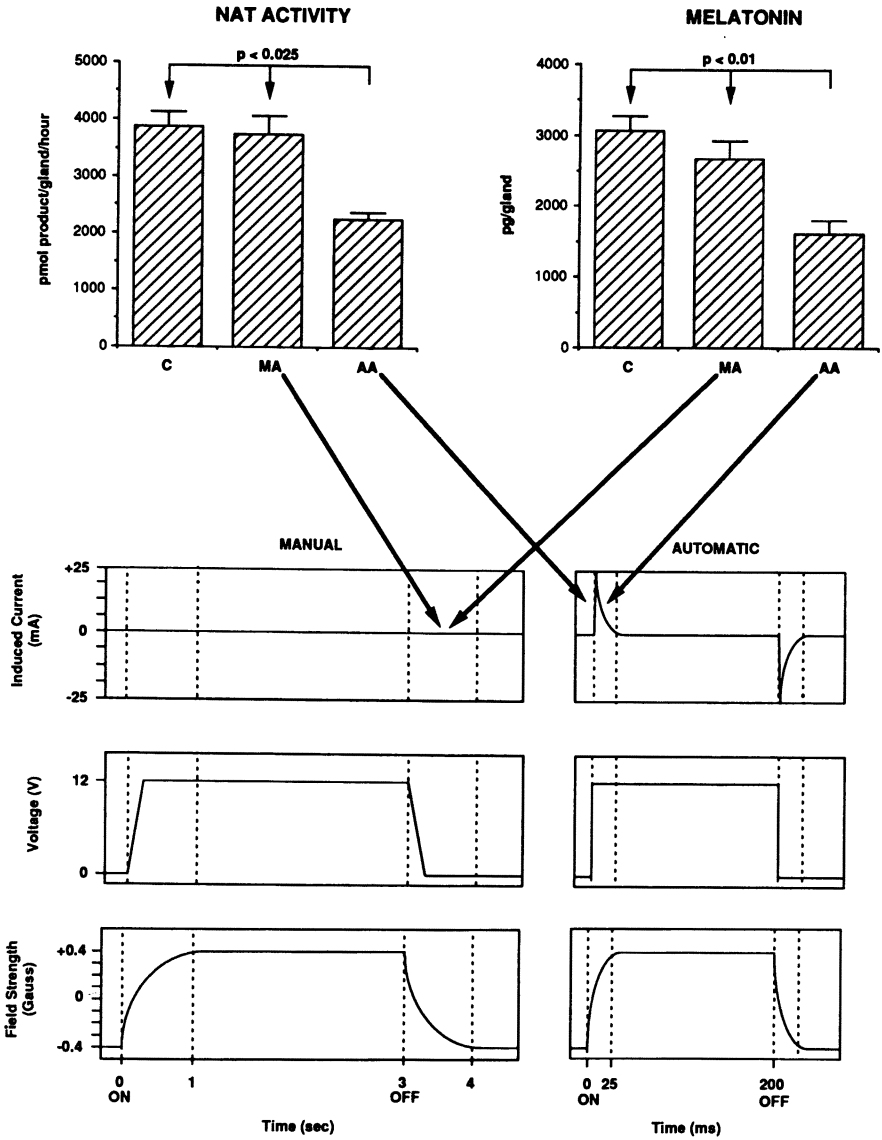

*Figure 3. Pineal NAT and melatonin reductions following the exposure of rats to intermittent magnetic (*B*) fields at night. The fields were either inverted "rapidly" (AA) or "slowly" (MA). Only the rapidly inverted fields reduced pineal serotonin metabolism. Also shown is the voltage required to invert the geomagnetic* B *field as well as the field strength. These findings suggest that the induced electric current may be causative in the observed pineal changes. (Reproduced with permission from reference 19. Copyright 1992.)*

and 5-HIAA. None of these changes were seen when rats were exposed to repeatedly inverted fields in which the inversions occurred slowly. The implication of these findings is that induced electric currents may be the mechanism that couples *B* fields to the organism (Figure 3). This report (*28*) is one of the first to be designed to specifically test for a coupling mechanism of perturbed *B* fields, and if confirmed in similar studies in other laboratories, it could represent a very significant finding.

Some of the variability in the response of the melatonin-generating system to *B* fields may relate to the timing of exposure. According to Yaga et al. (*29*), the pineal gland at night becomes progressively more sensitive to *B* field perturbations as the night is prolonged. Thus, when rats were exposed to rapidly inverted *B* fields either early in the night, at mid-dark, or late in the dark period, only at the mid- or late-dark times was melatonin significantly depressed; furthermore, the suppression was greater at late dark than at mid-dark. Hereafter, studies designed to test the ability of *B* fields to change pineal gland activity should take into account the time the exposure is done

Many of these studies implied that the eyes may serve as the magnetoreceptors for the *B* field effects on the pineal gland. However, isolated cultured pineal glands also responded to *B* field changes with a reduced melatonin synthesis (*30*). This finding does not prove, however, that in intact animals the eyes are not important coupling site for *B* fields.

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Time-Varying B *Field Effects on Melatonin*

Because most electromagnetic fields to which humans and animals are exposed are sinusoidal, interest in time-varying fields in reference to possible changes in physiology are of special importance. Surprisingly, however, rather few studies have used time-varying *B* fields in the experimental setting to examine their effect on the pineal gland.

According to Kato and colleagues (*31, 32*), 50-Hz circularly polarized *B* fields, but not 50-Hz horizontal or vertical *B* fields, reduce plasma and pineal melatonin levels in rats. The study in which circularly polarized fields were used was very complete in the sense that a range of field strengths was used (0–120 μT) on a large number of rats. Also, both daytime and nighttime pineal and blood melatonin levels were examined. The animals were continually exposed to these fields for 6 weeks (except for twice weekly 2-h intervals). Examination of blood melatonin levels estimated by radioimmunoassay indicated that fields strengths of 1 μT or greater altered melatonin levels. Unexpectedly, daytime pineal levels of this hormone were actually slightly, albeit statistically significantly, elevated. This unexpected finding detracts somewhat from a report that otherwise is of considerable interest. Although melatonin levels were lower in animals exposed to a variety of field strengths, no dose–response relationship was observed.

In contrast to these 50-Hz rotating vector *B* fields, as already mentioned, neither 50-Hz horizontal nor vertical field exposure influences melatonin in rats

(*32*). These studies seem to have been well-controlled. Without specifying why these fields failed to depress melatonin whereas the 50-Hz circularized polarized fields did, the authors merely assumed that the outcomes differed because of differences in magnetic field characteristics.

As part of a study related to the ability of 50-Hz *B* fields to promote mammary carcinogenesis in rats, Mevissen et al. (*33*) also measured a significant reduction in the nocturnal serum concentrations of rats. The field strength in this experiment was 0.3–1 μT, and the duration of exposure was 91 days. Melatonin was measured in these studies because it is generally considered to be an oncostatic agent (*6*) and thus, if lowered by the field exposure used, a high incidence of tumorigenesis would be expected.

Perhaps the most unique sinusoidal *B* field exposure to reportedly curtail melatonin production is that used by Yellon (*34*). This worker used a 60-Hz, 1-G horizontal magnetic field during the day to test its effect on the subsequent night's melatonin rhythms. In this case the species employed was the Djungarian hamster (*Phodopus sungorus*), and the exposure duration was 15 min, beginning 2 h before darkness onset. The control animals were appropriately sham-exposed. On the night following the daytime exposure, both pineal and serum melatonin levels were lower in the experimental compared to the control animals in the first study. When the experiment was replicated, similar results were obtained, with the melatonin values again being depressed. A second replicate, however, failed to confirm the results of the previous two experiments. These perplexing observations have no obvious explanation, but presumably some exposure parameter must have varied among the experiments or some technical failure must have occurred. Interestingly, the suppression of melatonin that Yellon (*34*) observed in the first two studies has been confirmed in an independent laboratory (*35*).

Although only a preliminary report, Graham et al. (*36*) claimed that in humans as well blood melatonin levels may be lowered by time-varying *B* field exposure. The fields used were 60-Hz, 200-mG fields that were intermittently turned off and on during the night. The suppression of circulating melatonin was noted only in individuals who had an inherently dampened nighttime melatonin rise (Figure 1). Also, maximal suppression occurred later in the dark phase, similar to earlier observations made in rats (*29*). These important studies on humans require replication and extension.

Combined Time-Varying E *and* B *Field Effects on Melatonin*

A small number of studies have examined potential melatonin changes following the exposure of animals to a combined sinusoidal *E* and *B* field. In a very thorough and well-controlled study, 10 female Suffolk lambs were caged under a 500-V transmission line between the ages of 2 and 10 months (*37*). The respective mean *E* and *B* fields were 6 V/m and 40 mG. Control lambs were housed in similar pens 229 m from the transmission lines, where the ambient *E* and *B* fields were <10 V/m and <0.3 mG, respectively. During the course of the 8-

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month study, blood melatonin levels were measured at eight different 48-h intervals. Serum progesterone levels were also measured to assess the onset of puberty. At each 48-h blood collection, the 24-h patterns of melatonin were similar between experimental and control animals. Likewise, the animal caged under the high-voltage line reached puberty at the same average age as the cage-controlled ewes. The results of these studies obviously do not support the contention that the continuous exposure of sheep to combined low-intensity *E* and *B* fields has an influence on either the circadian production and synthesis of melatonin or on pubertal onset.

Contrasting results were obtained when rapidly pulsed combined *E* and *B* fields were employed in a study using baboons. When baboons were exposed to a 30-kV/m and 1-G electromagnetic field that was rapidly turned on and off [in the manner used by Lerchl et al. (*27, 28*)] at intermittent intervals, this exposure caused a highly significant attenuation of the nocturnal rise in blood melatonin compared to that seen during a control period when the animals were not exposed to either an *E* or a *B* field (*38*). The authors' interpretations of their data was that the rapid on–off nature of the pulsed fields was likely causative in the induction of the reduced melatonin levels. This possibility is reasonable considering another study in which the *E* and *B* fields were slowly ramped to their predetermined exposure levels was without an effect on circulating melatonin (*28*).

Clearly, few studies have used combined *E* and *B* fields in experiments related to possible melatonin suppression. This state of affairs may not be totally unexpected because interest in the potential effects of *E* fields has decreased relative to the possible changes resulting from *B* field exposure. Thus, most studies currently under way are concerned with only the latter fields. Also, most studies have as at least one goal to determine whether the physiological consequences observed are due to the *E* or the *B* field exposure; by using combined fields this determination cannot be made.

Coupling Mechanisms

How electromagnetic fields are coupled to the organism generally and to the pineal gland in particular remains, for the most part, unknown. Lerchl and colleagues (*27, 28*) have proposed that induced electric currents that arise as a consequence of the rapid dB/dt are causative in terms of the suppression of melatonin. This explanation was also used in later experiments by others (*31, 38*) to explain how electromagnetic fields suppress the production of melatonin. Although this hypothesis is attractive, the induced electric current concept requires additional experimental support before it will be widely accepted.

The eyes are often said to serve as the site for magnetoreception. This assumption is based on the claim that a low level of background red illumination is required before a perturbed geomagnetic field will induce an alteration in melatonin production (*22–24*). Again, however, little agreement exists that this claim is, in fact, true; this parameter needs additional investigation, and if retinal stimulation is required, the mechanism could involve the activation of rhodopsin (*25*).

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The coupling of electromagnetic fields to organisms would seem likely to involve the nervous system inasmuch as it normally generates and conducts electrical impulses. However, no direct proof for this hypothesis exists although a number of neuronal changes have been shown (*39–41*) to occur as a consequence of especially *B* field exposures. Also, magnetite, which exists in the brain, could theoretically be involved in responding to magnetic signals; directional changes in magnetite deposits could serve as an intracellular transduction mechanism for *B* field perturbations (*42*).

Considering that organ cultured pineal glands exhibit a reduction in melatonin synthesis when exposed to a pulsed *B* field (30), the pineal gland might be directly responsive to the fields, possibly because of changes in the cell membrane of the pinealocyte (*39, 41*) The in vitro studies, however, do not preclude the possibility that under in vivo conditions, cells distant from the gland serve as the primary magnetoreceptor.

Although changes in pineal melatonin synthesis have been reported (*43*) as a consequence of electromagnetic field exposure, it is difficult to biophysically explain the results observed because of the extremely low energy input to the organism during such exposures. However, failure to explain the biophysical mechanisms of field interactions with cells or organisms is not sufficient justification for concluding that changes do not occur.

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Consequences of Melatonin Suppression

Although the data are equivocal, if we accept the observations that melatonin levels are suppressed by at least some electromagnetic field exposures, the question remains as to what might be the consequences of a reduction that is of the magnitude that has been described. Of special interest has been whether the lower melatonin values could have any connection to the alleged increased incidence of cancer that some epidemiologists claim may be a consequence of living in the vicinity of high-voltage transmission and distribution lines (*44, 45*). Certainly such a suggestion has been formally made (*46*), and experimentally melatonin has been linked to both cancer initiation (*47*) as well as to cancer progression (*6*).

Melatonin is a highly potent hydroxyl radical scavenger (*48*); as such it serves to protect DNA from damage due to exposure to the highly toxic hydroxyl radical (*49, 50*). Damaged DNA, if it goes unrepaired, can mutate with the subsequent initiation of a tumor. Clearly, any factor, such as electromagnetic field exposure, that lowers endogenous melatonin levels would at least theoretically put the DNA in higher jeopardy for damage and, as a result, would increase the likelihood of cancer. Thus, *E* and *B* field exposures may indirectly increase the possibility of cancer initiation. Although these potential associations are plausible, they remain to be experimentally documented.

As already noted, besides protecting DNA from hydroxyl radical damage and thereby possibly reducing the likelihood of cancer initiation, melatonin also is often an effective inhibitor of tumor growth once a tumor is initiated (*6*). Thus

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even a partial loss of endogenous melatonin, such as that following *E* or *B* field exposure, could hasten tumor progression and promotion. However, no studies have actually been performed to determine whether an induced cancer would grow more rapidly if the nocturnal melatonin rise was only partially attenuated, such as seen in animals exposed to experimental electromagnetic fields.

Concluding Remarks

The fact that changes in mammalian pineal melatonin production are slowed under some cases of electromagnetic field exposure presents an enigma because the energy input would seemingly not overcome thermal noise of cells, and as a result no physiological change should occur (*43*). However, factors may amplify the coupling mechanisms and thereby allow the fields to induce physiological changes (*41*). One of these alterations seems to be a reduction in melatonin synthesis and secretion by the pineal gland (*8, 9, 19, 20*). Whereas a change in melatonin has been documented in many studies in which electromagnetic fields have been used as an experimental variable, the outcomes of all studies have not been uniform in that reports of no effects of such fields on melatonin have also been documented. The major factor that makes the melatonin results of special interest and potential importance is that, when a change has been seen, it has always been a reduction (*8, 20*). Furthermore, all associated changes that have been observed in the pineal as a result of field exposure are consistent with a reduced conversion of serotonin to melatonin (*51*). Such a change could account for the increases in cancer that have been reported in epidemiological studies (*44, 45*). However, this association is by no means proven.

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Acknowledgments

Research by the author was supported in part by contracts form the Electric Power Research Institute, Palo Alto, CA; the Department of Natural Resources, State of Maryland; and the National Institute of Environmental Health Sciences.

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Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch025

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RECEIVED for review July 6, 1994. ACCEPTED revised manuscript May 2, 1995.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch026

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Effects of Radio-Frequency Radiation on Mammalian Cells and Biomolecules In Vitro

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In vitro biological systems, such as mammalian cells or biomolecules, provide the most direct means of assessing the biological effects of electromagnetic fields. Such systems were exposed to radio-frequency electromagnetic radiation (RFER) under conditions that permitted differentiating indirect effects due to RFER-induced heating from direct exposure effects. This distinction is of importance because current RFER health protection guidelines were designed primarily on the basis of limiting thermally induced physiological alterations. Studies reviewed here include RFER in vitro effects on cell proliferation, neoplastic transformation, cell membrane cation transport and binding, energy metabolism, and neuroelectrical activity. Whereas the basic RFER biological interaction mechanisms remain elusive, in vitro data provide insight and direction for future studies. Such data also provide a perspective for emerging issues related to health effects of exposure to environmental electromagnetic fields.

IN VITRO SYSTEMS afford a unique opportunity to investigate biological effects of radio-frequency electromagnetic radiation (RFER) under conditions of precise experimental control of variables including (1) induced electric (*E*) or magnetic (*B*) field strength, (2) modulation, (3) dose rate or specific absorption

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rate (SAR = $0.5\sigma E^2$, where σ is the effective conductivity), (4) temperature, and (5) composition of cell exposure medium. Temperature control is essential in studies designed to differentiate between direct effects of RFER on biological systems as contrasted to indirect effects due to RFER-induced heating. Physical aspects of the interaction of RFER with mammalian cells and biomolecules are also amenable to theoretical determination of the magnitude and spatial distribution of induced *E* and *B* fields and hence cell or molecular level SAR distributions. Consequently, mammalian cells and biomolecules provide the most direct approach to determining basic interaction mechanisms of RFER with biological systems. In vivo systems do not afford this opportunity because of inherent dosimetric and densitometric complexities that introduce significant uncertainty regarding the accuracy of *E* field, *B* field, or SAR determinations in tissue. The highly interactive nature of various organs and organ systems also impedes assessments of cause–effect relationships in in vivo systems exposed to RFER. Although the relationship between SAR and tissue heating is complex, as a general rule intensities somewhat greater than 1 W/kg are associated with some degree of temperature elevation (*1*). In in vitro exposures active heat transfer systems may be used to control or eliminate field-induced temperature elevations over intensity ranges of 100 W/kg or greater (*2*).

In vitro studies of the effects of RFER on cell physiological end points include (1) cell proliferation and neoplastic transformation, (2) membrane cation transport and binding, (3) energy metabolism, (4) molecular–biochemical effects, and (5) effects on membrane ion channels. The results of these studies have been the subject of review articles (*1–4*). This chapter will review recent in vitro studies that provide additional insight regarding possible RFER cellular interaction mechanisms and that present evidence of direct or athermal RFER cellular effects. The potential relevance of such in vitro studies to human health effects, such as reported associations of RFER exposure and cancer incidence, will be discussed. The purpose of this chapter is not to provide a comprehensive review of in vitro cellular effects of RFER (for more comprehensive reviews, *see* references 1–4).

The results of the studies reviewed here indicate that under certain exposure conditions RFER directly alters mammalian cell physiology in the absence of indirect thermal effects. Although basic interaction mechanisms are uncertain, the data suggest that the most likely interaction site for most of the reported cellular effects of RFER is the plasma membrane. Further insight regarding in vivo effects of RFER, such as cancer induction or promotion or effects on reproduction and development, may be provided by appropriately designed in vitro cell studies.

Cell Proliferation and Neoplastic Transformation

A variety of in vitro functional and genomic cellular alterations have been attributed to direct effects of RFER exposure. Cleary et al. (*5–7*) reported altered proliferation of normal resting human peripheral lymphocytes and human or rat

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch026

glioma following a 2-h exposure to 27- or 2450-MHz continuous-wave (CW) or pulse-modulated (PM) RFER at SARs in the range of 0.5 to 200 W/kg. Altered cell proliferation persisted for up to 5 days after RFER exposure. The effect was biphasic; maximum increased proliferation occurred at 25 W/kg, whereas exposure at 50 W/kg or higher generally suppressed proliferation (*7*). Dose fractionation studies indicated exposure effects were persistent for at least 1 day, which suggested the possibility of cumulative or additive exposure effects on cell proliferation. Because cells were exposed under isothermal conditions (37 ± 0.2 °C), altered proliferation was attributed to a direct effect of RFER. A direct effect of pulsed RFER on increased lymphoblastoid transformation was reported following a 5-day exposure to 2450-MHz RFER at a maximum SAR of 12.3 W/kg (*8*).

The relevance of RFER-induced alterations in cell proliferation to health effects is suggested by the following: (1) increased in vitro proliferation of cancer cells, such as glioma, is consistent with tumor promotion; (2) decreased in vitro proliferation of immune cells, such as lymphocytes, is consistent with immunosuppression; and (3) recent studies of cell-cycle control mechanisms indicate that alteration of the mammalian cell cycle per se may be associated with increased cancer incidence (*9*). Altered cell proliferation occurred under conditions that did not directly involve heating. The principle of dose reciprocity suggests the possibility that long-term low-intensity RFER exposure could alter cell proliferation in vitro. Time-intensity thresholds for RFER-induced alterations in cell proliferation in vitro or in vivo have not been determined.

Neoplastic cell transformation has been reported as a direct effect of low-intensity RFER exposure. Mammalian embryonic fibroblasts were exposed for 24 h to 0.1-, 1-, or 4.4-W/kg, 2450-MHz RFER pulse modulated at 120 Hz (*10*). In the absence of the tumor promoter 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA), RFER did not affect cell survival or the rate of neoplastic transformation. Cells treated with TPA and RFER experienced a statistically significant dose-dependent increase in neoplastic transformation rate. Exposure to 4.4-W/kg RFER in the presence of TPA had a neoplastic transformation effect equivalent to exposure to 1.5 Gy of X-radiation. It was determined that RFER and X-rays acted independently in inducing neoplastic transformation (*10*). Evidence of direct genomic effects of RFER on human somatic cells has also been reported (*11, 12*) and includes chromosomal aberrations (acentric fragments and dicentric chromosomes), micronuclei formation, and mutagenic characteristics typical of chemical mutagens. Studies of effects of RFER on cell proliferation and the genome are summarized in Table I.

Membrane Cation Transport and Binding

Studies of cell membrane cation permeability provided the first indication of direct nonthermal effects of RFER. Exposure of human, rabbit, and canine erythrocytes to 2450-, 3000-, and 3950-MHz CW microwave radiation at SARs of up to 200 W/kg resulted in intracellular K^+ leakage and osmotic lysis. Temperature control studies conducted over the temperature range of 26 to 44 °C suggested

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch026

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch026

Table I. Effects of RFER on Cell Proliferation and the Genome

Microwave Source Descriptors	*Typical Effect*	*Field Parameters/ Thresholds*	*Comments*
27 MHz CW or pulsed; 2450 MHz CW or pulsed	Altered proliferation of human or rat glioma and human lymphocytes	0.5–200 W/kg 2-h isothermal (37 °C) exposure	Proliferation altered for 1–5 days postexposure; evidence of cumulative effect; similar effects of CW or pulsed fields; biphasic dose rate effect (refs. 5–7)
2450 MHz pulsed	Transformation of C3H/10T ½ mouse embryo fibroblasts	4.4 W/kg 37.2 ± 0.1 °C 24-h exposure 120-Hz pulse modulation	Latent transformation revealed by TPA treatment of cells exposed to X-radiation and microwaves (ref. 9)
7700 MHz CW	Chromosomal aberrations; micronuclei formation in human lymphocytes	0.5, 10, and 30 mW/cm^2 10, 30, and 60 min	Higher frequency of chromosomal aberrations in all exposed samples; increased frequency of micronuclei in exposed samples correlated with specific chromosomal aberrations (refs. 10 and 11)
2450 MHz CW or pulsed	Lymphoblastoid transformation of human lymphocytes	Max SAR 12.3 W/kg 5-day exposure 1-μs pulse 100–10^3 pps	Temperature-dependent increase in control and CW microwave-exposed samples; pulsed microwaves increased lymphoblastoid transformation without heating (ref. 8)

NOTE: RFER is radio-frequency electromagnetic radiation; CW is continuous wave; TPA is 12-*O*-tetradecanoyl-phorbol-13-acetate; max SAR is maximum specific absorption rate; pps is pulses per second.

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that the effects were due to RFER-induced heating (*13*). In a subsequent study of passive cation (Na^+ and Rb^+) efflux from rabbit erythrocytes exposed to 2450-MHz RFER at SARs of 100, 190, and 390 W/kg, statistically significant increases were detected, but only at a temperature of 22.5 °C (*14*). In agreement with the results of Liu et al. (*13*), who had not investigated permeability changes at temperatures of <26 °C, RFER had no direct effect at temperatures >22.5 °C. Additional evidence of direct RFER-induced membrane permeability changes in the range of 17.7–19.5 °C was reported (*14–17*). The effect of RFER (2450 MHz) was enhanced when erythrocytes were exposed under hypoxic conditions or when exposed in the presence of plasma (*16*). Increasing the cell membrane cholesterol content or treatment with antioxidants, on the other hand, inhibited the effect of RFER exposure (*16*).

A lack of RFER frequency specificity for effects on cell membrane cation permeability was indicated by the results of a study of the effect of higher-frequency microwave (8420-MHz) RFER on K^+ release (*18*). Permeability was again increased when the steady-state temperature was maintained at 24.6 °C during exposure. RFER exposure at lower or higher temperatures did not detectably alter K^+ transport when compared to thermal controls. The results of these experiments (*13–18*) suggested an interaction between RFER exposure, cation transport, and a gel-to-liquid crystal membrane phase transition (*18*). Because temperature-specific effects on erythrocyte cation transport could not be induced in the absence of RFER exposure, they were interpreted as direct effects on the red blood cell membrane (*3*). The slight variation in the temperature at which RFER affected K^+ transport was attributed to differences in species as well as methods of erythrocyte preparation.

Allis and Sinha-Robinson (*19*) suggested a molecular interaction mechanism for the effect of RFER on membrane cation transport. They exposed human erythrocyte membranes to 6-W/kg, 2450-MHz RFER at 1 °C temperature increments between 23 and 27 °C. When membrane Na^+/K^+-adenosinetriphosphatase (Na^+/K^+-ATPase) activity was monitored spectrophotometrically, it was determined that enzyme activity was inhibited only at 25 °C (*19*). The authors concluded that inhibition resulted from a direct interaction of RFER with the ATPase enzyme. This interaction mechanism is consistent with reported effects of RFER on erythrocyte cation (K^+, Na^+, and Rb^+) transport at the membrane phase transition (*13–18*).

Evidence of direct RFER effects on cells was also revealed by a series of studies of effects on Ca^{2+} binding to cell membranes. In contrast to effects on membrane cation transport, which resulted from either CW or pulse-modulated (PM) RFER exposure, effects on Ca^{2+} binding were strongly dependent upon extremely low frequency (ELF) RFER modulation, most prominently at frequencies of 15 or 16 Hz (*20*). The Ca^{2+} efflux response went through a series of maxima as the modulation rate or RFER intensity was varied (*21, 22*). These responses, referred to as frequency or power "windows", occurred under condi-

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch026

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tions not associated with RFER-induced heating. The generality of the effect was indicated by similar ELF RFER modulation responses of synaptosomes (*23*) and neuroblastoma cells (*24*).

Energy Metabolism

The frequency dependence of low-intensity RFER exposure on cell energy metabolism was investigated by Sanders and co-workers (*25*). They reported a dose-dependent increase in nicotinamide adenine dinucleotide (NADH) fluorescence in rat brain cells immediately after a 2-min exposure to 200- or 591-MHz CW RFER. The SAR threshold for altered energy metabolism was on the order of 8×10^{-3} W/kg, well below the level associated with tissue heating. Exposure of brain cells to 2450-MHz CW RFER at this SAR, under the same experimental conditions, had no effect on NADH fluorescence. ATP brain cell concentrations were decreased following exposure to 200- or 591-MHz RFER, with no effect of 2450-MHz exposure. Cell concentration of creatine phosphate decreased following exposure to 591 MHz; other RFER frequencies were ineffective (*25*).

These results were interpreted by Sanders et al. (*25*) as RFER frequency-dependent effects on specific mitochondrial enzymes or electron-transport proteins that function to maintain cellular ATP pools. They proposed a mechanism for the inhibitory effect that involved RFER-induced dipole oscillations of divalent metal ions at active enzyme sites during either catalytic or transport activity. Mechanistically this hypothesis was similar to that proposed by Allis and Sinha-Robinson (*19*) for RFER effects on Na^+/K^+-ATPase. A subsequent study by Sanders et al. (*26*) of the effects of CW, sinusoidal amplitude-modulated, and pulsed square-wave-modulated 591-MHz radiation on brain energy metabolism provided additional evidence of direct molecular-level interactions of RFER with rat brain cells. The sensitivity of brain cells to low-intensity RFER is consistent with numerous reports of neurological and behavioral alterations in experimental animals and human beings (*27*).

Molecular–Biochemical Effects

The basic interaction mechanisms for molecular-level biochemical effects of RFER are uncertain. However, insight is provided by studies of a specific biomolecular effect conducted in different molecular microenvironments. Fisher et al. (*17*) detected a 40% reduction in ouabain-sensitive Na^+ efflux from erythrocytes exposed at 23–24 °C to 2450-MHz RFER at an SAR of 3 W/kg. Reduced efflux was attributed to a field-induced effect on membrane Na^+/K^+-ATPase. At this same frequency of RFER, Allis and Sinha-Robinson (*19*) induced a 35% decrease in Na^+/K^+-ATPase activity in erythrocyte membrane fragments at an SAR of 6 W/kg at a steady-state temperature of 25 °C. Brown and Chattopadhyay (*28*), on the other hand, reported a 23% decrease in Na^+/K^+-ATPase activity at 24.9 °C when the enzyme was exposed in solution to 9140-MHz CW RFER at an SAR of 20 W/kg. In view of previous evidence that the effect of

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch026

RFER on Na^+/K^+ membrane transport was not highly frequency dependent (*18*), the results of these studies suggest that the RFER intensity needed to alter enzyme activity may depend upon the microenvironment of the Na^+/K^+-ATPase. The fact that the minimum RFER intensity required to affect Na^+/K^+-ATPase activity occurred in intact membranes suggests an interaction between RFER energy and metabolic energy. Maximum RFER intensity was required for Na^+/K^+-ATPase suppression in solution in the absence of cell metabolic energy sources. This hypothesis is supported by the results of previous studies of the effects of low-intensity RFER on biomolecules in solution, which have, in general, yielded negative results (*29*). Attention must thus be focused on the nonequilibrium status of living systems in developing interaction mechanisms for RFER-induced alterations.

Additional evidence of direct biochemical interactions of RFER was reported by Phelan et al. (*30*), who investigated effects on the structure of cell or liposome membranes. RFER (2450-MHz) exposure at intensities as low as 0.2 W/kg caused a shift from a fluid to gel state in membranes that contained melanin. In the absence of melanin, RFER exposure had no detectable effect on membrane structure as determined by electron spin resonance spectroscopy. The fact that the RFER effect was inhibited in the presence of superoxide dismutase indicated the involvement of free-radical generation (*30*). The results and conditions for the molecular–biochemical studies of RFER are summarized in Table II.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch026

Membrane Ion Channels

Evidence of direct athermal interactions of RFER with biomolecules other than enzymes was provided by studies of effects on membrane ion channels and excitable membranes. Sandblom and Theander (*31*) investigated effects of 10,000-MHz pulsed RFER on gramicidin A channel kinetics in artificial lipid bilayer membranes. A 1-min exposure to short (1-μs), high instantaneous power (350-W/kg) RFER pulses had no effect on the lifetime or conductance of gramicidin A channels. However, RFER exposure decreased significantly the rate of channel formation (*31*).

An effect of RFER on ion channels was also reported by d'Inzeo et al. (*32*), who exposed chick myotubes to CW 9750-MHz RFER at low intensities (~1–2 $\mu W/cm^2$) for periods of 30–120 s. RFER exposure decreased the frequency of single-channel openings of acetylcholine-induced channels (*32*).

Another direct effect of low-intensity RFER on an excitable membrane was recently reported (*33*). The rate of rapid, burstlike changes in the firing rate of molluscan neurons was increased by exposure to PM 900-MHz RFER at SARs of 0.5 W/kg or higher. At the same SAR, CW RFER had no detectable effect on neuronal firing rates. The specificity of the effect on membrane firing rate was indicated by the lack of effect of CW or PM RFER on mediator-induced activation of acetylcholine, dopamine, serotonin, or γ-aminobutyric acid membrane receptors (*32*). Effects of RFER on ion channels are summarized in Table III.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch026

Table II. Molecular–Biochemical Effects of RFER

Microwave Source Descriptors	*Typical Effect*	*Field Parameters/ Thresholds*	*Comments*
9140 MHz CW	Altered Na^+/K^+-ATPase enzyme activity	20 W/kg	Enzyme activity increased as *T* was increased from 4 to 43.8 °C, except at 24.9 °C at which point it unexpectedly decreased; inhibitory effect of ouabain significantly reduced by microwave exposures at $T > 24.9$ °C; effect attributed to direct molecular level interaction of microwaves (ref. 28)
2450 MHz CW	Altered Na^+/K^+-ATPase activity in erythrocyte membrane fragments	6 W/kg	Enzyme activity decreased 35% at 25 °C only, not at other *T* in range 23–27 °C; ouabain-insensitive Ca^{2+} ATPase activity also altered (ref. 19)
2450 MHz pulsed	Altered Na^+/K^+-ATPase activity in erythrocytes	3 W/kg	40% decrease in ouabain-sensitive Na^+ efflux at 23 and 24 °C (ref. 17)
2450 MHz pulsed	Altered membrane structure in melanoma cells or melanin-containing liposomes	0.2 W/kg	Microwaves induced shift from fluid to gel state in membrane in presence of melanin; effect inhibited by SOD; microwave effect may be mediated by temperature-dependent generation of O_2 radicals (ref. 30)

NOTE: ATPase is adenosinetriphosphatase; *T* is temperature; SOD is superoxide dismutase.

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Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch026

Table III. Membrane Ion Channel Effects of RFER

Microwave Source Descriptors	*Typical Effect*	*Field Parameters/ Thresholds*	*Comments*
10,000 MHz pulsed	Single channel kinetics of gramicidin A channels in lipid bilayers	1-μs pulse, 350 W/kg 10^5 V/m instantaneous 10^3 pps 1-min exposure	No effect on channel conductance or lifetime; significant decrease in rate of channel formation; opposite from heating effect; due to direct interaction with channel-forming molecules (ref. 31)
9750 MHz CW	Single-channel kinetics of acetylcholine-induced channels in chick myotubes	~1–2 $\mu W/cm^2$ 30–120 s	Decreased frequency of single-channel openings (ref. 32)
900 MHz pulsed	Firing rate of molluscan neurons	0.5-W/kg threshold 0.5–110 pps 2-min exposure	Increased rates of rapid, burstlike firing; lesser effect of CW microwaves at same SARs; direct effect on neuronal membrane (ref. 33)

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Summary and Conclusions

In vitro cellular and molecular level studies provide evidence of direct athermal effects of RFER on cellular processes including (1) membrane ion transport and binding, (2) membrane structure, (3) membrane single-ion-channel kinetics, (4) neuronal excitability, (5) proliferation/activation, and (6) neoplastic transformation. Such alterations occurred under a variety of exposure conditions: (1) SARs from 8×10^{-3} to greater than 100 W/kg, (2) frequencies of from 27 to 10,000 MHz, and (3) CW and PM RFER fields. RFER in vitro studies generally involved short exposure durations (periods of a few hours or less). The diverse cell physiological end points directly affected by wide ranges of RFER intensity, frequency, and modulation suggest the possibility of more than one basic interaction mechanism. For the most part, in vitro RFER studies have involved single end points such as ion-channel kinetics, or a group of directly related end points such as bioenergetic changes. Consequently, it is not possible to interrelate RFER in vitro exposure effects on a given cell system to determine if more than one interaction mechanism may be involved or if multiple interrelated end points are affected. In order to do this type of analysis, integrated studies must be conducted involving the effect of a given RFER parameter set (i.e., intensity, frequency, and modulation) on an array of related cell physiological end points.

The existing data are insufficient to define time or intensity thresholds or RFER frequency dependence for the majority of the reported in vitro effects, and this lack places limitations on the application of data to the development of interaction mechanisms or to the assessment of health effects. In spite of such limitations, some general conclusions are possible: (1) RFER can directly induce cell physiological alterations in vitro under conditions that do not involve temperature elevations; (2) although detailed mechanisms are unknown, the cell plasma membrane is the most likely RFER interaction site; (3) RFER directly affects a variety of biomolecular systems with no clear indication of specific molecular sensitivities; and (4) effects of RFER on mammalian cells in vitro are generally consistent with reported in vivo exposure effects including increased cancer incidence, effects on reproduction and development, and neurological and behavioral changes. A well-defined need exists for additional data to more effectively relate in vitro to in vivo effects of RFER exposure.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ch026

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RECEIVED for review April 6, 1994. ACCEPTED revised manuscript January 18, 1995.

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Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ix002

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ix002

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Advances in Chemistry; American Chemical Society: Washington, DC, 1995.

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ix002

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Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ix002

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ix002

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Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ix002

In Electromagnetic Fields; Blank, M.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1995.

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Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ix002

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ix002

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ix002

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ix002

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Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ix002

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Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ix002

Publication Date: May 5, 1995 | doi: 10.1021/ba-1995-0250.ix002