

Electromagnetic field interactions with biological systems¹

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ABSTRACT This is a report on Symposia organized by the International Society for Bioelectricity and presented at the 1992 FASEB Meeting. The presentations summarized here were intended to provide a sampling of new and fruitful lines of research. The theme topics for the Symposia were cancer, neural function, cell signaling, pineal gland function, and immune system interactions. Living organisms are complex electrochemical systems that evolved over billions of years in a world with a relatively simple weak magnetic field and with few electromagnetic energy emitters. As is characteristic of living organisms, they interacted with and adapted to this environment of electric and magnetic fields. In recent years there has been a massive introduction of equipment that emits electromagnetic fields in an enormous range of new frequencies, modulations, and intensities. As living organisms have only recently found themselves immersed in this new and virtually ubiquitous environment, they have not had the opportunity to adapt to it. This gives us, as biologists, the opportunity to use these electromagnetic fields as probes to study the functioning of living systems. This is a significant opportunity, as new approaches to studying living systems so often provide the means to make great leaps in science. In recent years, a diversity of biologists have carried out experiments using electromagnetic fields to study the function of living cells and systems. This approach is now becoming quite fruitful and is yielding data that are advancing our knowledge in diverse areas of biology.—Frey, A. H. *Electromagnetic field interactions with biological systems. FASEB J.* 7: 272–281; 1993.

Key Words: electromagnetic • cancer • brain • pineal • immune

IF ONE USED ELECTROMAGNETIC ENERGY sensors to view the world from space 100 years ago, the world would have looked quite dim. Now the world glows with electromagnetic (em)³ energy emissions at most frequencies of the nonionizing portion of the spectrum, such as power line fields, radio waves, microwaves, etc.

Living organisms are complex electrochemical systems that evolved over billions of years in a world with a relatively simple weak magnetic field and with few em energy emitters. As is characteristic of living organisms, they interacted with and adapted to this environment of electric and magnetic fields. One example of this adaptation is the visual system, which is exquisitely sensitive to emissions in the very narrow portion of the em spectrum that we call light. Organisms also adapted to another portion of the spectrum, the UV, by developing filtering systems in the eye and the skin to protect themselves from it.

A wide range of living organisms, including humans, adapted by using em energy to regulate various critical cellular systems; we see this in the complex of circadian rhythms.

Fish, birds, and even the duckbill platypus developed systems to use electromagnetic fields to sense prey and to navigate (1). Electromagnetic fields are involved in neural membrane function. Even protein conformation involves the interactions of electrical fields.

Thus, it is not surprising that the massive introduction of electromagnetic fields in an enormous range of new frequencies, modulations, and intensities in recent years has affected living organisms (2–5). In fact, it would be incredible and beyond belief if these electromagnetic fields did not affect the electrochemical systems we call living organisms.

As living organisms have only so recently found themselves immersed in this new and virtually ubiquitous environment, they have not had an opportunity to adapt to it. This gives us, as biologists, the opportunity to use these em fields as probes to study the functioning of living systems. This is exciting because new approaches to studying living systems so often provide the means to make great leaps in science. Energy in the power line frequency, radio, and microwave portions of the spectrum, as well as magnetic fields, penetrate the body and can be used as probes; the emitters are now available. The ubiquitousness of electromagnetic fields is also now a matter of concern to us as biologists; it is relevant to the question of controls needed in experiments. Look about the lab and consider the em fields now being imposed on test specimens by all the electrical devices in use. How do they influence the results of biological experiments? One of the Symposium speakers touched on this question.

¹A report on five symposia presented on April 7, 1992, in Anaheim, California at the Annual Meeting of the Federation of American Societies for Experimental Biology by the International Society for Bioelectricity. Symposium titles and their participants are: Cancer and Environmental Electromagnetic Fields (chaired by R. Stevens); participants: J. Phillips, G. Harrison, and W. O. Short; Nervous System Function: Interaction with Electromagnetic Fields (A. H. Frey). M. McGinnis, A. R. Sheppard, and J. Walleczek; Cell Signaling and Electromagnetic Field Interactions (B. R. McLeod). M. Blank, C. Cain, and R. A. Luben; Pineal Gland Response to Electric and Magnetic Fields: Evidence and Possible Consequences (L. Anderson). R. Reiter, S. Yellon, and B. Wilson; Immune System Interaction with Environmental Electromagnetic Fields (chaired by D. Lyle). R. P. Liburdy, A. Cossarizza, and W. D. Winters.

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³Abbreviations: em, electromagnetic; mf, magnetic field; CREB, calcium/cyclic AMP binding protein; SRE, serum response element; SRF, serum response factor; mw, microwave; TPA, 12-O-tetradecanoyl-phorbol-13-acetate; dc, direct current; CNS, central nervous system; MRI, magnetic resonance imaging; IBIs, interburst intervals; emf, electromagnetic field; DFS, dual-sample (or differential) fluorescence spectroscopy; PTH, cell parathyroid hormone; NAT, N-acetyltransferase; PBMC, peripheral blood mononuclear cells; PHA, phytohemagglutinin; NK, natural killer; MHC, major histocompatibility complex; Con A, concanavalin A.

Much of the literature on electromagnetic field interactions published before the middle 1980s is irrelevant to biologists. Most of it was generated by the engineering community's attempt to find out if their high-power equipment creates a hazard. Thus, little attention was paid to the variables that are important in biology. Instead, efforts were wasted in fruitless controversies such as whether the effects seen were thermal or nonthermal. As I noted in an earlier published paper (2), this is really a matter of semantics, not science. There was no common definition of thermal—to some it meant a change that could be measured with a rectal thermometer; to others it meant unmeasurable vibration of molecules at a cell membrane. Besides, it is a label, not an explanation.

In recent years, though, the convergence of a number of lines of research has led a diversity of biologists to carry out experiments using low-intensity electromagnetic fields to study the function of living cells and systems. This approach is now becoming quite fruitful and is yielding data that are advancing our knowledge in diverse areas of biology. Presentations at the Symposia summarized here were intended to provide a sampling of these new and fruitful lines of research. The theme topics for these Symposia were cancer, neural function, cell signaling, pineal gland function, and immune system interactions.

Cancer

In the first Symposium, chaired by Richard Stevens, reports of research were presented by Jerry Phillips, George Harrison, and Welland Short. Previous work by Phillips (6, 7) and others indicates that exposure of various cell systems to magnetic fields with a variety of signal characteristics produces general increases in gene transcription. The question addressed in Phillips presentation was what specific genes are altered at the transcriptional level. The speaker had previ-

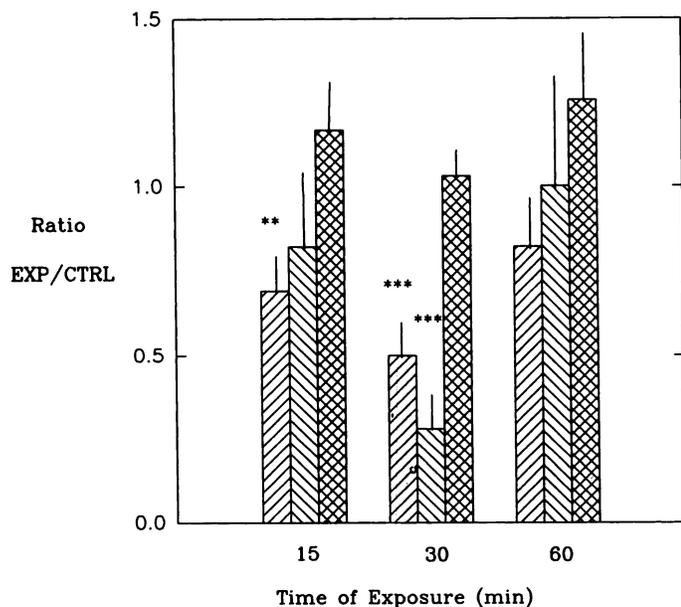


Figure 1. Effect of mf exposure on AP-1 and CREB DNA-binding activity. Results are expressed as the ratio of activity in exposed to control cell cultures, and are presented as the mean value of 9 separate experiments. Vertical bars represent SEMs. First bar in each group is AP-1 binding protein #1, second bar is AP-1 binding protein #2 and third bar is CREB. ***Indicates $P < 0.001$ and ** indicates $P < 0.01$.

ously used the nuclear run-off assay to study the effect of exposure of CCRF-CEM cells to a 1 gauss sinusoidal magnetic field (mf) at 60 Hz on the transcription of four genes: *c-fos*, *c-jun*, *c-myc*, and protein kinase C (β form). He found a time-dependent, and sometimes a cell density-dependent, change in the transcription of these genes. He then concentrated on the magnetic field-induced increase in *c-fos* transcription in order to determine the mechanism by which the mf induced changes to occur. His approach was to use transcription of the *c-fos* gene as an endpoint, and then move "upstream" one step at a time to determine which steps leading to gene transcription are influenced by mf exposure (6, 7).

Phillips used the mobility shift assay to study mf-induced changes in the DNA binding activity of specific transcription factors. Figure 1 summarizes the results of nine separate experiments in which the relative levels of two specific AP-1 complexes were measured, as was calcium/cyclic AMP (CRE) binding protein, CREB, in exposed compared with unexposed control cells. The mf exposure induced a significant decrease in the levels of both AP-1 complexes, reaching a nadir after 30 min of exposure. In contrast, mf exposure produced no change in the level of CREB capable of binding to its recognition sequence. Serum response factor (SRF) binding to the serum response element (SRE) was measured in four separate experiments. In two experiments SRF binding was unaffected by mf exposure, whereas in two other experiments SRF binding was increased substantially, peaking after 30 min of exposure.

The speaker stated that the data presented indicate that mf induced changes in the levels of DNA binding activity of specific transcription factors and are consistent with increased *c-fos* transcription and decreased *c-jun* transcription. He noted that because transcription of the *fos* gene is down-regulated by the *fos* protein, and because AP-1 consists of either *fos-jun* heterodimers or *jun-jun* hetero- or homodimers, decreased AP-1 may indicate decreased *fos* protein and thus a removal of repression to *fos* gene transcription. At the same time, decreased AP-1 signifies decreased levels of *jun* protein, and both are consistent with decreased *c-jun* transcription.

Phillips stated that the results of his em field studies of SRF binding to the SRE are also consistent with other reports that detail the mechanism of mitogen-induced changes in *c-fos* transcription. He noted that it has been shown that the SRF has two distinct activities: 1) a DNA binding activity, and 2) a transcriptional-initiation stimulatory activity. Consequently, he concluded that depending on the cell type or the state of a given cell, the DNA binding activity may be constitutively activated whereas the protein, already bound to the SRE, awaits activation of its transcriptional-initiation stimulatory function. Conversely, treatment of a cell with some agent may, through phosphorylation of the SRF, increase both its DNA binding and transcriptional-initiation stimulatory functions. A similar situation, he said, has been reported for CREB.

Harrison presented data from a series of studies (8, 9) of neoplastic transformation induced by 2.45 GHz microwave (mw) energy pulse-modulated at 120 Hz, which was followed by treatment with 0.1 μ M of the tumor promoter 12-O-tetradecanoyl-phorbol-13-acetate (TPA). He noted that this energy has been found to be a promoter by other investigators. In the reported study, microwaves were tested for their activity as an initiator or a complete carcinogen. The methodology of these experiments followed the original protocols, yielded dose-response data, and were intended to study the multi-step progression of tumor development culminating in the growth of fatal fibrosarcomas when fully transformed cells are injected into test animals. The possible cocarcino-

genic potential of microwaves combined with X rays or benzo[*a*]pyrene was also investigated.

The speaker reported that there was no mw transforming activity without postculturing with TPA. But when followed by TPA treatment, microwaves were additive with X ray-induced transformation. The dose-response curve resulting from mw exposure followed by TPA is shown in Fig. 2, along with similar X ray-induced responses for comparison. The speaker stated that the results were not due to heating.

Short was interested in determining the effect of high-strength static magnetic fields on the ability of human malignant melanoma cells to increase in number and remain viable. Normal human fibroblasts were also studied to determine whether any alterations were unique to tumor populations. Human cell lines were grown in monolayer culture in vitro and subjected to a static magnetic field using a 4.7 tesla superconducting magnetic resonance imaging (MRI) magnet with the gradient coils removed. The number of cells within the total population was determined by using an electronic particle counter. Cell viability was estimated by trypan blue exclusion and the cellular morphology of the attached cells was documented using microscopy. The results Short reported showed that the human malignant melanoma cells and the normal human cells were both unaffected by the presence of a high-strength magnetic field in terms of a change in their numbers or viability. He found, however, that the ability of the malignant melanoma cells to remain attached to the tissue culture surface was markedly but reversibly impaired by the presence of the static magnetic fields. Disattachment was not observed within the human normal fibroblasts. The speaker concluded that high field strength static magnetic fields may alter the ability of a tumor cell to successfully interact with its extracellular environment.

Neural function

In the second Symposium, chaired by Allan Frey, research reports were presented by Michael McGinnis, Asher Sheppard, and Jan Walleczek. McGinnis noted that small local endogenous direct current (dc) electric fields are associated with a variety of developmental and repair events. In addition, exogenously applied dc fields are able to alter the orientation, migration, and growth of cells in vitro. This led to the hypothesis that applied fields may be used to influence either

developmental or repair events in vivo. He tested this hypothesis in the mammalian central nervous system (CNS) by imposing dc fields across lesioned guinea pig spinal cords and assayed for the return of function in the simple spinal reflex (10, 11). He found a modest, yet consistent, effect of such fields on the percentage of animals showing recovery of this reflex months after the original injury.

McGinnis extended these findings in a recently completed veterinary clinical trial using dogs with complete paraplegia due to naturally occurring disk herniation. Specifically, he used 24 small breed dogs presenting with complete paraplegia due to intravertebral disc herniation, which were entered into the study at the Veterinary Teaching Hospital at Purdue University. Each animal received a neurological exam, a battery of electrodiagnostic tests, and radiographic examination before surgery. At surgery, the spinal cords were decompressed and an oscillating field stimulator was implanted with platinum electrodes placed rostral and caudal to the lesion. The stimulator delivered 200 mA of regulated dc current that alternated in polarity every 15 min. This regular reversal of polarity was based on the hypothesis that nerve fibers are induced to regenerate only by a distal cathode. Therefore, to affect both ascending and descending fibers, the polarity of the current was reversed at regular intervals. Evaluations of the neurological and evoked potential were repeated at 6 weeks and at 6 months after the stimulator was implanted.

There were more instances of recovery of function in the 13 electrically treated animals than in the 11 sham-treated controls. This difference in the degree of functional recovery was statistically significant for a combined neurological score. This incorporated deep pain appreciation, superficial pain appreciation, placing ability, and ambulatory ability. He noted that the data suggest that applied dc fields may be able to influence the response to trauma in such a way as to allow more functional recovery to occur than would normally be expected.

Sheppard noted that interactions of the nervous system with exogenous electric fields and currents are of significance to neurophysiology. He indicated there is evidence that optimal coupling of currents to cells occurs with currents parallel to nerve cell fibers for focal dipole fields and pulsed fields. However, it has been shown that very much weaker environmental electric fields (in the microvolt range) can be sensed by a number of aquatic animals. Magnetic fields of about 50 mT can be sensed by organisms as primitive as bacteria and as complex as marine mammals. He also discussed laboratory studies using a wide range of field strengths and frequencies that indicate effects on human cardiac rate, evoked brain potentials, animal behaviors such as learning and time estimation, nerve function, neurite growth, circadian rhythms—including brain transmitter and pineal melatonin rhythms, EEG rhythms, brain slice physiology—and on both active and passive properties of individual neurons.

The speaker indicated that shifts in membrane surface potential may be more important for situations in which transmembrane shifts are insignificantly small or observed phenomena are not directly related to membrane potential. As an example of such indirect effects, he presented data showing the influence of a strong 60 Hz magnetic field on intracellularly recorded interburst intervals (IBIs) of a pacemaker neuron of the marine snail *Aplysia*. The speaker concluded that the electric field (≤ 8 mV/m_{rms}) induced in the experimental chamber by the magnetic field was too weak to produce direct electrical effects on the cell, as there was a 6 min delay before effects were evident.

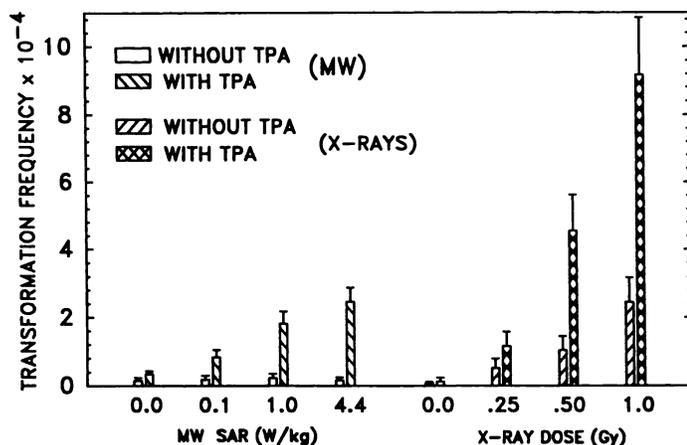


Figure 2. The response resulting from mw exposure followed by TPA and similar X-ray-induced responses for comparison.

Walleczek explained how membrane-mediated signal transduction pathways are thought to be an early target of electromagnetic field (emf) interactions with cellular systems, including cells of the immune and nervous systems. In particular, he noted that the early modulation of Ca^{2+} signals by electromagnetic fields is a plausible candidate for inducing later-stage emf effects on cellular function (12). Thus, one goal of his research has been to study the effects of 50/60 Hz emfs on Ca^{2+} regulation in electrically nonexcitable cells of the immune system compared with electrically sensitive neuronal cells. As model systems for studying emf influences on Ca^{2+} -mediated immune system signal transduction events, the human leukemic T cell line JURKAT as well as freshly isolated rat thymic lymphocytes were used. The speaker stated that a 22 mT, 60 Hz sinusoidal magnetic field enhances $^{45}\text{Ca}^{2+}$ uptake in concanavalin A (Con A)-treated rat lymphocytes, but not in resting cells, as determined after a 60 min exposure (13). He also stated that rat pheochromocytoma PD-12 cells, a neurosecretory cell line frequently used as a nerve cell model, were used to study cellular interaction mechanisms of emfs with the nervous system. Neurotransmitter secretion in PC-12 cells reportedly is mediated at least in part by changes in the free cytosolic calcium concentration, $(\text{Ca}^{2+})_i$. He thus suggested that emf-induced alterations in $(\text{Ca}^{2+})_i$ could affect neurotransmitter release, and subsequently nerve cell function.

To define these effects, Walleczek developed a new fluorimetric technique, termed dual-sample (or differential) fluorescence spectroscopy, or DFS, which he described. The design of the DFS instrument was based on the assumption that a reliable and efficient approach for probing emf phenomena would be to simultaneously monitor real-time fluorescent changes in an emf-exposed cell sample and an identical isothermal control sample. Commercially available fluorimetry systems ("one-sample" systems) are limited to measuring either the experimental or the control sample, and thus cannot be used in this manner. With DFS, it is now possible to record at the same time real-time changes in $(\text{Ca}^{2+})_i$ in the absence/presence of emfs.

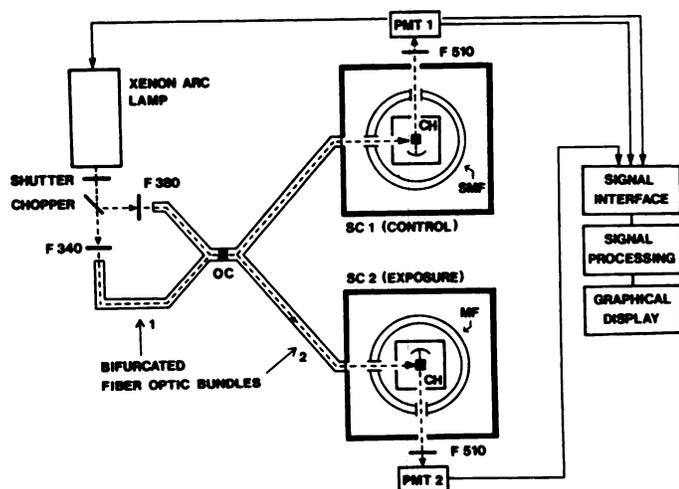


Figure 3. Dual sample FURA-2 fluorescence spectroscopy (DFS) electric and magnetic field-exposure system. Abbreviations are as follows: CH, nonmetallic, thermostated cuvette holder; F, interference filter (340, 380, or 510 nm bandpass filters); MF, water-cooled befilar Lee-Whiting magnetic field exposure coil; OC, optical coupler; PMT, photomultiplier tube; SC, nonmetallic sample compartment; SMF, sham magnetic field exposure coil (same as MF). Solid lines represent electrical pathways; dashed lines represent the excitation and emission light paths.

Figure 3 provides a schematic representation of the key components of the DFS electric and magnetic field exposure system. The cells were prevented from sedimenting to the bottom of the cuvette by a custom-made, nonmagnetic cell mixing system. This was found to be necessary because the magnetic flux densities associated with magnetic cell stirrers, as used in conventional fluorimeters, are on the order of 1 to 10 mT. This has implications for quite a few labs.

Cell signaling

In the third Symposium, chaired by Bruce McLeod, research reports were presented by Martin Blank, Christopher Cain, and Richard Luben. Blank explored em field effects on Na,K-ATPase activity and biosynthesis. The processes he focused on range from relatively simple ion transport due to the Na,K-ATPase to the complex stages of biosynthesis. These systems also represent links in a possible mechanistic chain that couples events outside the cell to processes in the cell.

The speaker reported that ATP splitting by the membrane Na,K-ATPase changes when electric currents are imposed with electrodes or induced by magnetic fields. The effect depends on the basal level of enzyme activity. Under optimal conditions, electric currents decrease the activity; when the basal activity is lowered by inhibitors or by lowering temperature, electric currents increase the activity. The speaker noted that both effects have a broad frequency dependence that is similar (and probably related) to the one calculated for ion concentration changes at membrane surfaces.

It was stated that both inhibition and stimulation, as well as the observed dependences on the electric field and level of enzyme activity, can be explained by increased binding of activating cations to the Na,K-ATPase. When the enzyme is at optimal activity, an increase in ion concentration lowers activity. Below optimal activity, an increase in ion concentration increases the enzyme activity. The threshold for inhibition by induced electric currents is about an order of magnitude higher than that for currents imposed by electrodes. The additional stray magnetic field in his induced current experiment appears to have an effect on Na,K-ATPase function which is opposite to that of the electric field. Studies of transcription suggest similar effects of electric and magnetic fields. Blank thinks his results indicate that em signal transduction can occur at ion activation sites on the outside surface of the membrane Na,K-ATPase. The changes affect enzyme function and alter the composition of the cell. He noted that the observed therapeutic effects of em signals on damaged tissues correlate with the enhanced activity of the enzyme, due to electric currents, when it is far from its optimum (14, 15).

Blank also noted that changes in biosynthetic response to em fields are complicated, but stated that he found similarities in the patterns of the biosynthetic responses of *Sciara coprophila* salivary gland cells to two kinds of stress: em fields and heat shock. He converted data from two-dimensional gels into protein distribution curves (% mass vs. mw, % mass vs. pI) and found new peaks that were characteristic for both types of stress. He indicated that changes in the two peaks in the pI distribution as a function of em field strength define a dose-response curve and the range of effective field intensities. Em fields and heat shock are quite different in their effects on cells, but similar biosynthetic responses indicate that both stimuli affect the same final common pathway. The cell appeared to respond in an undifferentiated way to what it sees as a stress. Also, the apparent absence of variations in the response resulting from variations in em field intensity suggest an all-or-none response.

Cain addressed the question of mechanisms for em fields and carcinogenesis. He noted that the effects of em fields on biological systems are consistent with the concept that the cell membrane is a primary site of interaction with em fields. Thus, he chose a model that focuses on membrane-related events and not on genotoxic and mutagenic events in studying the relation between power frequency em fields and carcinogenesis in vitro. He stated that in multistage carcinogenesis, membrane-related events mediated through receptor mechanisms are involved in tumor promotion that is reversible and environmentally modulated. He noted that it has been suggested that plausible biological mechanisms by which environmental em fields may influence carcinogenesis include epigenetic events of tumor promotion such as transmembrane signal transduction and intercellular communication. However, he felt that there was a need for direct experimental evidence that fields influence tumor promotion per se, although higher-frequency em fields have been shown to influence carcinogenesis in vitro in C3H/10T1/2 fibroblasts. The question he specifically addressed in the reported experimentation was whether 60 Hz em field exposure and a chemical tumor promoter, (TPA), are copromoters.

With his in vitro model system, he measured the focus formation of mutant cells (UV-TDT10e) in coculture with parental C3H/10T1/2 fibroblasts. Specifically, he said C3H/10T1/2 cells suppress growth of the transformed phenotype of their daughter UV-TDT10e cells by preventing UV-TDT10e cells from growing in multilayered foci, as occurs when UV-TDT10e cells grow alone. In coculture, TPA promotes focus formation of mutant UV-TDT10e cells cocultured with parental C3H/10T1/2 cells.

Disruption of communication between transformed cells and normal cells is involved in tumor promotion. Cain tested the hypothesis that 60 Hz em field exposure and TPA are copromoters enhancing focus formation. Em field exposures alone did not affect the growth of parental C3H/10T1/2 fibroblasts or daughter mutant cells, UV-TDT10e (16). Furthermore, em field exposure alone did not promote focus formation of mutant cells in coculture with parental cells under the conditions tested. However, em field exposure copromoted with TPA by increasing focus formation in coculture, as shown in Fig. 4. Video image analysis of three replicate experiments showed that field exposed cultures produced 1.91-fold more foci than sham exposed cultures when treated with TPA. The total area of foci per dish increased 2.22-fold and the number of cells in stained foci increased 2.34-fold.

Cain concluded that his experiments suggest that chronic intermittent exposures to a 60 Hz em field and a chemical tumor promoter influence membrane-related events by copromoting focus formation. Thus, a coculture system provides a method to investigate biological mechanisms by which em fields may influence carcinogenesis.

Luben discussed the effects of low-intensity electromagnetic fields on signal transduction by G protein-linked receptors. In previous studies of bone cell parathyroid hormone (PTH) receptors, he noted, pulsed em fields decreased the efficiency of signal transduction by the PTH receptor (i.e., at a concentration of PTH, which should have been effective in inducing bone cells to decrease their bone forming activities, exposure to pulsed em fields decreased that response by as much as 90%). These changes in response to PTH are consistent with the known in vivo effects of pulsed fields, i.e., an increase in the rate of bone formation relative to bone resorption. As the observed decreases in responsiveness to PTH were not accompanied by decreases in hormone binding to the receptor, he hypothesized that pulsed fields had

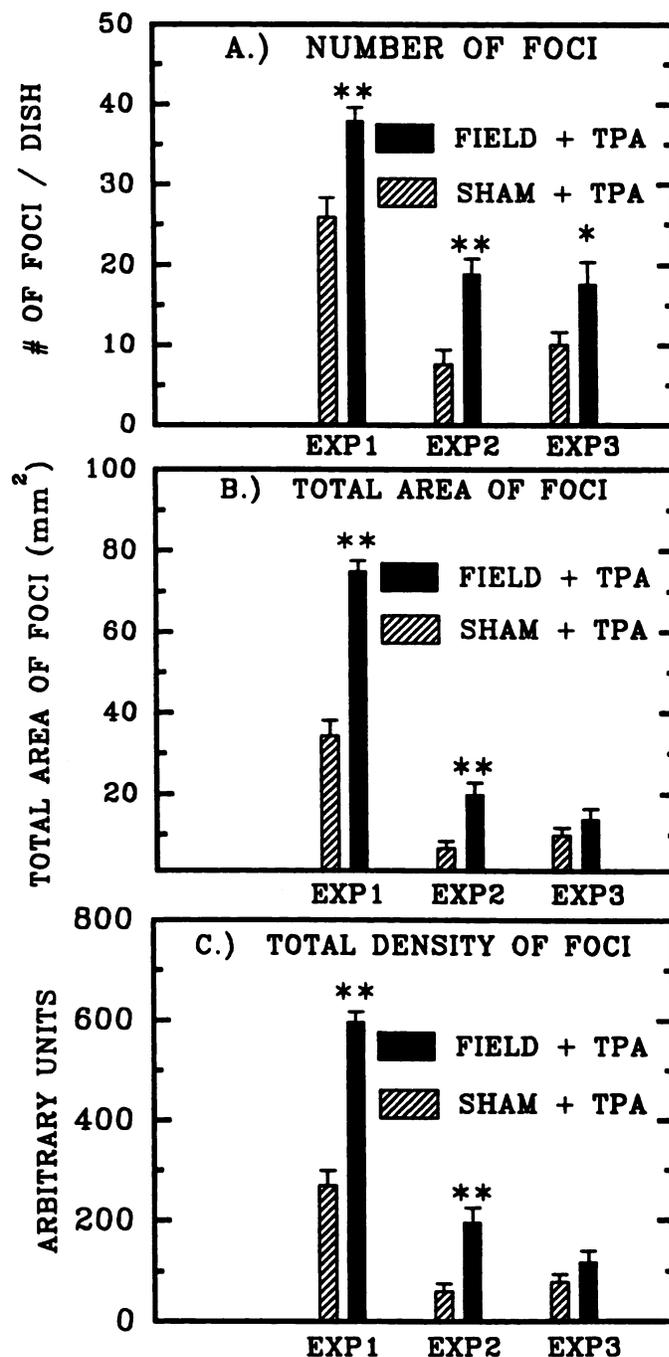


Figure 4. 60 Hz em field exposures and TPA co-promoted focus formation. A) foci were counted using video analysis software for cocultures with TPA, 50 ng/ml. B) from the same video analysis data set, the total area of foci per dish was calculated. C) the total optical density of stained foci was calculated. For cocultures with TPA: in experiment 1, *n* was 10 (sham) and 13 (field); in experiment 2, *n* was 10 (sham) and 11 (field); in experiment 3, *n* was 19 (sham) and 16 (field). The mean values \pm SEM for TPA-treated cocultures are depicted for A-C. **Indicates $P < 0.001$ and * indicates $P < 0.05$ using the *t* test.

modified the coupling of receptor to membrane signal transduction processes, most likely the interaction of membrane G proteins with the receptor. He stated that studies with membrane-specific probes for G proteins and adenylyl cyclase support this hypothesis. Further studies with specific monoclonal antibodies to the PTH receptor, he noted, have confirmed that pulsed fields caused change in the exposure

to the extracellular medium of individual receptor determinants. He thinks this indicates that the orientation or aggregation state of the receptor is actively modified by the fields (17, 18).

Luben reported on experiments derived from his hypothesis that other members of the G protein-linked receptor family might also be susceptible to em fields effects. He noted that the hypothesis is based on the observations mentioned above and the following: 1) the basic structure of the transmembrane signaling domains found in all of these receptors is very highly conserved through a variety of species and receptors (e.g., from bacterial rhodopsin through mammalian neurotransmitter receptors), 2) the only other clearly demonstrated receptor for electromagnetic energy is rhodopsin, a member of the G protein-linked family, 3) probable receptor organs for low-energy electromagnetic fields in monotremes are modified retinal/neural cells, which contain high levels of rhodopsin-like membrane proteins, and 4) em field effects on neural metabolism and behavior could be explained plausibly by effects on neurotransmitter and/or neurohormone receptors, many of which are members of the G protein-linked class of receptors (e.g., adrenergic, muscarinic, serotonergic, and several types of peptidergic neurons are known to function by G protein-linked receptors). He stated that he has expanded his studies to include β -adrenergic receptors because they are widely distributed, their properties are well characterized, and there is evidence that they may be involved in some responses to em fields in various systems.

In his experimentation, Luben measured activation of adenylyl cyclase and the binding of hormone analogs to PTH receptors and β -adrenergic receptors in various cultured cell types exposed to em fields (using exposure schedules previously demonstrated to be effective in modifying PTH receptor activity). He examined β -adrenergic receptor activities in cultured mouse bone cells, cultured mouse skin fibroblasts, and explanted mouse pineal glands in organ culture exposed to pulsed em fields. He did not observe significant effects on the β -adrenergic receptor with a 72 Hz field, although that was the most effective in studies of PTH receptor signal transduction. A 15 Hz pulse train em field, however, which also affects the PTH receptor after 4 h of exposure, inhibited adenylyl cyclase activation by the β -adrenergic agonist isoproterenol, but not by forskolin, a nonreceptor-dependent activator of adenylyl cyclase. Some results are shown in Table 1. Net levels of Gi and Gs proteins measured by ADP-ribosylation also were not changed by em fields. This, he believes, suggests that the observed effects were specific either for receptor/ligand or receptor/G protein interactions.

Calculations of the binding constants for the β -adrenergic ligand ^{125}I -labeled cyanopindolol indicate, in his view, that the total number of binding sites on the cells was not changed, i.e., the em field is not inhibiting expression of the

receptor, at least in these short time courses. Rather, he concluded that the affinity of the receptor for the hormone was apparently lowered. Exposure to em fields produced no effect on β -adrenergic receptor activities of mouse skin fibroblasts with the exposure conditions used for bone cell experiments. However, he reported that in preliminary experiments with explanted mouse pineal glands in short-term organ culture, 4 h of exposure to em fields at 72 Hz produced significant inhibition of isoproterenol-sensitive adenylyl cyclase and binding of ^{125}I -labeled cyanopindolol.

Luben suggested that the effects of em fields are specific for the hormone receptors rather than for other parts of the membrane signal transduction system for the following reasons: 1) there was no change in the overall level of adenylyl cyclase as measured by activation with forskolin, a reagent specific for the cyclase independently of other membrane components, and 2) there was no change in the incorporation of [^{14}C]ribose by ADP-ribosylation with cholera toxin into membrane proteins in preliminary experiments. This, he thinks, suggests that membrane levels of G protein were not affected. He believes these data indicate that β -adrenergic receptors can respond to em fields in a tissue-specific and signal-specific manner.

Pineal gland

In the fourth Symposium, chaired by Larry Anderson, research reports were presented by Russel Reiter, Steven Yellon, and Bary Wilson. Reiter discussed the important parameters of exposure and the potential mechanisms for the interaction of em fields and the pineal. He noted that the pineal gland produces melatonin almost exclusively at night. Extremely low-frequency electric fields and pulsed static magnetic fields have both been reported to depress pineal melatonin production. He reported use of weak pulsed magnetic fields at night to suppress the production of melatonin and associated constituents and to define some of the parameters of exposure that are required. He also suggested a biological mechanism to explain the findings.

In most of the studies reported, young adult male rats were exposed to a pulsed magnetic field (0.25–0.44 gauss) for 30–60 min during the dark phase of the light:dark cycle. The pineal content of melatonin and the activity of N-acetyltransferase (NAT) activity, which is rate-limiting in melatonin production, was measured. The initial study, conducted 4 h after the onset of darkness, showed that NAT was depressed by approximately 30% after exposure to a repeatedly (at 1 min intervals) inverted magnetic field. In a subsequent study, the fields were inverted either “instantaneously” by means of a relay switch, or more slowly by using an integrating potentiometer. Only when the fields were instantaneously inverted were both NAT and melatonin levels depressed. He noted that rapid inversions of magnetic fields

TABLE 1. Inhibition of β -adrenergic receptor responses by pulsed emfs in mouse bone cell cultures^a

Cell line tested	cAMP accumulation at 10^{-6} M isoproterenol	^{125}I -labeled CYP binding at half-maximal activity
Bone cells	0.64 \pm 0.13 ^b	0.52 \pm 0.09 ^b
HEP hepatoma	1.06 \pm 0.11	0.93 \pm 0.12
Skin fibroblasts	0.96 \pm 0.13	1.06 \pm 0.11
Pineal cells	0.59 \pm 0.14 ^b	0.71 \pm 0.15 ^b

^aCells were incubated for 6 h with a Bi-osteogen clinical fracture healing device, which produced a pulsed 10 gauss field. The last hour of incubation included 0.1 mM isobutylmethylxanthine. Cells were then treated with isoproterenol at 10^{-6} M for 10 min and cyclic AMP accumulation was measured by radioimmunoassay. Alternatively, binding of ^{125}I -labeled cyanopindolol was measured using standard radio ligand binding procedures. The values represent field-treated/untreated ratios. ^b $P < 0.05$ using Dunnett's multiple range test.

induce electric currents (eddy currents) in rats; thus, he suggested that eddy currents may have been the cause of melatonin suppression. In another study he found that only in the mid- and late-dark phase (but not early in the dark phase) is melatonin synthesis suppressed.

By way of explanation, Reiter noted that melatonin production in the pineal gland is normally low during the day because light impinging on the eyes inhibits pineal melatonin production. As pulsed magnetic fields, like light, inhibit melatonin production, he suggested that em field exposure may have similar mechanisms, possibly by causing the isomerization of 11-*cis*-retinal to all-*trans*-retinal in the photoreceptor of the retina. Retinal is associated with the rod photopigment rhodopsin. Another constituent potentially involved in the coupling of magnetic fields to the retina/pineal system may be dopamine, a neurotransmitter found at a number of neural sites and in the retina.

Yellon's presentation was concerned with his finding that acute 60 Hz magnetic field exposure suppresses the night melatonin rise in the pineal and blood of the adult djungarian hamster. He noted that in a variety of seasonal breeders, the pineal melatonin rhythm appears to transduce information about environmental photoperiod to control reproduction. In the djungarian hamster, the evidence suggests that the duration of the nighttime rise in circulating melatonin is the physiological signal that mediates the effect of long days to stimulate and short days to inhibit reproductive function.

To determine whether an acute 60 Hz mf exposure influences the nocturnal melatonin rhythm in the pineal and blood of the djungarian hamster, adult hamsters in long days (16L:8D, male and female) were exposed to a 60 Hz mf (+1 gauss horizontal component) for 15 min beginning 2 h before lights were turned off. The time of treatment was chosen because it is a window during the day when melatonin is most effective in suppressing gonadal function. Serum obtained from blood collected from the body trunk and pineal glands was assayed for melatonin using the Guildhay antisera. The speaker noted that this radioimmunoassay for melatonin has been validated for use in this species.

Melatonin concentrations in the pineal and blood increased in the controls to a peak 3 h after the light was off. Melatonin concentrations were significantly elevated compared with low day concentrations for the remainder of the dark period. The mf exposure significantly reduced the duration of increased melatonin in the pineal at night and blunted the nocturnal rise in serum melatonin. Because the duration of increased melatonin in blood codes information about day length, the mf exposed hamsters had an inappropriate photoperiod cue, i.e., an extremely long day that is analogous to 20 h of light. The speaker suggested that mf exposure influences the mechanism of photo-periodic time measurement, which may indicate a disruption of the timekeeping capabilities of an endogenous biological clock.

Wilson, in his presentation, stated that alterations in neuronal and neuroendocrine system function have emerged as the most consistently observed *in vivo* effects of extremely low-frequency electromagnetic field exposure in laboratory studies. Electrophysiological, neurochemical, and behavioral endpoints have all been used to study the effect of emf exposure. He noted that em field exposure has been shown to affect electroencephalograms, electrocardiograms, and magnetoencephalograms in humans. Such exposure also changed the concentration of neurotransmitters in both human and animal studies (19, 20).

Wilson noted that a striking aspect of the effect of em fields on the pineal is a reduction or phase shifting of the nightly rise in melatonin. He said it has been implicated as a possi-

ble etiologic factor in cancer, neurobehavioral effects, and reproduction epidemiologic studies. **Figure 5** depicts the possible importance of em field-induced alterations in melatonin circadian rhythms as related to the health outcomes of interest in em field epidemiologic studies.

He noted that in the case of cancer, there appears to be some correspondence between those neoplasms against which melatonin has been shown to be protective in laboratory studies and those that appear associated with em fields in epidemiologic studies. Melatonin has been shown to be protective against leukemia, breast cancer, prostate cancer, and melanoma. According to Wilson, melatonin infusion has been reported to be successful in the treatment of lymphomas and breast cancer, as well as leukemia and several other neoplasms in humans. Epidemiologic studies have identified leukemia, breast cancer, melanoma, prostate cancer, brain cancer, and lymphoma as neoplasms associated with em field exposure.

Wilson said that in 1987 Stevens hypothesized that if pineal gland function was affected by em field exposure in humans, then increased breast cancer risk could be one consequence detectable in epidemiologic studies. Wilson pointed out that studies to determine the effect of em field exposure on breast cancer risk in chemically initiated rats showed a small, but statistically significant, increase in number of tumors per tumor-bearing animal. Three subsequent epidemiologic studies that assessed breast cancer showed an excess risk for this disease in occupationally exposed males. Results from other epidemiologic studies, which are suggestive of increased risk of depressive symptoms and miscarriage, are also consistent with the hypothesis that em field exposure may influence risk of certain disorders in humans via changes in melatonin.

Immune system

In the last Symposium, chaired by Dan Lyle, research results were presented by Robert Liburdy, Andrea Cossarizza, and Wendell Winters. Liburdy reported on tests of two hypotheses. In the first he asked if the cell membrane, specifically the calcium ion channel, is directly involved in em field interactions. He considered this important because receptor sites and ion channels are located in the cell membrane and these are the first structures involved in the signal transduction process. In the second he asked whether alterations in calcium ion flux are propagated down the signal

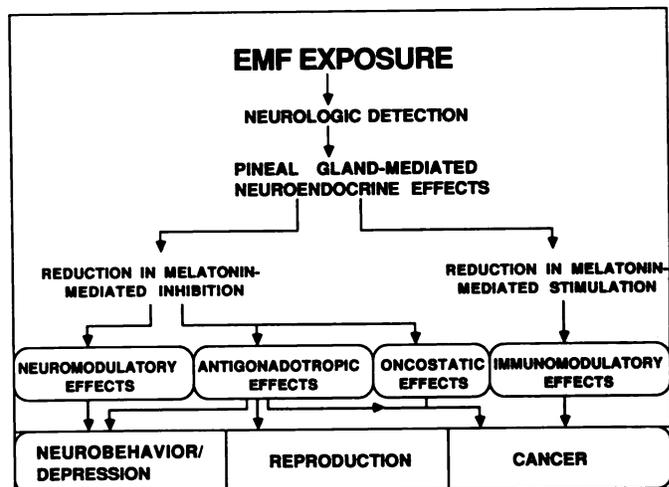


Figure 5. Neuroendocrine effects model depicting possible consequences of EMF exposure as mediated via the pineal gland.

transduction cascade to alter events such as gene activation. This question follows from his interaction model in which em fields alter calcium ion flux, and thereby influence subsequent cellular events in the signal transduction cascade such as gene activation (21). He considered his results to indicate that 1) the cell membrane is a site of em field interaction, 2) the early signal transduction markers of calcium ion influx and intracellular calcium are altered by em fields, and 3) these changes in calcium ion second messenger are linked to induction of *c-myc* mRNA, a mid-stage signal transduction marker. The latter, he believes, suggests that field-induced changes in calcium ion flux are propagated down the signal transduction cascade to alter gene activation events.

Some of the data that led to his conclusions are shown in Fig. 6. This figure shows a typical time course for $[Ca^{2+}]_i$ during Con A activation in the absence or presence of a 1.7 mV/cm 60 Hz electric field. When Con A (1 μ g/ml) was added at 200 s, $[Ca^{2+}]_i$ increased during the early phase of calcium signaling to a value of approximately 300 nM at 800 s. In the presence of a 60 Hz field, when Con A was added at 200 s, an increase in $[Ca^{2+}]_i$ was observed which was identical to that shown for unexposed cells during the first 100 s. However, $[Ca^{2+}]_i$ increased at a greater rate than for cells in the absence of the field. By 800 s the plateau phase of $[Ca^{2+}]_i$ reached approximately 380 nM. As he sees it, alteration of the plateau phase of calcium signaling implicates the calcium ion channel of the outer plasma membrane as a site of field interaction, in contrast to structures associated with calcium release from intracellular stores.

Liburdy separated the early and plateau phase of calcium signaling in time by placing cells in calcium-free media and then added calcium to the buffer. At approximately 200 s, Con A was added and $[Ca^{2+}]_i$ increased to approximately 200 nM at 600 s, corresponding to the early phase, with release of calcium from intracellular stores. When extracellular calcium was added to the cells at approximately 670 s, the plateau phase of $[Ca^{2+}]_i$ reached 390 nM at 800 s. In the absence of Con A, when a 60 Hz electric field was applied no change in $[Ca^{2+}]_i$ was detected. When Con A was added to control cells in calcium-free buffer, an increase in $[Ca^{2+}]_i$ oc-

curred. An essentially identical increase was observed for Con A-treated cells exposed to a 60 Hz electric field. He concluded that the electric field does not influence the early phase of calcium signaling. He believes his findings indicate that an em field influences calcium movement across the outer cell membrane, which implicates the cell membrane as a site of field interaction.

Liburdy hypothesized that such alterations in early signal transduction influence subsequent "downstream" cellular events in the signal transduction pathway such as gene activation and cell proliferation. To test this, he carried out experiments to simultaneously quantify Ca^{2+} influx (early ST marker) and *c-myc* mRNA (mid-stage ST marker) in the same cell preparation exposed to 60 Hz em fields.

He carried out three separate Northern analyses of the same sample that were imaged using a cooled CCD camera. There were three bands for each Northern blot, which correspond to: -Con A, +Con A (1 mg/ml), and +Con A plus 60 Hz. He performed a multivariate analysis of variance for mean pixel intensity of each band across the three Northern analyses. Liburdy stated that the results were statistically significant and consistent with mRNA findings. He believes they support a linkage between an em field interaction with the cell membrane involving Ca^{2+} signaling and "downstream" ST pathway processes such as gene activation.

Cossarizza, on a somewhat different tack, described his experiments in which electromagnetic fields affected cell proliferation and cytokine production in human cells. His previous studies indicated that in vitro exposure of human peripheral blood mononuclear cells (PBMC) to pulsed em fields increased in vitro phytohemagglutinin (PHA)-induced lymphocyte proliferation, as measured by (3 H-TdR) and bromo-deoxy-uridine incorporation assays (22). Microtiter plates containing the cell cultures were placed between a pair of Helmholtz coils (maintained parallel to the plates) and powered by a pulse generator. The effect was much more evident, he reported, when cells from aged donors or from subjects affected by a syndrome of precocious aging such as Down's syndrome were used. He reported that these fields were devoid of genotoxic effects as assessed by the cytokinesis block micronucleus method and they did not interfere with DNA repair capability of human lymphocytes exposed to genotoxic agents. The exposure system was given to investigators at two other laboratories and they were able to reproduce the results. He reported that in the course of several years he obtained the same increased cell proliferation in pulsed em field-exposed, PHA-stimulated PBMC from aged people.

Further experiments were carried out in order to assess 1) the reproducibility of these results, 2) the mechanism (or mechanisms) by which em fields are able to act as comitogens in PHA-stimulated PBMC, and 3) the kind of cells affected. Cossarizza noted that the proliferation of human PBMC is regulated and modulated by several cytokines. He stated that previously he demonstrated that IL-2 is more efficiently utilized in pulsed em field exposed cells, as a higher percentage of such lymphocytes expressed low-affinity IL-2 receptor (CD25 antigen), as shown by cytofluorimetric analysis. He then presented data suggesting that the production of other cytokines is also effected by pulsed fields. When PBMC from healthy donors were exposed to the fields, he reported a dramatic increase in the production of IL-1b and IL-6 compared with unexposed controls, as shown in Table 2 and Table 3. He reported that this phenomenon was noted not only in mitogen-stimulated cultures, but also in unstimulated PEMF exposed cultures. He thinks these data are interesting for two main reasons. First, they help explain the

60Hz Electric Field Enhances Plateau Phase of Calcium Signaling in Activated Thymocytes

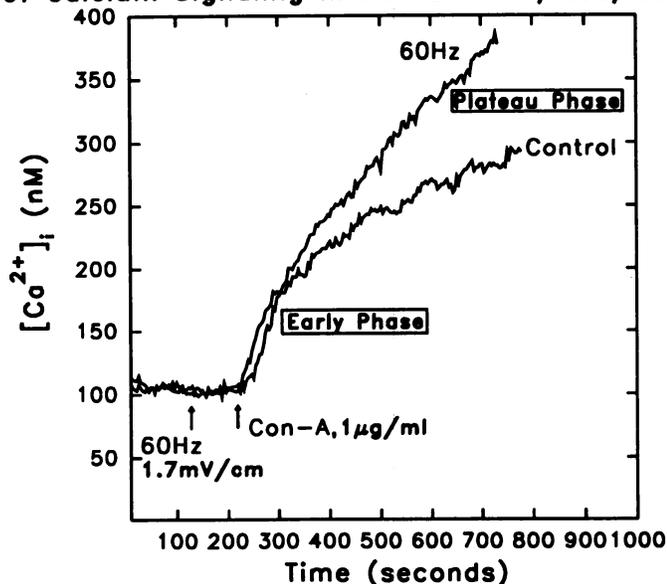


Figure 6. Real time measurements of intracellular calcium in mitogen activated thymic lymphocytes during 60 Hz field exposure.

increased cell proliferation he has reported, as IL-1 is critical in the early events of T cell activation and proliferation. Second, assuming that such a phenomenon could occur in vivo, it could explain other effects of pulsed fields, such as those described in fibroblasts and bone cells, in which beneficial effects with the same type of fields have been reported (23). He noted that IL-1 and IL-6 are the most pleiotropic cytokines, and are able to exert profound effects on nonimmune cells including cells of connective tissues and the neuroendocrine system.

He also reported studies of the genotoxic potential and capability of a 50 Hz electric field to affect important immune responses, such as the capability of human lymphocytes to respond to polyclonal stimuli and of human natural killer (NK) cells to kill tumor target cell lines. Seventy-five subjects were studied. Human PBMC proliferation was assessed by stimulating cells with different doses of PHA or with 10 ng/ml anti-CD3 monoclonal antibody (which he stated selectively stimulates T lymphocytes). Cell proliferation was evaluated by measuring the ³H-TdR incorporation during the last 6 h of a 72 h culture. The data show that the main effects of the electric field were on the proliferation of PBMC from 21 aged subjects and 12 young donors. He reported that there was a slight decrease of ³H-TdR incorporation, which was particularly evident with optimal and supraoptimal PHA doses and in aged subjects. He concluded that his data show that the age of the donor is of critical importance in the biological effects of electric fields on human cells.

Winters also spoke about the immune system. He noted that the biological activities of cells of the immune system, particularly those of lymphoid lineage, have repeatedly been reported to be sensitive to in vitro exposure to a variety of types and strengths of environmental electromagnetic and magnetic fields (emf/mf). He stated that a wide range of bioeffects, such as significant changes in cellular synthesis of macromolecules, receptor functions, release of ions, and regulatory factors, have been found in clinical and basic science research studies to be associated with specific populations of immune system cells obtained from normal donors and from patients with immune cell disorders, infectious diseases, or neoplasia after their exposure to extremely low-frequency emf/mf. He pointed out that the immune cell populations studied have included those cells responsible for initiating and maintaining cellular and humoral immune responses, immunosurveillance, and resistance against pathogenic organisms.

Winters said that the lymphoid cell populations most often studied in emf/mf exposure investigations have been mixed subpopulations of human lymphocytes of the B type plus those of regulatory cell types, i.e., T helper cells regulating B and T cell proliferation and functions and bearing clusters of differentiation marker 4 (CD4) and class II major histocompatibility complex (MHC) antigens. Lymphocytes

TABLE 2. The production of IL-1 β in the supernatants of 12-O tetradecanoylphorbol 13-acetate-stimulated PBMC cultures^a

Stimulus	Control	Exposed	P
None	11.3 \pm 1.3	54.4 \pm 13.3	<0.015
TPA, 1 ng/ml	62.7 \pm 26.4	119.1 \pm 31.6	<0.009
TPA, 10 ng/ml	159.0 \pm 60.5	282.1 \pm 63.9	<0.015

^aMeasurement was performed by ELISA. Data are expressed in pg/ml, at 24 h, and refer to 10 healthy young donors. Statistical analysis was done with a two-tail paired *t* test.

TABLE 3. Production of IL-6 in the supernatants of phytohemagglutinin-stimulated PBMC cultures^a

Stimulus	Control	Exposed	P
None	0.50 \pm 0.22	0.58 \pm 0.32	ns
PHA, 1 μ l/ml	3.24 \pm 0.75	3.97 \pm 0.68	<.03
None	0.49 \pm 0.25	0.94 \pm 0.37	<.04
PHA, 1 μ l/ml	3.88 \pm 0.54	4.49 \pm 0.52	<.018

^aMeasurement was performed by ELISA. Data are presented in ng/ml, at 24 h for the first two sets and at 48 h for the second two sets, and refer to six healthy young donors. Statistical analysis was done with a two-tail paired *t* test.

of the effector-killer cell types, including T cytotoxic cells bearing CD8 and class I MHC antigens and NK cells, have also been studied. He said that the results from such studies of mixed T and B lymphocyte populations, collected and prepared from human peripheral blood specimens, provided a rational basis for additional studies of emf/mf exposure responses in the other immune cell subpopulations most often studied, such as neutrophils, macrophages, and monocytes.

Winters said that mitogen-stimulated lymphocyte blastogenesis assays most frequently have been used to measure bioresponses in immune cells after emf/mf exposures. He believes the results from the laboratories of Conti, Grattarola, Chiabrera, Cadossi, Emilia, Cantini, Franceschi, Bersani, Winters, Cossarizza and their co-workers (see ref 24) over the past few years, taken as a whole, indicate significant emf/mf-induced changes in blastogenesis, in DNA and RNA synthesis, and in release of interleukins and calcium ions. He said several major factors have been identified as important for the successful induction of significant biological changes in immune cells by emf/mf exposure. These include the duration, type, strength, frequency, and modulation of the exposure as well as the type, source, and concentration of the challenge mitogen. Other factors that have been found to be of consequence are the sequence of the mitogen challenge relative to emf/mf exposure. Additional influential factors relating only to the source of the immune target cells, he reported, include the species of the donor of the immune cells, the age of the immune cell donor, and the health status of the immune cell donor (24).

Conclusions

The foregoing is a brief summary of the research reported at the Symposia organized by the International Society for Bioelectricity and presented at the 1992 FASEB Meeting. As may be seen, recent research with weak electromagnetic fields is yielding new insights into biological processes. Clearly, the use of electromagnetic fields as a probe provides new means to understand basic biological processes at many levels of biological investigation. There are also significant implications for therapy.

It is also quite clear that the toxicology model used by investigators in earlier years was not the appropriate model on which to design experiments. It was assumed that electromagnetic fields are a foreign substance to living organisms, like lead or cyanide. With foreign substances in a toxicology model, the greater the dose, the greater the effect—a dose-response relationship. Thus, experiments tended to be designed with high doses and with little regard for other parameters such as modulation and frequency. This is one reason why those earlier studies yielded so little useful information.

As noted in the introduction, electromagnetic fields are not a foreign substance. Living organisms are electrochemical systems that use emfs in everything from protein folding through cellular communication to nervous system function. A more appropriate model of how living organisms can be expected to respond to em fields would be to compare them to a radio receiver.

An electromagnetic signal a radio detects (let us call it signal x) and transduces into the sound of music is almost unmeasurably weak. Yet the radio is immersed in a sea of em signals from power lines, radio stations, TV stations, radars, etc. The radio doesn't notice the sea of signals because they are not the appropriate frequency or modulation. Thus, they don't disturb the music we hear. If we expose the radio to an appropriately tuned em signal or harmonic, however, even if it is very weak compared to signal x, it will interfere with the music. Similarly, if we expose a living system to a very weak em signal, if the signal is appropriately "tuned," it could facilitate or interfere with normal function (25). This is the model that much biological data and theory tell us to use, not a toxicology model. And this is the model that is now starting to be used so fruitfully. FJ

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