

## Biological Effects of Electromagnetic Fields

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**Abstract** Life on earth has evolved in a sea of natural electromagnetic (EM) fields. Over the past century, this natural environment has sharply changed with introduction of a vast and growing spectrum of man-made EM fields. From models based on equilibrium thermodynamics and thermal effects, these fields were initially considered too weak to interact with biomolecular systems, and thus incapable of influencing physiological functions. Laboratory studies have tested a spectrum of EM fields for bioeffects at cell and molecular levels, focusing on exposures at athermal levels. A clear emergent conclusion is that many observed interactions are not based on tissue heating. Modulation of cell surface chemical events by weak EM fields indicates a major amplification of initial weak triggers associated with binding of hormones, antibodies, and neurotransmitters to their specific binding sites. Calcium ions play a key role in this amplification. These studies support new concepts of communication between cells across the barriers of cell membranes; and point with increasing certainty to an essential physical organization in living matter, at a far finer level than the structural and functional image defined in the chemistry of molecules. New collaborations between physical and biological scientists define common goals, seeking solutions to the physical nature of matter through a strong focus on biological matter. The evidence indicates mediation by highly nonlinear, nonequilibrium processes at critical steps in signal coupling across cell membranes. There is increasing evidence that these events relate to quantum states and resonant responses in biomolecular systems, and not to equilibrium thermodynamics associated with thermal energy exchanges and tissue heating. Published 1993 Wiley-Liss Inc.

**Key words:** cell membrane, electromagnetic fields, cooperative processes, nonequilibrium thermodynamics, free radicals, athermal interactions

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In cellular aggregates that form tissues of higher animals, cells are separated by narrow fluid channels that take on special importance in signaling from cell to cell. These channels act as windows on the electrochemical world surrounding each cell. Hormones, antibodies, neurotransmitters and chemical cancer promoters, for example, move along them to reach binding sites on cell membrane receptors [Adey, 1992a]. These narrow fluid "gutters," typically not more than 150 Å wide, are also preferred pathways for intrinsic and environmental electromagnetic (EM) fields, since they offer a much lower electrical impedance than cell membranes. Although this intercellular space (ICS) forms only about 10 percent of the conducting cross section of typical tissue, it carries at least 90 percent of any imposed or intrinsic current, directing it along cell membrane surfaces.

Numerous stranded protein molecules protrude from within the cell into this narrow ICS. Their glycoprotein tips form the glycocalyx, which senses chemical and electrical signals in surrounding fluid. Their highly negatively charged tips form receptor sites for hormones, antibodies, neurotransmitters, and for many metabolic agents, including cancer promoters. These charged terminals form an anatomical substrate for the first detection of weak electrochemical oscillations in pericellular fluid, including field potentials arising in activity of adjacent cells or as tissue components of environmental fields.

### OBSERVED SENSITIVITIES TO IMPOSED EM FIELDS

As a perspective on the biological significance of this cell-surface current flow, there is evidence from a number of studies that extremely low frequency (ELF) fields in the range 0-100 Hz and radiofrequency (RF) fields amplitude-modulated in this same ELF range, producing tissue gradients in the range  $10^{-1}$ - $10^{-1}$  V/cm, are involved in essential physiological functions

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in marine vertebrates, birds, and mammals [see Adey, 1981, for review]. In vitro studies have reported similar sensitivities for cerebral  $\text{Ca}^{2+}$  efflux, and in a wide spectrum of calcium-dependent processes that involve cell membrane functions, including bone-growth, modulation of intercellular communication mechanisms that regulate cell growth, reduction of cell-mediated cytolytic immune responses, and modulation of intracellular enzymes that are molecular markers of signals arising at cell membranes and then coupled to the cell interior.

The steady membrane potential characteristic of most cells is approximately 0.1 V in the resting state. Since this potential exists across the 40 Å of the very thin plasma membrane, it creates an enormous electric barrier of 105 V/cm, many orders of magnitude greater than these intrinsic and imposed electric oscillations in fluid surrounding cells; Nevertheless, these sensitivities have been confirmed for many cell types, including lymphocytes, ovary cells, bone cells, fibroblasts, cartilage cells, and nerve cells [Adey, 1992a].

These observations have been viewed cautiously by many biologists as beyond the realm of a possible physical reality. However, it is necessary to view them in the context of cooperative processes and associated nonlinear electrodynamics at cell membranes, revealed with imposed EM fields. As discussed below, these phenomena are in the realm of nonequilibrium thermodynamics, and are thus far removed from traditional equilibrium models of cellular excitation based on depolarization of the membrane potential and on associated massive changes in ionic equilibria across the cell membrane.

### **PRINCIPAL AREAS OF RECENT RESEARCH IN BIOELECTROMAGNETICS**

The main endeavors in this field have focused on limited but widely separated areas of biology and medicine. In many respects, they form an hierarchical sequence: (1) coupling mechanisms between fields and tissues at the cellular level [Adey, 1990, 1992a; Hoth and Penner, 1992; Luben, 1991; Moolenaar et al., 1986]; (2) field effects on embryonic and fetal development [Delgado et al., 1982; McGivern et al., 1990]; (3) modulation of central nervous and neuroendocrine functions [Lerchl et al., 1990; Reiter, 1992; Wilson and Anderson, 1990]; (4) modification of immune functions [Byus et al., 1984; Lyle et al., 1983, 1988]; (5) regulation of cell growth, and EM field action in tumor promotion [Wilson et al., 1990]; (6) modulation of gene expression [Goodman and Henderson, 1988; Phillips et al., 1992]; and (7) from pioneering

therapeutic applications in healing ununited fractures, there is a vista of much broader future therapies that may involve joint use of pharmacological agents and EM fields, each tailored for optimal dosage in these specific applications [Mir et al., 1992; Weaver, 1992].

Research in tumorigenesis now emphasizes epigenetic mechanisms, focusing on dysfunctions at cell membranes, rather than on damage to DNA in cell nuclei [Pitot and Dragan, 1991; Yamasaki, 1991]. Steps in tumor formation are described in the initiation-promotion-progression model [Weinstein, 1988]. Initiation typically involves a single event, damaging nuclear DNA, as through action of ionizing radiation or chemicals. Tumor formation does not occur unless there is subsequent, repeated intermittent exposures to promoters, with many known to act at cell membranes. There is minimal evidence that EM fields act as initiators. Current and planned research is directed to possible promoter actions of EM fields at cell membranes, acting either alone or together with chemical agents [Adey, 1990b, 1991, 1992b].

### **THE TRANSDUCTIVE STEP; EM FIELD DETECTION IN BIOMOLECULAR SYSTEMS**

As evidence has mounted confirming occurrence of bioeffects of EM fields that are not only dwarfed by much larger intrinsic bioelectric processes, but may also be substantially below the level of tissue thermal noise, there is a mainstream of theoretical and experimental studies seeking the first transductive steps.

### **CYCLOTRON RESONANCE AND CA COORDINATION COMPOUND MODELS OF LOW-FREQUENCY EM FIELD SENSITIVITIES**

In a cyclotron oscillator, charged particles are exposed to a static magnetic field and to an oscillating magnetic field at right angles to one another. The particles will move in circular orbits at right angles to the two imposed fields when the frequency of the imposed oscillating field matches the particle gyrofrequency, determined by its mass, charge, and the intensity of the static magnetic field.

Free (unhydrated) Ca ions in the earth's geomagnetic field would exhibit cyclotron resonance frequencies around 10 Hz, with cyclotron currents as

much as five orders of magnitude greater than the Faraday currents [Polk, 1984]. Liboff [1985] hypothesized that EM fields close to cyclotron frequencies may couple to ionic species, transferring energy selectively to these ions. Criticism of this model has been directed at its requirements for ions to be stripped of hydration shells that would presumably alter gyrofrequencies, and to the presumed direction of ion motion in the magnetic field.

Lednev [1991] has proposed a quite different explanation of the same experimental conditions. Considering an ion inside a Ca-binding protein as a charged oscillator, a shift in the probability of an ion transition between different states of vibrational energy occurs when there is a combination of static and oscillating magnetic fields. This in turn affects the interaction of the ion with surrounding ligands. This effect is maximal when the frequency of the alternating field is equal to the cyclotron frequency of this ion or to some of its harmonics or subharmonics.

These models do not address the question of transductive coupling of these weak EM fields at energy levels substantially below the thermal energy of living tissues. Answers to that important question are currently sought in EM field interactions with free radicals.

### A POSSIBLE ROLE FOR FREE RADICALS

McLauchlan [1992] has proposed a role for chemically reactive free radicals at 50 and 60 Hz electric power frequencies. In McLauchlan's model, very low static magnetic fields cause triplet pairs to break and form singlets. But as the field is increased, typically to a level of about 8 mT, two of the three triplet states become entirely decoupled from the singlet state. Thus, at this field level two-thirds of the radical pairs may not react as they would in a weaker field, "an enormous effect of a small magnetic field on a chemical reaction, *and the effect begins at the lowest applied field strength, even at levels below thermal (U) noise* .... The all-important interaction has an energy very much less than the thermal energy of the system, and is effective exclusively through its influence on the kinetics; this is counter-intuitive to most scientists." The effect is quite general and does not depend on any specific chemical identity of the radicals.

Addition of an oscillating field to this system introduces intensity *windows* that cause triplets to return to singlet states that react with one another. The

major effect of the field is to remove degeneracies of sublevels of the triplet pair, whose energies are equal in zero field, but differ progressively in an increasing field as a Zeeman splitting effect.

### COOPERATIVE MODELS OF FREE RADICAL BEHAVIOR IN EM FIELD BIOEFFECTS

Research at the other extreme in the EM spectrum also support concepts of free radical interactions. There may be special significance to biomolecular interactions with millimeter wave EM fields. At frequencies within the range 10-1,000 GHz, resonant vibrational or rotational interactions, not seen at lower frequencies, may occur with molecules or portions of molecules [Illinger, 1981]. Biomolecular and cell research in this spectral region has been meager. Studies in solutions of DNA and of growth effects in bacteria have yielded conflicting results that may relate to extreme technical difficulties not encountered at lower frequencies. There are major problems in the engineering of suitable exposure systems, in ensuring biocompatible exposure devices, and in evaluation of experimental data for physical and biological artifacts [see Adey, 1990a, for review].

Studies of yeast cell growth by a team of German scientists over the past 15 years using athermal millimeter wave fields [Grundler and Keilmann, 1978; Grundler and Kaiser, 1992] have shown that growth appears finely "tuned" to applied field frequencies around 42 GHz, with successive peaks and troughs at intervals of about 10 MHz. In recent studies, they noted that the sharpness of the tuning increases as the intensity of the imposed field decreases; but the tuning peak occurs at the same frequency when the field intensity is progressively reduced. Moreover, clear responses occur with incident fields as weak as 5 picowatts/cm<sup>2</sup>.

In a recent synthesis emphasizing nonthermal interactions of EM fields with cellular systems, Grundler et al. [1992] present models of the sequence of EM field transductive coupling, based on magnetic field-dependent chemical reactions, including cytochrome-catalyzed reactions that involve transient radical pairs, and production of free radicals, such as reactive oxygen or nitric oxide, leading to further highly cooperative amplification step. Based on Frohlich's [1986] model of interactions between an imposed field and high-frequency (1012 Hz) intracellular van der Pol oscillators, they conclude

that "imposed fields can be active even at intensities near zero." In other words, a threshold might not exist in such a system.

### **DETERMINATION OF THE ROLE OF IRON-CONTAINING MOLECULES IN SENSITIVITIES TO EM FIELDS**

There is a wealth of evidence that iron-containing molecules are widespread in the chemistry of cellular transductive mechanisms. They include transferrins with specific receptors on cell surfaces, heme-containing G proteins that couple receptors to enzymes at cell membranes, and cytochrome P-450 enzymes that are key elements in cellular respiration. Transferrin mechanisms are sensitive to 60-Hz EM fields [Phillips, 1986]. These iron atoms are not arranged in ferromagnetic configurations and are too scattered to form magnetic dipoles. Nonetheless, future research may focus on their interactions with biomolecules that exhibit paramagnetic properties.

Neuromelanin exhibits paramagnetism, due to the presence of large numbers of unpaired electrons, qualifying this molecule as a stable free radical. It is suspected of a role in Parkinson's disease. These molecules influence proton relaxation times in NMR studies. Although shortened T1 relaxation time in these studies were attributed to paramagnetism of melanin free radicals, it has now been shown that this shortening requires interactions of paramagnetic ferric iron with melanin [Tosk et al., 1992]. Future developments may allow MRI studies of nigrostriatal regions of the brain in Parkinson's disease, based on compromised status of neuromelanin and ferric iron.

### **BIOPHYSICAL MODELS OF TRANSMEMBRANE SIGNALS; INWARD SIGNALING ALONG CELL MEMBRANE RECEPTOR PROTEINS**

McConnell [1975] noted that intrusion of a protein strand into an artificial phospholipid bilayer induces coherent states between charges on tails of adjoining phospholipid molecules, with establishment of energetic domains determined by joint states of intramembranous proteins and surrounding phospholipid molecules. Resulting states of dielectric strain in these lipoprotein interactions may determine optical properties within their cooperative domains. As in fiberoptic systems, these optical properties may depend on states of membrane excitation, and may determine stability of *dark soliton* propagation as a means of transmembrane signaling [Christiansen, 1989].

These dark solitons suggest analogies with the sharp changes in optical properties of living vertebrate and invertebrate axons accompanying polarizing currents [Tobias and Solomon, 1950], and with the highly cooperative movements of about 18 Å reported with laser interferometry at the axon surface within 1 ms of excitation [Hill et al., 1977]. A sensitivity of the traveling waves of the Belousov-Zhabotinski chemical reaction to weak electric fields with reversal and splitting has been noted [Sevcikova et al., 1992] and also ascribed to possible solitonic phenomena.

### **POSSIBLE ROLE OF HIGHLY COOPERATIVE ELECTRON TRANSFER IN PROTEINS AS A BASIS FOR EM FIELD INTERACTIONS**

In 1966, pioneering studies by Chance [DeVault and Chance, 1966] disclosed the physical nature of first steps in activation of cytochrome c-bacteriochlorophyll complex by millisecond electron transfer. Since the transfer was temperature-independent from 120K to 4K, they deduced that the reaction proceeded through a quantum mechanical tunneling mechanism, perhaps taking place over distances as large as 30 Å. Their studies coincided with Mitchell's development of his Nobel award winning chemiosmotic model, which proposed that key electron-transfer steps of respiration operated across the full 35 Å width of the cell membrane.

Extension of these early concepts by Moser et al. [1992] has suggested options for much further research on detection and coupling of EM fields at cell membranes. They found that a variation of 20 Å in the distance between donors and acceptors in protein changes the electron-transfer rate by 10<sup>7</sup>-fold. In the time domain, there is also a strong dependence on distance. Thus, in considering electron transfer across the full thickness of the cell membrane, with edge-to-edge distances between 25 and 35 Å, the optimal electron transfer rate is pushed into time scales of seconds and days, respectively.

Here, protein behaves like an organic glass, presenting a uniform electronic barrier to electron tunneling and a uniform nuclear characteristic frequency. Using this technique to study biological membranes, it would be sufficient to select distance, free energy and reorganizational energy, in order to define rate and directional specificity of biological electron transfer, thus

meeting physiological requirements in a wide range of cytochrome systems.

### **INWARD SIGNALING ALONG CELL MEMBRANE RECEPTOR PROTEINS**

In their simplest forms, membrane receptor proteins appear to cross the plasma membrane only once. In a second group, the strand crosses the membrane seven times or more. The most striking feature of these proteins is in the structure of the extremely short chain of 23 amino acids that lies within the cell membrane at each consecutive membrane crossing. These short segments are composed of hydrophobic amino acids (and therefore nonconducting), in contrast to the hundreds of hydrophilic amino acids form- the rest of the strand inside and outside the membrane.

Is there evidence that this transmembrane movement of ions is mediated by these receptor protein segments, despite their hydrophobicity and their location in this highly hydrophobic environment? Ullrich et al. [1985] concluded that a hydrophobic segment as short as 23 amino acids is probably too short to be involved in conformation changes; and that its hydrophobic character makes unlikely its participation in either ionic or proton movement by coulombic forces.

Direct measurement of receptor-mediated inward Ca currents in epidermal cells [Moolenaar et al., 1986] and mast cells [Hoth and Penner, 1992] indicates that it is not voltage-activated and would thus be consistent with ion translocation by molecular vibrational modes. Binding of one epidermal growth factor (EGF) to its specific receptor is followed by a fourfold increase in intracellular Ca within 30 sec. All of this increment is derived from extracellular sources, and there is no change in membrane potential. In mast cells, it shows a characteristic inward rectification. For mast cells, Hoth and Penner have proposed that this may be the mechanism by which electrically nonexcitable cells maintain raised intracellular Ca<sup>2+</sup> stores after receptor stimulation.

### **EFFECTS OF EM FIELDS ON RECEPTOR-G PROTEIN COUPLING**

As a representative model of receptor coupling to intracellular enzymes, Luben [1991] has examined EM field effects on coupling of the parathyroid hormone (PTH) receptor to adenylate cyclase via a G protein. Osteoblasts exposed to a 72-Hz pulsed magnetic field for as little as 10 min show a persistent desensitization to the effects of PTH on adenylate cyclase. This does not result from decreased total adenylate cyclase levels,

nor from reduced numbers of hormone receptor sites, nor reduced affinity of the hormone for the receptor, nor from reduced fractional occupancy of the receptor by the hormone.

Rather, the ability of bound hormone-receptor complex to activate G protein alpha-subunits is impaired by this treatment of osteoblasts with a pulsed magnetic field; and in consequence of this desensitization of the PTH receptor, there is increased collagen synthesis by osteoblasts and decreased bone resorption by osteoclasts. Luben has identified potential clues to this EMF induced desensitization. Using monoclonal antibodies specific for the PTH receptor, exposure to the pulsed field changes certain PTH receptor determinants similar to changes induced by PTH analogs. These determinants modified by the EM field appear homologous to the signaltransduction domains of other G protein-linked receptors, viz., the transmembrane helices denoted as 5, 6, and 7 attached to intracellular loops 2 and 3. These are known sites of interaction of G proteins in rhodopsin and the adrenergic receptors.

### **FLUORESCENCE SPECTROMETRY AND MICROSCOPY OF INTRACELLULAR CA FLUXES INDUCED BY EM FIELDS**

A first indication of the nonequilibrium character of tissue interactions with EM fields came from studies of cerebral Ca efflux. Frequency and amplitude windows were observed at lowfrequency field frequencies, typically centered around 16 Hz [Bawin et al., 1975; Bawin and Adey, 1976; Blackman et al., 1979]. Ion-sensitive fluophores display the spatiotemporal distribution of Ca<sup>2+</sup> in single cells and in domains of cultured cells. In confluent cell cultures, aggregate levels of intracellular Ca<sup>2+</sup> may rise over large domains of the culture while simultaneously decreasing in others [Tsien, 1986].

Such highly cooperative behavior over many hundreds of neighboring cells may be mediated by a wavelike pattern of diffusion of ATP, rapidly released into the intercellular medium in the absence of gap junction communication and detected by cell-surface purinergic receptors [Osipchuk and Cahalan, 1992]. It is likely to be a manifestation of an important modulating

behavior within tissue [see Adey, 1992c]. Initial fluorescence microscopy studies with EM fields have measured Ca uptake as an aggregate behavior in cultures of lymphocytes and other cells as a function of field frequency and intensity [Walleczek, 1992].

## DISCUSSION

There is a reasonable prospect that bioelectromagnetics may emerge as a separate biological discipline, having developed unique tools and experimental approaches in a search for essential order in living systems. Future research on submolecular transductive coupling will be diversified and increasingly dependent on new technologies, such as high-resolution magnetic resonance spectroscopy and electro-optical techniques. These approaches may answer such challenging problems as structural modifications during receptor-ligand binding, vibration modes in cell membrane lipoprotein domains during excitation [Christiansen et al., 1992], and possible coherent millimeter wave emissions accompanying enzyme action.

In little more than a century, our biological vista has moved from organs to tissues, to cells, and most recently to the molecules that are the exquisite fabric of living systems. There is now a new frontier, more difficult to understand, but of vastly greater significance. It is at the atomic level that physical processes, rather than chemical reactions in the fabric of molecules, appear to shape the transfer of energy and the flow of signals in living systems [Trullinger, 1978].

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