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Joint Actions of Environmental Nonionizing Electromagnetic Fields and Chemical Pollution in Cancer Promotion

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Studies of environmental electromagnetic (EM) field interactions in tissues have contributed to a new understanding of both normal growth and the biology of cancer in cell growth. From cancer research comes a floodtide of new knowledge about the disruption of communication by cancer-promoting chemicals with an onset of unregulated growth. Bioelectromagnetic research reveals clear evidence of joint actions at cell membranes of chemical cancer promoters and environmental electromagnetic fields. The union of these two disciplines has resulted in the first major new approach to tumor formation in 75 years, directing attention to dysfunctions in inward and outward streams of signals at cell membranes, rather than to damage DNA in cell nuclei, and to synergic actions of chemical pollutants and environmental electromagnetic fields.

We are witnesses and, in great measure, participants in one of the great revolutions in the history of biology. In little more than a century, we have moved from organs, to tissues, to cells, and finally to the molecules that are the elegant fabric of living tissues. Today, we stand at a new frontier. It may be more difficult to comprehend, but it is far more significant; for it is at the atomic level, rather than the molecular, that physical, rather than chemical, processes appear to shape the flow of signals that are at the essence of living matter.

To pursue these problems in the environment and in the laboratory, our needs for further research with appropriate budgets are great. We recognize the importance of studies that address the effects of long-term, recurrent, intermittent exposures to environmental electromagnetic fields, which is an area where simplistic concepts of cumulative dose effects do not apply.

These epidemiologic and laboratory studies emphasize the growing impact of environmental chemical pollution and the rapidly increasing deployment of an almost infinite variety of environmental electromagnetic fields as possible joint factors in cancer promotion. As we move towards the twenty-first century, elucidation of mechanisms underlying these interactions at the cellular and molecular level will become matters of urgency. At the same time, implementation of public policies that would mitigate risks from these exposures may impact heavily on existing industrial practices and on important aspects of environmental planning in housing and urban development. At this stage, it is of paramount importance that the significance of these issues no longer be ignored.

Introduction

There has been an exponential increase over the past half century in the use of devices and systems employing electromagnetic (EM) energy in the workplace, in the home, and in external environments. Although all life on earth evolved in a sea of weak natural EM fields that ranged in frequency from a few cycles per second (Hz) to the extremely short wavelengths and high energies of cosmic rays, EM fields from man-made devices and systems are typically far above levels of this background radiation in our urban environments. These systems cover an increasingly broad frequency spectrum that ranges from electric power distribution systems, which operate at 50 or 60 Hz; through the radiofrequency region up to 300 MHz; and the microwave region that now increasingly uses millimeter microwave and far-infrared radiation (*1*).

This development is expected to continue into the twenty-first century. Even though electric power generation may not increase significantly on a nation-wide basis in this time, it is likely that long-term, intermittent, and recurrent human exposure will increment appreciably because of pervasiveness of electrical and electronic equipment that generates a complex spectrum of low-level EM fields in proximity to man.

Do these EM fields constitute a health hazard? Based on available epidemiological data and laboratory studies, it has become increasingly clear that these fields acting either alone or in conjunction with chemicals that occur as environmental pollutants may constitute a potential health hazard. Much has been accomplished in the past decade in establishing a firm base of new knowledge, despite a grave and growing lack of research funds and also entrenched and often self-serving attitudes among

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influential groups who have denied the possibility of adverse effects, based simply on their *a priori* positions.

Potential hazards of these fields relate to *athermal tissue* interactions where tissue heating is not the significant factor in the observed effects. By definition, ionizing radiation is not involved. From a public health aspect, we should recognize at the outset the principal nay-sayers against the potential health hazards of athermal EM fields.

Engineers have taken the view that if it cannot fry the subject, it cannot hurt him. Physiologists have maintained that equilibrium phenomena, which they have believed to be the prime determinants of excitation at cell membranes, would not be influenced by such weak fields and therefore that they are of no physiological significance. Physicists not sufficiently informed in nonequilibrium long-range interactions and the vast burgeoning of new knowledge in quantum mechanics in their chosen field have stoutly maintained that these fields are too weak to disrupt even a hydrogen bond by an increase in thermal energy. All three views are erroneous because they ignore the fundamental importance of highly cooperative processes in biomolecular systems, based on long-range atomic interactions in nonlinear, nonequilibrium electrodynamics, and the quantum physical interactions that now emerge as the key phenomena in biomolecular systems.

Tissue interactions with electromagnetic fields have been widely studied in terms of two quite different endpoints: their thermal effects and ionization of atoms in biomolecular systems. Only recently has it become apparent that major biological interactions also occur in the absence of either significant heating or ionization. Ionizing radiation poses hazards to living organisms through its destructive effects on key macromolecular systems. It is implicit for ionizing radiation that it have sufficiently high photon energies to disrupt the atomic organization of the exposed macromolecular systems. Since EM radiation with wavelengths longer than the ultraviolet region of the spectrum (photon energies less than about 12 eV) does not possess sufficient energy to cause ionization, there has been a persistant view in certain areas of the physical sciences that nonionizing EM fields are incapable of inducing bioeffects other than by heating (*2,3*).

This inadequate view overlooks the existence of cooperative organization in biomolecular systems and the profoundly important role that cooperativity appears to play in the detection of tissue components of nonionizing EM fields (*4*). The nature of these interactions is so far removed from the concepts and models that have guided research in ionizing radiation that expertise in the latter area can offer little in the search for underlying mechanisms. Equilibrium thermodynamics and the classical models of the statistical mechanics of matter appear equally inappropriate in their applications to most key questions on the biological effects of nonionizing EM fields.

Moreover, far from a limited biological significance restricted to considerations of potential hazards, the imposition of weak, nonionizing EM fields has proved a powerful tool in understanding both the sequence and the energetics of transmembrane signals initiated by hormones, antibodies, neurotransmitters, and chemical cancer promoters at cell-surface receptor sites. We shall note that cell membranes function as powerful amplifiers of their first weak interactions with both EM fields and humoral stimuli, and that, as revealed by field effects, these interactions are nonequilibrium in character. They are consistent with quantum processes involving long-range interactions between electric charges on cell surface macromolecules. Since these studies have shown similar sensitivities in a wide range of tissues and cell types, we conclude that these electrochemical sensitivities may be a general biological properties of all cells.

Cells in body tissue initiate weak electrical and chemical signals by which they can "whisper together" in a private language necessary for the normal health of the tissue. *If this normal pattern of communication is interrupted, unregulated cell growth may result.* On the other hand, unregulated growth of cancer cells in culture is arrested when they make contact with normal cells; they may then differentiate once more into normal cells (*5–8*).

Electromagnetic Environment and Its Impact on Man

In the U.S., in addition to the obvious peak at power line frequencies in the extremely low-frequency (ELF) range, most suburban environments exhibit peaks in the AM radio broadcast band; FM broadcast stations are the main contributors in the very high-frequency (VHF) spectrum. Exposure to microwave sources is less common. The more common of these sources include low-powered telephone relays; certain security alarm systems; and a variety of radar sources, ranging from low-powered highway surveillance devices to major airport and military installations. In domestic environments and in the workplace there has been an ever-increasing complexity in the EM environment. Microwave ovens, video display terminals, electric hair dryers, electric blankets, and personal radio communication systems are all associated with EM fields that are substantially above natural backgrounds. Occupational exposures cover an enormous range that includes RF heaters and sealers; electric welders; TV servicing; electric power distribution systems; and radio, TV, and microwave transmitters (Table 1).

What are the resulting field levels induced in body tissues? This will depend on body geometry, with respect to field orientation and field frequency. For ELF fields, a simple capacitance model of coupling suffices, and in consequence, coupling is poor at these low frequencies. Thus, a 10 kV/m field from a 60 Hz power line would only produce tissue gradients in man of about 0.1 mV/cm. On the other hand, radio frequency fields couple

Table 1. Typical environmental field levels.

Electric blanket	200 V/m
High-voltage power lines	10,000 V/m
Hair dryer	30 Gauss
Microwave oven (at door)	5 mW/cm^2, 130 V/m
Hand-held radio transmitter (at head)	5 mW/cm^2, 130 V/m
U.S. city/suburbia radio fields (mainly from FM broadcast stations)	1–4 microwatts/cm^2, 2–4 V/m

much more efficiently, particularly where the body dimensions approximate the wavelength of the incident field. At frequencies of 100 to 400 MHz, field intensities of 1.0 mW/cm^2 (61 V/m in air) are associated with tissue gradients of 10 to 100 mV/cm in mammalian organisms, with precise levels depending on body configuration and orientation with respect to the field, and on the organism's size in relation to the wavelength of the incident field.

Bioelectric Sensitivities to Intrinsic and Induced EM Fields

As a perspective on the biological significance of these induced fields, there is evidence from a number of studies that ELF fields producing tissue gradients around 10^{-7} V/cm are involved in essential physiological functions in marine vertebrates, birds, and mammals, including man (Table 2) (*1*).

By contrast with extremely weak gradients induced by these ELF field exposures, radio frequency (RF) and microwave fields are far more strongly coupled to tissues. At incident energy levels of 1.0 mW/cm^2 (61 V/m in air), these fields induce tissue gradients in the range 10 to 100 mV/cm. At these levels, they are in the same amplitude range as intrinsic oscillations generated biologically, such as those seen in the electroencephalogram. These microwave fields also exhibit a wide range of biological interactions when amplitude-modulated at ELF frequencies. They exert strong effects on cell membrane functions in nonnervous tissue, including modulation of cytolytic immune responses (*9,10*) and regulation of intracellular enzyme responses to signals initiated at cell membranes and then coupled to the cell interior (*11–13*). Microwave and ELF fields both interact at cell membranes with chemical stimuli from hormones (*14*), antibodies (*15*), neurotransmitter molecules (*16*), and chemical cancer promoters (*17*).

Table 2. Bioelectric sensitivities to ELF fields.

Life form	Function	Tissue gradient	Imposed field
Sharks and rays	Navigation and predation	10^{-8} V/cm	Dc to 10 Hz
Birds	Navigation	10^{-7} V/cm	0.3 Gauss
Birds	Circadian rhythms	10^{-7} V/cm	10 Hz, 2.5 V/m
Monkeys	Subjective time estimation	10^{-7} V/cm	7 Hz, 10 V/m
Man	Circadian rhythms	10^{-7} V/cm	10 Hz, 2.5 V/m
Comparison with intrinsic cell and tissue neuroelectric gradients			
Membrane potential		10^5 V/cm	
Synaptic potential		10^3 V/cm	
Electroencephalogram		10^{-1} V/cm	

As discussed later, there are two emergent conclusions from these studies. First, tissue heating is not an essential aspect of mechanisms in these interactions, since they are not associated with a biologically significant temperature rise (0.1°C or less). Second, a consideration of interactions based only on possible temperature changes overlooks evidence for sharp low frequency and intensity dependencies (windowed responses) in many field effects that are discussed later.

Epidemiological Studies Relating Nonionizing EM Fields to Cancer Promotion and Developmental Abnormalities

Many recent reports from different countries indicate a significant increase in malignant diseases, particularly leukemia and lymphoma, in association with exposure to nonionizing EM fields. These studies are in two broad categories: one concerned with an increased incidence of childhood malignant diseases with emphasis on domestic exposures to ELF fields of high-voltage power lines, and the other relating to industrial exposures to a wide range of ELF and RF/microwave fields. Initial reports by Wertheimer and Leeper (*18*) of increased childhood leukemia and lymphoma and certain brain tumors have now been replicated in the same geographic area by Savitz et al. (*19*), in a different cohort and covering different years, with similar findings of increased risk. A conclusion of this study conducted in behalf of the New York State Power Authority is that 10 to 15% of childhood cancer may relate to environmental EM field exposure. Speculation by Wertheimer that magnetic, rather than electrical components of these environmental fields may be significant factors are supported by engineering studies in the U.S. (*20*) and Sweden (*21*). Developmental abnormalities have also been reported from exposure to environmental EM fields. An increased incidence of spontaneous abortion has been reported in women sleeping on electrically heated water beds or using electric blankets during pregnancy (*22*). Reports of pregnancy abnormalities associated with exposure to EM fields from computer terminals have their counterpart in increased fetal deaths and teratological abnormalities noted in Swedish studies of mice exposed to VDT fields (*23,24*). Swine and rats exposed to 60Hz high-voltage power line fields have also shown a higher incidence of developmental abnormalities (*25,26*).

Studies of death certificates of 486,000 electrical workers covering the years 1950–1979 have shown higher proportional mortality ratios (PMRs) for myelogenous leukemia, non-Hodgkin's lymphoma, and in pancreatic, lung and brain malignancies (*27*). Studies from the National Cancer Institute indicate that microwave workers with more than 20 years' job experience,

and who are also exposed to soldering fumes and/or electronic solvents, have a 10-fold risk of brain tumors (astrocytomas). The risks from chemical exposures without microwaves in a cohort was 2 to 3, and the risk was related to duration of cumulative microwave exposure (*28*).

Although all these epidemiological studies have been considered flawed for differing reasons, their collective impact is an indication of urgently needed further research in both epidemiology and in cellular and molecular mechanisms underlying bioeffects at athermal field levels. Laboratory studies of joint actions of chemical substances and EM fields in mechanisms of cancer promotion (see later discussion) emphasize the importance of their possible joint actions in the observed increased cancer incidence. These studies also indicate a requirement for evaluating these joint actions in future epidemiological studies (*15,16*).

Structural and Functional Substrates of EM Field Interactions with Biomolecular Systems

At the molecular level, RF and microwave fields may interact with biological systems in two quite different ways. At wavelengths longer than the millimeter region, spectroscopy indicates only collision-broadened, relaxation spectra (*31*). In the millimeter wave and far-infrared regions, resonant interactions occur with portions of biological macromolecules (*32,33*), which were predicted by Frohlich (*34*). At lower frequencies (longer wavelengths), absorbed energy results in tissue heating as a function of dielectric properties, principally in water molecules (*35*).

Little is known about any role for these direct interactions of tissues with unmodulated carrier waves of RF and microwave fields in carcinogenesis. On the other hand, ELF fields and RF/microwave fields amplitude-modulated at ELF frequencies have been shown to influence growth in normal cells and to modulate their transformation and initiation as cancer cells. We have identified cell membranes as prime sites of these ELF-mediated responses (*29,36,37*), including synergic actions of EM fields and cancer-promoting phorbol esters (*13,14,29,38*).

We will now consider the role of the cell membrane in detection and amplification of weak EM fields in the surrounding pericellular fluid. Our studies have used EM fields with ELF components to study the sequence and energetics of events that couple chemical stimuli from cell surface receptor sites to the cell interior; stimuli that arise in binding of hormones (*14*), antibodies (*9,15*), neurotransmitters (*39*) and chemical cancer promoters (*13,17,29*). Intercellular fluid surrounding cells forms a preferred pathway in electrical current flow through tissues. Specific resistance of this fluid (typically 50 ohm·cm^{-1}) is three orders of magnitude or more lower than transmembrane resistance (in the range 2000–100,000 ohm/cm^2).

Fluid Mosaic Model of Cell Membranes: Transductive Coupling from Surface Receptors to Cell Interior

Concepts of cell membrane structure have changed considerably since 1952 when the Davson-Danielli model was one of the first to consider the membrane as a lipoprotein complex (*40*). Recent research has focused on the arrangement of proteinaceous material on and within the lipid bilayer of the cell membrane, with a general acceptance of the fluid mosaic model of Singer and Nicolson (*41*). Glycoprotein strands protrude outward from intramembraneous particles (IMPs) to form a polyanionic surface layer by reason of their amino sugar (sialic acid) terminals. IMPs relate internally to a submembrane plexus of microfilaments (*42*), some of which exhibit an actin-like periodicity and are extremely calcium-sensitive in their spectral characteristics. It has been hypothesized that internal strands of IMPs may have further functional connections with elements of the cytoskeleton (*42*), thus providing relatively direct communication from receptor sites on the cell surface to intracellular organelles, including the nucleus.

Initial stimuli associated with weak pericellular EM oscillations and with binding of humoral molecules at their membrane receptor sites elicit a highly cooperative modification of calcium binding to glycoproteins along the membrane surface. This longitudinal spread is consistent with the direction of flow of extracellular currents associated with physiological activity and from imposed EM fields, as discussed previously; it is also consistent with the spreading activation of Ca-dependent protein kinase-C that is also a specific receptor for cancer-promoting phorbol esters (*43*).

This cooperative modification of surface calcium binding is an amplifying stage, with evidence from concurrent manipulation of these initial events by imposed EM fields that there is a far greater increase or decrease in Ca^{2+} efflux than is accounted for in the energy of the imposed field or in the events of receptor-ligand binding (*44–46*). There is further striking evidence for the nonequilibrium character of this modification in calcium binding in its occurrence in quite narrow frequency and amplitude windows (*47–49*).

The pioneering observations of Bawin et al. (*44,45*) were the first to show tuning curves of altered Ca^{2+} efflux from cells and tissue as a function of low frequencies in imposed EM fields. Most studies have used cerebral tissue including cerebral cortex in awake cats (*50*), isolated chick cerebral hemispheres (*44,45,51,52*), cultured neurons (*53*), and cerebral synaptosome fractions (*46*). Studies in different laboratories are consistent in reporting shifts in Ca^{2+} efflux that depend on low-frequency characteristics and on field intensity.

These effects have been observed with RF fields amplitude-modulated at low frequencies; with low-frequency electric fields; with low-frequency EM fields; and with combined low-frequency EM fields and static magnetic fields. Effects are maximal around 16 Hz and

less at higher and lower frequencies. Neither size nor geometry are determinants of these interactions, which occur over an enormous range of physical dimensions, from intact cerebral cortex to cultured neurons and finally in cerebral synaptosomes with mean diameters around 0.7 μm. These fields induce electric gradients at physiological levels, including those of the EEG in fluid around brain cells. They are at least six orders of magnitude less than the electric barrier of the membrane potential, emphasizing the role of an amplifying function in their ultimate effects on intracellular mechanisms that are discussed later.

Signal Coupling along Transmembrane Proteins: Inward and Outward Signal Streams

There are inward and outward signal streams at cell membranes that are mediated by coupling proteins. Intramembranous protein particles (IMPs) that span the plasma membrane form pathways for signaling and energy transfer. As part of the inward signal stream, Luben (*14,54*) has identified a role for intramembranous proteins in conveying signals from hormone receptor sites on the membrane surface to the cell interior and vice versa.

These studies first examined effect of low-frequency pulsed magnetic fields on stimulation of adenylate cyclase in bone cells by parathyroid hormone (PTH). In bone cells, PTH binds to specific surface receptor sites; adenylate cyclase is located on the internal surface of the membrane. The receptor site outside the membrane and the catalytic subunit inside the membrane are coupled by the N protein. Stimulation of adenylate cyclase by PTH was inhibited by about 90% by induced pericellular electric gradients of only 1 to 3 mv/cm, which are eight orders of magnitude less than the gradient of the membrane potential. Moreover, the adenylate cyclase was not inactivated, nor did the fields interfere with binding of PTH to receptor sites. Thus, the evidence points to a protein that couples between the receptor and the adenylate cyclase as the probable site of field action. The cell membrane, as the site of field action, was further substantiated by differential effects of PTH (acting at the membrane) and vitamin D_3 (acting at the nucleus) on field modulation of collagen synthesis.

Structure of Membrane-Coupling Proteins and Nature of the Transmembrane Signal: EGF and NGF Receptor Proteins

Recent Nobel Award-winning studies of the epidermal growth factor (EGF) by Cohen and the nerve growth factor (NGF) by Levi-Montelcini have led to identification of their cell membrane receptor proteins. Much attention now focuses on these proteins as models of coupling proteins in studies of the nature of transmembrane signals. They share unexpected structural characteristics that appear to delimit aspects of their signaling capacities.

Ullrich et al. (*55*) have deduced the complete sequence of 1 210 amino acids of the EGF receptor. The molecule appears to cross the membrane only once. The salient and surprising feature is the extremely short length of the intramembranous segment of 23 amino acids, predominantly hydrophobic, and with only a single amino acid having a side chain capable of hydrogen bonding. Radeke et al. (*56*) report a similar 23 hydrophobic amino acid sequence in the putative intramembranous segment of the NGF receptor. Ullrich et al. (*55*) point out that this segment is probably too short to be involved in conformation changes; its hydrophobic character makes its participation unlikely in either ion or proton translocation. Despite the hydrophobic character of this transmembrane segment, addition to EGF to human A431 epidermal cell cultures causes a 2-fold to 4-fold increase in cytoplasmic-free Ca^{2+} within 30 to 60 sec (*57*). This rise is completely dependent on extracellular Ca^{2+} and is not accompanied by a change in membrane potential. This EGF-induced signal appears to result from Ca^{2+} entry via a voltage-independent channel. The response is inhibited by cancer-promoting phorbol esters that have a specific membrane receptor, Ca^{2+}-dependent protein kinase-C.

We have hypothesized that this transmembrane signaling may involve nonlinear vibration modes in helical proteins and generation of Davydov-Scott soliton waves (*58*). Solitons may be considered as traveling packets of a vibrational state, forming quasiparticles that pass along the triple amide spines of the protein molecule. They are relatively uninfluenced by random vibrations of particles through which they pass or even by other solitons. It is now recognized that the clustering of atoms exchanging vibrational and electrical energy in creation of these quasiparticles and solitary waves would confer hitherto unsuspected properties in conveyance of information and in storage and transmission of energy. Although evidence for soliton waves in DNA and helical proteins is inconclusive, we have strong supporting evidence for nonlinear, nonequilibrium processes at critical steps in transmembrane coupling, based on windows in EM field frequency, amplitude, and time of exposure that determine stimulus effectiveness.

Immune Surveillance; Modulation of Lymphocyte Cytotoxicity and Protein Kinase Activity by Amplitude-Modulated Microwave Fields, *In Vitro*

Allogeneic T lymphocytes targeted against cultured lymphoma cells showed a 20% reduction in cytolytic capacity when exposed to a weak (450 MHz, 1.0 Mw/cm^2) microwave field when modulated at 60 Hz. Interactions were less at higher and lower modulation frequencies.

Unmodulated fields were without effect (*9*). Similar effects occurred with the same cell lines in 60 Hz EM fields in the range 0.1 to 10 mV/cm, with clear evidence of a threshold and field intensity effects (*15*).

Intracellular enzymes that are sensitive to EM field exposure include certain protein kinases. These are activated by signals arising in cell membranes that do not involve the cAMP pathway by which adenylate cyclase converts ATP to cAMP. This group includes the phosphatidylserine protein kinase (kinase-C) that is a specific receptor for cancer-promoting phorbol esters. These cAMP-independent protein kinases in human lymphocytes exposed to the same 450 MHz microwave fields showed windowed activity with respect to exposure duration and modulation frequency (*12*). Reduced enzyme activity only occurred at modulation frequencies between 16 and 60 Hz, and only for the first 15 to 30 min of field exposure. Unmodulated fields produced no effects.

Intercellular Communication and Cancer Promotion: Enzymatic Markers of RF Field Interactions with Chemical Cancer Promoters at Cell Membranes

Tumor formation as a manifestation of abnormal control of cell growth is now widely modeled as a multistep process, based on animal tumor models. These models of carcinogenesis envisage initial damage to the DNA genome within the nucleus. This stage of initiation involves actions of mutagenic substances or agents such as ionizing radiation. Initiated or transformed cells may remain indefinitely in this condition without tumor formation. Tumor formation occurs as a second step requiring promotion by agents which are not mutagenic and thus are not cancer initiators by an action on DNA in the nucleus.

Tumor promoters include insecticides such as DDT, polychlorbiphenyls (PCBs) that are used as electrical insulators and coolants, saccharin, and plant lectins now used as cancer promoters in laboratory studies, the phorbol esters. Phorbol esters have a specific receptor at cell membranes. For that reason, we have examined cancer promotion in cultured cells in studies of joint actions of phorbol esters and environmental EM fields, *since both act at cell membranes* (*13,29,59*).

Available evidence indicates that nonionizing EM fields do not act as classical initiators in the etiology of cancer by causing DNA damage and gene mutation. The biology of cancer invites the consideration of promotional processes occurring at cell membranes, rather than at the cell nucleus. We have observed strong interactions at cell membranes between cancer-promotion phorbol esters and EM fields. We hypothesize that cancer promotion may involve a distorted inward signal stream from cell membranes directed to the nucleus and other organelles (*6*). At the same time, cell to cell communication through gap junctions is disrupted by chemical cancer promoters and is indicative of a disrupted outward signal stream from cell membranes (*60*). Moreover, normal cells can exert control over those of their number that have been transformed into cancer cells. For example, differentiation is restored to carcinomatous keratinocytes by contact with normal skin fibroblasts and the development of carcinomas is inhibited (*5*). The following evidence implicates modified intercellular communication through distorted inward and outward signal streams at cell membranes in cancer promotion.

Cancer Promoting Phorbol Esters at Cell Surfaces: Enzyme Activities of Protein Kinases and Ornithine Decarboxylase

As previously noted, phorbol esters have a specific receptor, protein kinase-C, that occurs widely in cell membranes and functions as a receptor and an enzyme. It is Ca^{2+}-dependent and normally activated by diacylglycerol formed from inositol phospholids by the action of cell surface stimuli. In invertebrate neurons, activation of protein kinase-C enhances voltage-sensitive inward Ca^{2+} currents (*61*). A single molecule of diacylglycerol may activate one molecule of kinase-C. Thereafter, there appears to be a spreading domino effect that activates all kinase-C molecules around the whole membrane surface (*43*); an event concurrent with the spreading wave of altered Ca binding along the membrane surface discussed above that accompanies actions of humoral stimuli and EM fields at cell surface receptor sites (*36,37*).

Ornithine decarboxylase (ODC) occurs in all eukaryotic cells and is essential for cell growth and DNA synthesis; it is also involved in synthesis of polyamines. Clinically, ODC activity in cultures of suspected cancer cells (for example, human prostatic cancer) has proved a useful index of malignancy. All agents that stimulate ODC are not cancer-promoting, but all cancer promoters stimulate ODC. Its activation pathways are not well defined. However, we have shown that binding of phorbol esters at membrane receptor sites induces ODC and that 450 MHz fields amplitude-modulated at 16 Hz increase ODC activity by 50% in cultured liver, ovary, and skin cells for 3 hr following a 1-hr exposure (*13*). Similar sensitivities were noted with 60 Hz fields (*19*).

Synergic Actions of Phorbol Esters and EM Fields on ODC Activity and Cell Transformation Frequencies

We have shown that induction of ODC following stimulation of liver and ovary cells with a phorbol ester is sharply enhanced by 450 MHz fields (1.0 mW/cm^2) sinu-

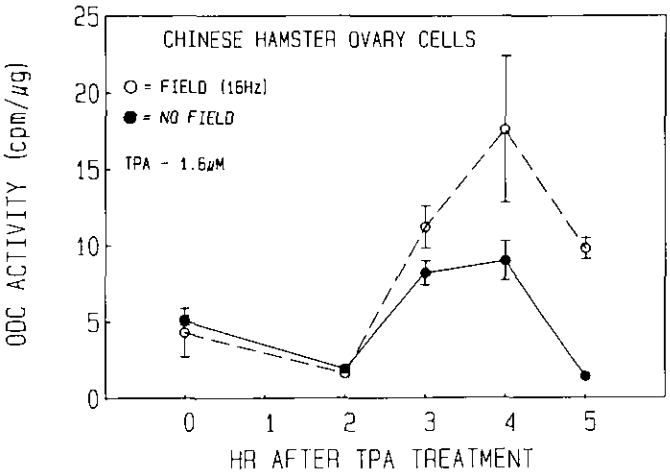

FIGURE 1. Ornithine decarboxylase activity (ODC) activity in CHO hamster ovary cells stimulated by a phorbol ester (tetradecanoyl phorbol acetate, TPA), with and without concurrent exposure to a 450-MHz field (1.0 mW/cm^2, amplitude-modulated at 16 Hz). From Byus et al. (*13*).

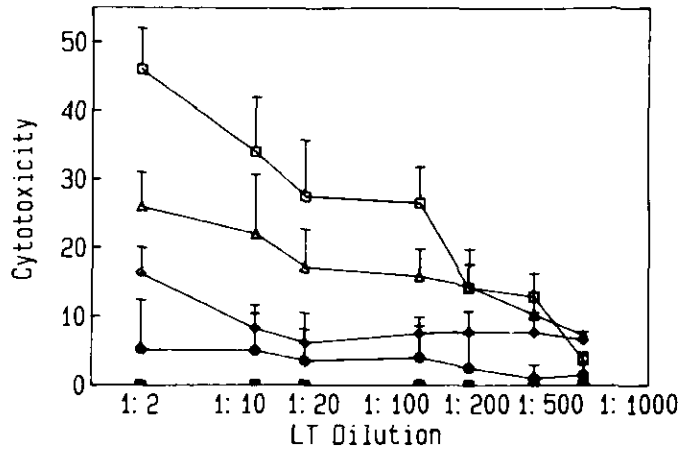

FIGURE 2. Effects of cancer-promoting phorbol ester (TPA) on α-lymphotoxin-mediated cytolysis of CHO hamster ovary cells treated with increasing doses of TPA in the presence of graduated concentrations of lymphotoxin. Results are cumulated from two to four experiments (n = 8-16). (●) CHO-F + LT only, culture of CHO-F cell line with α-lymphotoxin only; (◇) same cell culture with phorbol ester promoter TPA (4800 nM) and α-lymphotoxin; (Δ) same cell culture with phorbol ester promoter TPA (1600 nM) and α-lymphotoxin; (□) same cell culture with phorbol ester promoter TPA (480 nM) and α-lymphotoxin; (■) same cell culture with phorbol ester promoter TPA (9600 nM) and α-lymphotoxin. Redrawn from Fletcher et al. (*59*).

soidally amplitude-modulated at 16 Hz (*13*) (Fig. 1). Similar enhancement of ODC activity was noted with 60 Hz EM fields at fluid gradients of 0.1 to 10 mV/cm (*19*), which was similar to fields induced by high-voltage power line exposure. Also, phorbol ester treatment of embryonic fibroblasts previously irradiated with X-rays and microwaves increased transformation frequencies above rates previously irradiated only with X-rays (*38*). The findings are consistent with persistent membrane effects from prolonged microwave exposure (24 hr in this study), and these may enhance promoter action.

Disruption of Intercellular Communication by Phorbol Esters and Microwaves in Tumor Promotion: Outward Signals through Gap Junctions

Cell to cell communication between normal cells occurs through gap junctions, characterized by a thin protein band between adjoining cell membrane surfaces (*62*). During embryonic development, cells grow rapidly until contact occurs, but growth slows or ceases in response to chemical messages that pass between touching cells (*60,63*). Antibodies to gap-junction proteins selectively disrupt junctional communication early in embryonic development (*64*).

We have examined the separate and combined actions of phorbol esters and microwave fields on gap-junction communication (*65*). In CHO hamster ovary cells, the entry of alpha-lymphotoxin is dependent on their ability to form gap junctions (Fig. 2). This capacity varies widely in different cell strains (*59*). Entry is facilitated in strains unable to form gap junctions. In cells with well developed gap junctions, the ability to exclude alpha-lymphotoxin is disrupted by phorbol esters, an action enhanced by 450 MHz fields (1.0 mW/cm^2, 16 Hz modulation). No effects were noted with 450 MHz fields alone.

These findings imply that microwave fields at athermal levels may act synergically with chemical promoters to disrupt normal intercellular communication through gap junctions, leading to autonomous cell growth.

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REFERENCES

1. Adey, W. R. Tissue interactions with nonionizing electromagnetic fields. Physiol. Rev. 81: 435–514 (1981).
2. Foster, K. R., and Guy, A. W. The microwave problem. Sci. Amer. 255(3): 32–39 (1986).
3. Foster, K. R., and Pickard, W. F. Microwaves: the risks of risk research. Nature 330: 531–532 (1987).
4. Adey, W. R. Effects of microwaves on cells and molecules. Nature 333: 401 (1988).
5. Newmark, P. Oncogenes and cell growth. Nature 327: 101–102 (1987).
6. Adey, W. R. Physiological signalling across cell membranes and cooperative influences of extremely low frequency electromagnetic fields. In: Biological Coherence and Responses to External Stimuli (H. Frohlich, Ed.), Springer Verlag, Heidelberg, 1988, pp. 148–170.
7. Trosko, J. E. Mechanisms of tumor promotion: possible role of

inhibited intercellular communication. Eur. J. Cancer Clin. Oncol. 23: 599–601 (1987).
8. Yamasaki, H. The role of cell-to-cell communication in tumor promotion. In: Nongenotoxic Mechanisms in Carcinogenesis (B. E. Butterworth and T. J. Slaga, Eds.), Banbury Report 25, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1987, pp. 297–309.
9. Lyle, D. B., Schechter, P., Adey, W. R., and Lundak, R. L. Suppression of T-lymphocyte cytotoxicity following exposure to sinusoidally amplitude-modulated fields. Bioelectromagnetics 4: 281–292 (1983).
10. Lyle, D. B., Ayotte, R. D., Sheppard, A. R., and Adey, W. R. Suppression of T-lymphocyte cytotoxicity following exposure to 60-Hz sinusoidal electric fields. Bioelectromagnetics 9: 303–313 (1988).
11. Luben, R. A., Cain, C. D., Chen, M.-Y., Rosen, D. M., and Adey, W. R. Effects of electromagnetic stimuli on bone and bone cells *in vitro:* inhibition of responses to parathyroid hormone by low-energy, low-frequency fields. Proc. Natl. Acad. Sci. USA 79: 4180–4183 (1982).
12. Byus, C. V., Lundak, R. L., Fletcher, R. M., and Adey, W. R. Alterations in protein kinase activity following exposure of cultured lymphoyctes to modulated microwave fields. Bioelectromagnetics 5: 34–51 (1984).
13. Byus, C. V., Kartun, K., Pieper, S., and Adey, W. R. Increased ornithine decarboxylase activity in cultured cells exposed to low energy modulated microwave fields and phorbol ester tumor promoters. Cancer Res. 48: 4222–4226 (1988).
14. Luben, R. A., Chen, M.-Y., Rosen, D. M., and Mohler, M. A. Effects of electromagnetic stimuli on bone and bone cells *in vitro:* inhibition of responses to parathyroid hormone by low-energy, low-frequency fields. Proc. Natl. Acad. Sci. USA 79: 4180–4183 (1982).
15. Lyle, D. B., Wang, X. H., Ayotee, R. D., Sheppard, A. R., and Adey, W. R. Calcium uptake by leukemic and normal T-lymphocytes exposed to magnetic fields under cyclotron resonance conditions. In: Proceedings of the U.S. Department of Energy Annual Contractors' Conference, Phoenix, AZ, 1988, p. 5.
16. Dixen, R., and Rein, G. ^{3}H-Noradrenaline release potentiated in a clonal nerve cell line by low-intensity pulsed magnetic fields. Nature 296: 253–255 (1982).
17. Byus, C. V., Pieper, S. E., and Adey, W. R. The effects of low-energy 60-Hz environmental electromagnetic fields upon the growth-related enzyme ornithine decarboxylase. Carcinogenesis 8: 1385–1389 (1987).
18. Wertheimer, N., and Leeper, E. Electrical wiring configurations and childhood cancer. Am. J. Epidemiol. 109: 273–284 (1979).
19. Savitz, D. A., Wachtel, H., Barnes, F. A., John, E. M., and Tvdrik, J. G. Case-control study of childhood cancer and exposure to 60-Hertz magnetic fields. Am. J. Epidemiol. 128: 21–38 (1988).
20. Barnes, F., Savitz, D., Wachtel, H., Fuller, J., Van Feldt, W., and Brown, K. On the relationship between wiring configuration and endogenous 60-Hz fields. In: Proceedings of U.S. Dept. of Energy Contractors' Review, Denver, CO, 1986, p. 31.
21. Tomenius, L. 50-Hz electromagnetic environment and the incidence of childhood tumors in Stockholm County. Bioelectromagnetics 7: 191–208 (1986).
22. Wertheimer, N., and Leeper, E. Possible effects of electric blankets and heated water beds on fetal development. Bioelectromagnetics 7: 13–22 (1987).
23. Frolen, H., Svedenstal, B. M., and Bierke, B. Repetition of a Study of the Effect of Pulsed Magnetic Fields on the Development of Fetuses in Mice (Swedish). Final Report to Swedish National Institute of Radiation Protection, Project 346,86, March 1987.
24. Tribukait, B., Cekan, E., and Paulsson, L.-E. Effects of pulsed magnetic fields on embryonic development in mice. In: Work with Display Units (B. Knave and P.-G. Wideback, Eds.), Elsevier North Holland, 1987.
25. Rommereim, D. N., Kaune, W. T., Buchsbom, R. L., Phillips, R. D., and Sikov, M. R. Reproduction and development in rats chronologically exposed to 60-Hz electric fields. Bioelectromagnetics 8: 243–275 (1987).
26. Sikov, M. R., Rommereim, D. N., Beamer, J. L., Buchsbom, R. L., Kaune, W. T., and Phillips, R. D. Developmental studies of Hanford miniature swine exposed to 60-Hz electric fields. Bioelectromagnetics 8: 229–242 (1987).
27. Milham, S. Mortality in workers exposed to electromagnetic fields. Environ. Health Perspect. 62: 297–300 (1985).
28. Thomas, T. L., Stolley, P. D., Stemhagen, A., Fontham, E. T. H., Bleecker, M. L., Stewart, P. A., and Hoover, R. N. Brain tumor mortality risks among men with electrical and electronic jobs: a case-controlled study. J. Natl. Cancer Inst. 79: 233–237 (1987).
29. Adey, W. R. The sequence and energetics of cell membrane transductive coupling to intracellular enzyme systems. Bioelectrochem. Bioenerg. 15: 447–456 (1986).
30. Adey, W. R. Cell membranes, the electromagnetic environment and cancer promotion. Neurochem. Res. 13: 671–677 (1988).
31. Illinger, K. H. Electromagnetic-field interaction with biological systems in the microwave and far-infrared region. In: Biological Effects of Nonionizing Radiation (K. H. Illinger, Ed.), American Chemical Society Symposium Series, No. 157, American Chemical Society, Washington, DC, 1981, pp. 1–46.
32. Kremer, F., Poglitsch, A., and Genzel, L. Picosecond relaxations in proteins and biopolymers observed by mm-wave spectroscopy. In: Nonlinear Electrodynamics in Biological Systems (W. R. Adey and A. F. Lawrence, Eds.), Plenum Press, New York, 1984, pp. 177–186.
33. Keilmann, F., and Grundler, W. Nonthermal resonant action of millimeter microwaves on yeast growth. In: Nonlinear Electrodynamics in Biological Systems (W. R. Adey and A. F. Lawrence, Eds.), Plenum Press, New York, 1984, pp. 59–64.
34. Frohlich, H. Coherence and the action of enzymes. In: The Fluctuating Enzyme (G. R. Welch, Ed.), John Wiley and Sons, New York, 1986, pp. 421–449.
35. Schwan, H. P. Electrical properties of bound water. Ann. N.Y. Acad. Sci. 125: 344–354 (1965).
36. Adey, W. R. Molecular aspects of cell membranes as substrates for interactions with electromagnetic fields. In: Synergetics of the Brain (E. Basar, H. Flohr, H. Haken, and A. J. Mandell, Eds.), Springer Verlag, Berlin, 1983, pp. 201–211.
37. Adey, W. R., and Lawrence, A. F. (Eds.). Nonlinear Electrodynamics in Biological Systems. Plenum Press, New York, 1984.
38. Balcer-Kubiczek, E. K., and Harrison, R. G. Evidence for microwave carcinogenesis *in vitro*. Carcinogenesis 6: 859–864 (1985).
39. Kaczmarek, L. K., and Adey, W. R. Weak electric gradients change ionic and transmitter fluxes in cortex. Brain Res. 66: 537–540 (1974).
40. Davson, H., and Danielli, J. R. The Permeability of Natural Membranes. Cambridge University Press, Cambridge, England, 1952.
41. Singer, S. J., and Nicolson, G. L. The fluid mosaic model of the structure of cell membranes. Science 175: 720–731 (1972).
42. Hyden, H. A calcium-dependent mechanism for synapse and nerve cell membrane modulation. Proc. Natl. Acad. Sci. USA 71: 2965–2968 (1974).
43. Nishizuka, Y. Protein kinase C as a possible receptor protein of tumor-promoting phorbol esters. J. Biol. Chem. 258: 11442–11445 (1983).
44. Bawin, S. M., and Adey, W. R. Sensitivity of calcium binding in cerebral tissue to weak environmental electric fields oscillating at low frequency. Proc. Natl. Acad. Sci. USA 73: 1999–2003 (1976).
45. Bawin, S. M., Kaczmarek, L. K. and Adey, W. R. Effects of modulated VHF fields on the central nervous system. Ann. N.Y. Acad. Sci. 247: 74–81 (1975).
46. Lin-Liu, S., and Adey, W. R. Low-frequency amplitude-modulated microwave fields change calcium efflux rates from synaptosomes. Bioelectromagnetics 3: 309–322 (1982).
47. Adey, W. R. Ionic nonequilibrium phenomena in tissue interactions with electromagnetic fields. In: Biological Effects of Non-

ionizing Radiation (K. H. Illinger, Ed.), American Chemical Society Symposium Series, No. 157, American Chemical Society, Washington, DC, 1981, pp. 271–297.

48. Bawin, S. M., Sheppard, A. R., and Adey, W. R. Possible mechanisms of weak electromagnetic field coupling in brain tissue. Bioelectrochem. Bioenerg. 5: 67–76 (1978).
49. Blackman, C. F., Elder, J. A., Weil, C. M., Benane, S. G., Eichinger, D. C., and House, D. E. Induction of calcium ion efflux from brain tissue by radio frequency radiation. Radio Sci. 14: 93–98 (1978).
50. Adey, W. R., Bawin, S. M., and Lawrence, A. F. Effects of weak amplitude-modulated microwave fields on calcium efflux from awake cat cerebral cortex. Bioelectromagnetics 3: 295–307 (1982).
51. Blackman, C. F., Benane, S. G., Kinney, L. S., Joines, W. T., and House, D. E. Effects of ELF fields on calcium-ion efflux from brain tissue *in vitro*. Radiation Res. 92: 510–520 (1982).
52. Blackman, C. F., Benane, S. G., Rabinowitz, J. R., House, D. E., and Joines, W. T. A role for the magnetic field in the radiation-induced efflux of calcium ions from brain tissue *in vitro*. Bioelectromagnetics 6: 327–338 (1985).
53. Dutta, S. K., Subramoniam, A., Ghosh, B., and Parshad, R. Microwave-induced calcium efflux from brain tissue, *in vitro*. Bioelectromagnetics 5: 71–78 (1984).
54. Luben, R. A., and Cain, C. D. Use of bone cell hormone response systems to investigate bioelectromagnetic effects on membranes, *in vitro*. In: Nonlinear Electrodynamics in Biological Systems (W. R. Adey and A. F. Lawrence, Eds.), Plenum Press, New York, 1984, pp. 23–34.
55. Ullrich, A., Coussens, L. Hayflick, J. S., Dull, T. J., Gray, A., Tam, A. W., Lee, J., Yarden, Y., Libermann, T. A., Schlessinger, J., Downard, J., Mayes, E. L. V., Whittle, N. Waterfield, M. D., and Seeburg, P. H. Human epidermal growth factor receptor cDNA sequence and aberrant expression of the amplified gene in A431 epidermoid carcinoma cells. Nature 309: 421–428 (1985).
56. Radeke, M. J., Misko, T. P., Hsu, C., Herzenberg, L. A., and Shooter, M. Gene transfer and molecular cloning of the rat nerve growth factor receptor. Nature 325: 393–397 (1987).
57. Moolenaar, W. H., Aerts, W. J., Tertoolen, L. G. J., and DeLast, S. W. The epidermal growth factor-induced calcium signal in A431 cells. J. Biol. Chem. 261: 279–285 (1986).
58. Lawrence, A. F., and Adey, W. R. Nonlinear wave mechanisms in interactions between excitable tissue and electromagnetic fields. Neurol. Res. 4: 115–153 (1982).
59. Fletcher, W. H., Shiu, W. W., Ishida, T. A., Haviland, D. L., and Ware, C. F. Resistance to the cytolytic action of lymphotoxin and tumor necrosis factor coincides with the presence of gap junctions uniting target cells. J. Immunol. 139: 956–962 (1987).
60. Yotti, L. P., Chang, C. C., and Trosko, J. E. Elimination of metabolic cooperation in chinese hamster cells by a tumor promoter. Science 206: 1089–1091 (1979).
61. DeRiemer, S. A., Strong, J. A., Albert, K. A., Greengard, P., and Kaczmarek, L. K. Enhancement of calcium current in Aplysia neurons by phorbol ester and kinase C. Nature 313: 313–316 (1985).
62. Loewenstein, W. R., Junctional intercellular communication and the control of growth. Biochim. Biophys. Acta 560: 1–65 (1979).
63. Fletcher, W. H., Byus, C. V., and Walsh, D. A. Receptor-mediated action without receptor occupancy: a function for cell-cell communication in ovarian follicles. Adv. Exper. Med. Biol. 219: 299–323 (1987).
64. Warner, A. E., Guthrie, S. C., and Gilula, N. B. Antibodies to gap-junctional protein selectively disrupt junctional communication in the early amphibian embryo. Nature 311: 127–131 (1984).
65. Fletcher, W. H., Shiu, W. W., Haviland, D. A., Ware, C. R., and Adey, W. R. A modulated-microwave field and tumor promoters similarly enhance the actions of alpha-lymphotoxins. In: Proceedings of the Bioelectromagnetic Society, 8th Annual Meeting, Madison, WI, 1986, p. 12.