PLENARY PAPERS

IN VITRO STUDIES: LOW FREQUENCY ELECTROMAGNETIC FIELDS

Prepared by: Stephen F. Cleary, Ph.D.
Professor of Physiology and Biophysics
Department of Physiology
Medical College of Virginia
Richmond, Virginia

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INTRODUCTION

In vitro studies of effects of low-frequency (LF) electromagnetic (EM) fields have revealed a variety of sensitive cell-physiologic end-points. Effects have been reported on: (1) DNA, RNA and protein synthesis; (2) cell proliferation; (3) cation fluxes and binding; (4) immune responses; and (5) membrane signal transduction (i.e. hormones, enzymes, and neurotransmitters). Typically such effects occurred as a result of short-term exposure of cells to EM at frequencies of 100 Hz or less and at low field intensities. The dependency on frequency or modulation, as well as the apparent weak cellular interaction of these LF EM fields, lacks theoretic explanation. It has not been determined whether effects are induced by electric or magnetic fields.

Confounding the interpretation of the results of such studies are associated phenomena such as: (1) transient or time-delayed responses; (2) modulation- and intensity-specific effects, referred to as modulation or intensity "windows"; and (3) general lack of dose-(or dose-rate) response data or EM field thresholds. Consequently, although it is well-established that LF EM fields affect biological systems *in vitro* use of these data to assess human health effects is limited.

Purpose

The purpose of this plenary paper is to review selected representative published reports of LF EM fields on *in vitro* systems. This is not intended to be an exhaustive review. *In vitro* studies that did not detect EM field effects were not reviewed in detail since they provide no guidance for the direction of future research. This does not imply that

ment of effects of EM fields on *in vitro* systems. However, the limited number of published reports of EM field effects, be they positive or negative, precludes the generation of a consensus view at this time.

To the extent possible, relevance of the findings to occupational exposures will be assessed. Principally, this will be attempted by considering the consistency of *in vitro* and *in vivo* EM exposure effects and comparison of EM field intensities that affect *in vitro* systems with occupational EM exposure intensities. Finally, suggestions will be made for the direction of future *in vitro* research of direct pertinence to potential occupational exposure problems.

Definitions

In the context of this article *in vitro* studies are defined as experimental or theoretical studies of the effects of low frequency (i.e. frequencies less than 1000 Hz) electromagnetic (EM) fields on individual cells or explanted tissue, exposed and assayed outside of human or animal bodies. *In vitro* studies may involve: (a) normal mammalian cells or tissue, such as lymphocytes or other blood cells, obtained from donors prior to exposure, or (b) transformed mammalian cells that are maintained indefinitely in culture. Type (a) cells, derived from a specific donor in limited quantities, have a finite lifespan of up to a week or so. Type (b) cells, on the other hand, may be propagated indefinitely in large numbers and exposed to EM radiation, or other agents, for extended periods of time in various laboratories. Cells of either type can be maintained under controlled conditions in defined composition culture media supplemented with various growth factors and antibiotics.

Advantages and Limitations

The primary advantages of *in vitro* studies include: 1) the potential for the precise control of experimental variables such as electric or magnetic field strength, temperature, culture medium composition, etc., 2) accurate and detailed dosimetric and densitometric information, 3) exposure replication (essentially unlimited replication for type (b) cells), 4) relatively simple cell geometry, amenable to theoretical modeling of electric and/or magnetic field interactions with cells or cell constituents, such as the plasma membrane, 5) significant reduction in cost relative to *in vivo* studies. Taken as a whole, *in vitro* studies afford the opportunity to determine basic mechanisms of interaction of EM fields with living systems. This distinction, relative to *in vivo* systems, is attributed to the complex nature of EM field interactions and induced field distributions within the body of experimental animals or humans which impede the establishment of precise dose-effect relationships. The advantages of *in vitro* systems may be exploited in investigations of co-factor interactions of EM fields with other physical or chemical agents. Such studies should prove of value in screening for potentially adverse interactions of EM fields with other agents in the workplace.

In vitro systems thus provide a versatile means of investigating direct EM cellular interactions or co-factor interactions. However, there are limitations on their use in developing guidelines or standards for occupational exposure to EM fields due to the complex interactive nature of integrated physiological systems comprising an organism which preclude direct extrapolation of *in vitro* data to *in vivo* responses. *In vitro* data, including EM cellular effects thresholds and dose-response relationships can, however, provide the basis for the design of *in vivo* studies by defining critical physiological end-points, and EM field parameters. Understanding basic cellular-level interaction mechanisms will provide a general basis for extrapolation of *in vitro* to *in vivo* exposure effects, as well as inter-species exposure effects of EM effects on mammalian systems.

IN VITRO EFFECTS OF LF EM FIELDS

Biomolecular Synthesis

Low intensity LF electric and magnetic fields affect rates of synthesis of DNA, RNA and proteins *in vitro*. Liboff et al. (1984), for example, reported increased DNA synthesis in human fibroblasts exposed to low intensity sinusoidal magnetic fields (15 to 4000 Hz). Weak ELF electric or magnetic fields affected collagen and/or glycosaminoglycan synthesis in fibroblasts (Fitton-Jackson and Bassett, 1980; Kamrin, 1974; Farndale and Murray, 1985; Cleary et al., 1988). ELF electric fields increased DNA (Rodan et al., 1978) and glycosaminoglycan (Lee et al., 1982) synthesis in chondrocytes. Binderman et al. (1985) reported cell-specific bi-phasic stimulation of cyclic AMP levels and DNA synthesis in skeletal-derived cell cultures exposed to 3 Hz electric fields.

Goodman et al. (1987), detected increased rate of messenger RNA synthesis (transcription) in dipteran salivary gland cells exposed to pulse-modulated magnetic fields at frequencies of 15 to 72 Hz. Transcriptional activity was also increased following exposure to 72 Hz sinusoidal magnetic fields for periods of up to 45 min. The maximum induced magnetic and electric field strengths were less than approximately 4 mT (milliTesla) (40 Gauss) and 10 mV/m respectively. Although all of the magnetic fields affected transcriptional activity, there were quantitative and possible qualitative differences in the effects of the different magnetic field wave forms. Analysis of the X-chromosome transcription patterns indicated that pulsed magnetic fields augmented activation of pre-existing (normally active) chromosomal loci and activated inactive genes or gene sets. Subsequent studies demonstrated similar effects of LF modulated magnetic fields (including 60 Hz fields) on RNA transcription and protein synthesis in other cell types (Goodman et al., 1989) and in cell-free systems (Goodman, unpublished observations). The mechanism(s) for magnetic field effects on transcription are unclear. However, since this effect occurred in cell-free, as well as cell

systems, possible direct genomic interaction is suggested. This is of interest since most other *in vitro* cellular effects of LF electric or magnetic fields have been associated with interactions with the cell membrane, although here again mechanisms are uncertain.

Whereas ELF electric and/or magnetic fields of various intensities, frequencies and modulation affect cell biosynthetic processes, attempts to detect chromosomal alterations such as rearrangements, single strand breaks, point mutations, or sister chromatid exchange have proven negative (Cohen et al., 1986; Livingston, 1986; Benz, 1987; Reese et al., 1988).

Membrane Calcium Fluxes and Binding

The most extensively investigated and replicated *in vitro* effect of LF EM fields is altered calcium ion (Ca^{2+}) binding to chick brain tissue. These studies revealed tissue sensitivity to extremely low intensity LF electric and magnetic fields characterized by multiple modulation- and intensity-specific responses, referred to as modulation and intensity "windows", respectively. Multiple response windows, which have proven difficult to explain theoretically, present potentially perplexing problems with respect to the development of occupational exposure guidelines since they violate traditional dose-effect and threshold response concepts. To date there is limited *in vitro* data suggesting windowed responses for other cell end points such as cell proliferation (Cleary et al., 1988; Ross, 1990) or fibroblast protein synthesis (McLeod et al., 1987). This limitation may well be attributed to the small number of studies designed to detect windows.

Bawin et al., (1975, 1976) first reported modulation windows for Ca^{+2} efflux from chick brain exposed to 147 MHz radio frequency (RF) electromagnetic radiation, amplitude modulated (AM) at specific frequencies between 6 and 20 Hz. Blackman and co-workers, (1979, 1980a) reported intensity (power-density) windows for this phenomenon and subsequently found similar responses using modulated 50 MHz RF radiation (Blackman et al., 1980b). Sheppard et al. (1979)

also observed a Ca^{+2} efflux intensity window for modulated RF radiation. Dutta et al. (1984; 1989) reported multiple intensity windows for Ca^{+2} efflux from neuroblastoma cells in culture exposed to 915- or 147 MHz RF radiation amplitude modulated at 16 Hz.

Sinusoidal ELF fields induced intensity- and modulation-dependent windowed effects on Ca^{+2} efflux from chick brain tissue *in vitro* (Blackman, 1985a). Whereas a 16 Hz sinusoidal field enhanced Ca^{+2} efflux at 6 and 40 V/m, 1- or 30 Hz fields were ineffective, as was a 42 Hz field at 30-, 40-, 50- or 60 V/m. A 45 Hz field enhanced efflux at 40 V/m, of similar magnitude to the 16 Hz field. Field strengths between 45- and 50 V/m increased efflux at 45 Hz, whereas at 60 Hz, 35- and 40 V/m were effective intensities. Holding the field strength constant at 42.5 V/m and varying the frequency revealed Ca^{+2} efflux enhancement in a region around 15 Hz and another from 45 to 105 Hz (Blackman et al., 1985a).

Blackman et al. (1985b) observed that the local DC magnetic field at the site of ELF-exposed samples determined which electric and magnetic field frequencies were effective in inducing Ca^{+2} release from chicken-brain tissue *in vitro*. In this study the DC magnetic field was perpendicular to the plane containing oscillating electric and magnetic field components. In a subsequent study Blackman et al. (1990) observed that Ca^{+2} efflux occurred when the DC-magnetic field was perpendicular to the alternating magnetic field component of a 314 Hz, 15 V/m, 61 nT (nanoTesla) EM field, but not when the magnetic fields were in parallel alignment. They noted that this result is consistent with a magnetic resonance-like transduction mechanism for the conversion of EM energy into a physicochemical change, such as enhanced ion transport through helical membrane channels. It was also noted that the magnetic field alignment dependence was in direct contrast to the results of Smith et al. (1987), who demonstrated a resonance-like effect of an alternating ELF magnetic field on the mobility of diatoms.

Diatom mobility depends upon transmembrane transport of Ca^{+2}. Smith et al. (1987) exposed diatoms to combined DC and alternating magnetic fields they predicted would enhance Ca^{+2} transport on the basis of an ion cyclotron resonance theory advanced by Liboff (1985) and McLeod and Liboff (1986) (discussed below). In agreement with theory, a mobility maximum occurred at 16 Hz when the diatoms were exposed to a DC magnetic field of 20.9 μT and an AC field of 20.9 μT, when the static and AC magnetic fields were in parallel alignment. Perpendicular magnetic field alignment had no effect on diatom mobility. As noted by Blackman et al. (1990), the diatom experiments of Smith et al. (1987) and chick brain experiments were conducted under different conditions. Smith et al. (1987) exposed diatoms to a 1000-fold greater magnetic flux density than Blackman et al. (1985) and with different magnetic field alignment.

The results of Blackman et al. (1985a,b; 1990) and Smith et al. (1987), as well as observations of Thomas et al. (1986) on the effects of LF AC magnetic fields on rat behavior, provide evidence of intensity- and frequency-dependent responses having common features such as: (a) multiple windows at frequencies less than 1000 Hz; (b) intensity windows in a range of intensities well below levels at which cellular alterations can be accounted for by conventional, well understood physicochemical interaction mechanisms and (c) dependence on orientation of geomagnetic and applied EM field components.

In view of these complexities, and the limited number of studies that have been conducted, it is not surprising that the physiological significance of EM-induced alterations in membrane cation binding or transport has not been ascertained. The central role of Ca^{+2} fluxes in neural processes is well known. Effects of low-intensity LF EM fields on Ca^{+2} binding to brain tissue *in vitro*, reported by Bawin et al. (1975; 1976) and Blackman et al. (1979; 1980a,b; 1985a,b), suggest that such fields may affect the mammalian central nervous system (CNS) *in vivo*. The results of Thomas et al. (1986), and behavioral changes in monkeys induced by exposure to LF EM fields reported by Gavalas-Medici and Day-Magdaleno (1976) do, in fact, implicate

this phenomenon in EM-induced effects on the mammalian CNS. Further evidence derives from the observation that low-amplitude LF AM electromagnetic radiation induced Ca^{+2} release from the brain of a live cat (Adey et al. 1982). The potential physiological significance of LF EM field exposure has been reviewed in detail by Adey (1981).

Cell Proliferation

The most extensive body of information concerning cellular effects of LF EM radiation derives from studies of cell proliferation *in vitro*. Interest in effects on cell proliferation has been stimulated by clinical applications of such fields for the treatment of connective tissue disorders, such as bone nonunions (Bassett et al., 1981, 1982), fresh fractures (Wahlstrom, 1984) and tendinitis (Binder et al. 1985), as well as reported association of LF EM field exposure and cancer. Attempts to more fully characterize and quantitate *in vivo* responses, and to establish mechanisms, have led to a series of *in vitro* studies, many of which employed pulsed magnetic fields of the type reported to be clinically effective. The results of such *in vitro* studies of effects on nerve, muscle, fibroblasts, neural crest cells, and epithelial cells, reviewed by Robinson (1985), document effects of LF electric and/or magnetic fields on proliferation, intercellular communication and development.

In addition to effects of LF pulsed magnetic fields, the results of Liboff et al. (1984) indicated that sinusoidally varying magnetic fields at frequencies in the range 15 Hz to 20 kHz induced proliferative changes in human embryonic foreskin fibroblasts *in vitro*. Exposure to a 76 ± 4 Hz magnetic field, at an intensity of 1.6×10^{-5} T_{rms} (Tesla root-mean-square), induced statistically significant time dependent increases in DNA synthesis during exposures of up to 96 hours. In this series of experiments the maximum increase in proliferation, which occurred after 96 hours of exposure, was approximately 60%. Compared to sham-exposed cells, DNA synthesis in fibroblasts exposed to ten different LF EM frequency and amplitude combinations exhibited a time dependent maximum after 20 hours of exposure. Liboff et al. (1984) noted that this exposure

duration corresponded to the midpoint of the S-phase of the fibroblast cell cycle, suggesting that EM exposure effects may be related to specific cell cycle alterations. Cell proliferation data for various combinations of magnetic field intensity and frequency provided a means of testing the hypothesis that cell proliferation was directly stimulated by eddy currents induced by sinusoidal magnetic fields. According to Faraday's law the magnitude of the eddy currents, or induced electric fields, is proportional to the product of the magnetic field frequency and intensity. The data did not support this hypothesis, leading Liboff et al. (1984) to conclude that either the magnetic field effect on fibroblast proliferation was not due to induced eddy currents or that the effect was a saturable phenomenon, such as a self-limiting shift in the onset of S-phase. The threshold for sinusoidal magnetic field effects on fibroblast proliferation was in the range 5 to 25 μT/s (microTeslas per second). Liboff et al. (1984) noted that this value was similar in magnitude to the value of approximately 10 μT/s reported to interfere with development of chick embryos (Delgardo et al., 1982).

Liboff et al. (1984) also noted that threshold magnetic field magnitudes in their study were on the order of ambient 60 Hz fields in the vicinity of devices such as fluorescent lights, fans, or electric motors. Consequently, ambient fields must be measured and controlled to ensure against artifacts in *in vitro* studies.

Ross (1990) investigated the effect of 48 hour exposure of rabbit ligament fibroblasts *in vitro* to 16, 75, or 100 Hz sinusoidal magnetic fields. Variation of AC magnetic field amplitude, frequency, and vertical DC magnetic field strength resulted in either stimulation or inhibition of proliferation. Proliferation was inhibited at all three frequencies when the amplitude of the AC and DC magnetic fields corresponded to cyclotron resonance conditions. The bi-phasic nature of the effect of sinusoidal magnetic fields on proliferation was demonstrated by varying the amplitude of a 100 Hz signal from 0.1 to 1 mT (milliTesla), holding the DC magnetic field constant at 0.13 mT. Proliferation was inhibited at amplitudes of 0.5 mT or less but enhanced when the magnetic field intensity was increased to 0.7 mT

or greater, up to a maximum at 1 mT, the largest amplitude reported. By varying the amplitude of the DC field from 0.1 to 0.3 mT and the AC field from 0.5 to 1 mT, while holding the frequency of the AC field at 100 Hz, Ross (1990) detected a significant interaction between DC and AC magnetic fields and fibroblast proliferation. These data support the hypothesis of a cyclotron resonance-like phenomenon being associated with inhibition or stimulation of fibroblast proliferation.

Ross (1990) commented on the bi-phasic (stimulation/suppression) proliferative effect of varying the AC magnetic field intensity. He noted that cell proliferation is triggered synergistically by several biomolecular pathways (O'Keefe and Pledger, 1983; Roger et al., 1987; Van der Burg et al., 1988) which may be differentially affected by magnetic fields at different intensities. It was also noted that the AC magnetic field intensities used in this study were on the order of those encountered occupationally (Miller, 1974).

Evidence that in addition to effects of magnetic fields, electric fields per se affect cell proliferation was reported by Noda et al. (1987). DNA synthesis was increased 20% in rat osteosarcoma cells exposed for 34 hours to 60 Hz electric fields at current densities of 0.3 to 3 A_{rms} per m^2. Higher or low current densities were ineffective, indicating a current density "window". The results of this study are of interest since the electric field effect depended upon a number of variables including: (a) concentration of fetal calf serum (FCS); (b) cell seeding density; (c) "stage" or age of the cell population at the time of seeding. In general these data indicate that the 60 Hz electric field effect on proliferation depended upon the mitotic status of the cell population during exposure. This finding is potentially significant since it suggests specific interaction of LF EM fields with the mammalian cell cycle. The dependence of the proliferative effect of EM fields on factors such as (a) – (c), if not taken into account in the design of *in vitro* studies, could result in highly variable or contradictory results.

Further evidence that electric fields *per se*, of a different wave form than used by Liboff et al. (1984), Noda et al. (1987) or Ross (1990), affected cell proliferation was provided by Cleary et al. (1988) who exposed normal chicken tendon explants *in vitro* to low amplitude, unipolar, square wave pulsed electric fields. An electrical field parameter set consisting of 1 Hz, 1 millisecond duration pulses, having a time averaged current density of 7 mA/m^2 (maximum current density 7 A/m^2) induced a highly statistically significant 32% increase in fibroblast proliferation in tendon explants exposed for 96 hours. Exposure to the same pulsed field at a time averaged current density of 1.8 mA/m^2 did not affect fibroblast proliferation. Exposure to current densities of greater than 10 mA/m^2, on the other hand suppressed both proliferation and collagen synthesis, without affecting non-collagen protein synthesis.

The effect of the 1 Hz pulsed electric field on fibroblast proliferation was also dependent upon orientation of the explant with respect to the electric field. Fibroplasia was enhanced when the explant longitudinal axis was oriented parallel to applied E-fields having current densities of 3.5 or 7 mA/m^2. For perpendicular orientation there was no effect on proliferation. Fibroblast proliferation and collagen synthesis were inversely proportional to donor age for the 3 to 16 week old chickens used in this study. However, there was no interaction between donor age and the effect of ELF pulsed field exposure on these dependent variables. Subsequent studies revealed that the effect of pulsed electric fields on proliferation of explants from chickens aged 8 – 16 weeks depended upon extra-cellular Ca^{+2} and FCS concentration. This was not true for explants from chickens less than 3 weeks of age (Cleary, unpublished results).

It may be concluded that low intensity LF EM fields modulate proliferation of normal as well as transformed mammalian connective tissue cells *in vitro*. Intensity (current density) windows resulted from exposure to magnetic as well as unipolar or bipolar (AC) electric fields. The magnitude of the proliferative response was dependent upon EM field intensity, exposure duration, and cellular and extracellular factors.

Cell Surface Effects

Phillips et al. (1986a) investigated the effect of 60 Hz EM fields on the expression of the transferrin receptor on human colon carcinoma cells *in vitro*. Cells were exposed for 24 hours to either a 300 mA_{rms}/m^2 electric field; a 10^{-4} T_{rms} magnetic field; or combined E and H-fields at these intensities. The rationale for this study was the association of the transferrin receptor with the receptor of natural killer cells (cytotoxic lymphocytes), and the fact that expression of this receptor is correlated with proliferation of normal and malignant cells. Phillips et al. (1986b) reported that exposure of colon cancer cells *in vitro* to 60 Hz EM fields significantly increased colony formation in soft agar and increased the expression of tumor associated antigens.

Phillips et al. (1986a) reported a 24-fold increase in colony formation in colon cancer cells exposed to both E and H-fields; a 14-fold increase in magnetic field exposed cells; and an increase of 1.7 times in cells exposed to the 60 Hz E-field. The increased clonogenic capacity persisted for the 8 month study duration. The expression of transferrin receptors in cells exposed to the combined fields, or to the magnetic field alone, was maintained at maximal levels and was not under the normal cell density regulatory influence. The change in transferrin receptor expression was maintained in cells up to 8 months after EM exposure. Based on these data, Phillips et al. (1986a) suggested that EM exposure may affect normal cell proliferation control processes.

Luben et al. (1982) exposed osteoblast-like mouse bone cells to either a continuous pulse train magnetic field having a pulse burst repetition rate of 72 Hz or recurrent bursts modulated at 15 Hz. These fields induced extra-cellular electric field strengths of 0.1 V/m or less and current densities on the order of 10 mA/m^2 or less. Exposure to either EM signal for up to 90 hours significantly reduced the normal ability of bone cells to produce cyclic adenosine monophosphate (cAMP) in response to parathyroid hormone (PTH). There was no EM field

effect on adenylate cyclase activity. EM field exposure blocked the inhibitory effects of PTH on collagen synthesis. However, inhibition of collagen synthesis by 1,25-dihydroxyvitamin D_3 was not affected. PTH acts at the site of the plasma membrane, in contrast to 1,25-dihydroxyvitamin D_3, which acts primarily in the cell nucleus. Luben et al. (1982) concluded that their data supported the hypothesis that EM field effects are mediated primarily in the plasma membrane of osteoblasts, either by interfering with hormone receptor interactions or by blocking receptor cyclase coupling in the membrane. Support for hypothesized cell surface alterations induced by EM fields was provided by Marron et al. (1988) who used a chromatographic technique to demonstrate that both 60 Hz electric and magnetic fields altered the physical characteristics (surface charge, hydrophobicity) of the surface of the amoebae *Physarum*. The E and H-fields acted independently and in different ways. A 60 Hz, 1 V/m electric field exposure for 24 hours increased net negative surface charge, whereas magnetic field exposure at 0.1 mT decreased surface hydrophobicity.

Cancer Promotion

Membrane mediated alterations, induced by 60 Hz electric fields, have been implicated in cancer promotion. Byus and co-workers (1987) reported altered activity of ornithine decarboxylase (ODC), an enzyme intimately involved in induction of proliferation of normal and tumor cells. A 1 hour exposure to a 60 Hz 1 V/m electric field induced a 500 percent increase in ODC activity in human lymphoma cells and a 200 to 300 percent increase in mouse myeloma cells *in vitro*. The magnitude and duration of ODC activation depended upon cell type, E-field strength, and exposure duration. For example, a 1 hour exposure of hepatoma cells to a 60 Hz field strength of 10 mV/m induced a 30 percent increase in ODC. Exposure for 2 hours at 1 V/m had no effect, whereas a 3 hour exposure decreased enzyme activity. Based on a comparison of the effect of EM fields and the tumor promoting phorbol ester TPA on cellular ODC activity, Byus et al. (1987) indicated that 60 Hz EM fields may function as a tumor promoting stimulus. They noted, however, that there were significant differences in the magnitude of the effects of TPA and the EM fields

used in their study, on cellular ODC activity and that tumor promotion by TPA is highly dependent on the dosage time schedule. Thus direct comparisons of the tumor promoting potential of EM fields and TPA were not possible.

In addition to the possibility that LF EM fields may act as a tumor promoter, as suggested by Byus et al. (1987), there is *in vitro* evidence of an alternative, but not mutually exclusive mechanism to relate EM exposure to cancer, namely effects on immune surveillance. Lyle and co-workers (1988) detected a statistically significant 25 percent inhibition of allogeneic cytotoxicity of B-lymphoma target cells by murine cytotoxic T-lymphocytes that were exposed for 48 hours to a 1 V/m_{rms} 60 Hz sinusoidal electric field. The magnitude of cytotoxic inhibition was dependent upon E-field strength. Exposure of T-lymphocytes to 0.1–0.01 V/m resulted in 19 and 7 percent reductions in cytotoxicity, respectively. When the 4 hour cytotoxicity assay was conducted in the presence of a 1 V/m 60 Hz E-field, using previously unexposed T-lymphocytes there was a statistically nonsignificant 5 percent reduction in cytotoxicity. These results suggest that the EM field effect depended upon exposure duration and field strength. Lyle et al. (1988) indicated that the threshold for inhibition of cytotoxicity in clonal T-lymphocytes by exposure *in vitro* to a 60 Hz sinusoidal electric field is between 0.01 and 0.1 V/m.

Theoretical Studies

A theory that adequately accounts for the reported *in vitro* and *in vivo* effects of LF EM fields must address three major issues:

(1) How effects are induced by EM fields at intensities well below those known to induce recognized physical or physicochemical alterations in living systems;
(2) Why effects occur only in specific intensity ranges (i.e. intensity or power density windows);
(3) Why effects occur only at specific frequencies or modulations (i.e. frequency or modulation windows).

Whereas theories that partially explain LF EM field effects have been advanced, none provide an adequate quantitative basis encompassing these three issues. Failure to develop an adequate theoretical basis for low intensity LF EM field effects may be, in a general sense, attributed to the uniqueness of non-equilibrium living systems which render them not directly amenable to descriptions based on classical physical or biochemical principles. The need to consider living systems from different perspectives was discussed by Fröhlich (1984) Kaiser (1985), and others.

The general concept of cooperative and/or coherent interactions between elements in living systems, such as membrane constituents, has been invoked to explain effects involving weak coupling of EM fields. Theoretical models incorporating such concepts were described, for example, by Adey (1988a,b). Blackman et al. (1989) discussed specific implications of such theories with respect to the Ca^{+2} efflux from brain tissue *in vitro*.

Bawin et al. (1976), Blackman et al. (1989), and Smith et al. (1987) observed frequency windows in the efflux or transport of Ca^{+2} at or near 16 Hz. The observation that the position of the Ca^{+2} frequency window was dependent upon the magnitude and direction of the static geomagnetic field led Liboff (1985), and McLeod and Liboff (1986), to advance a theory that LF AC and DC magnetic fields coupled energy to cations, such as Ca^{+2} or Li^{+}, via a cyclotron resonance phenomenon. Whereas this theory predicted the observed occurrence of fundamental and harmonic frequency windows, unanswered questions remain about the cyclotron resonance phenomenon as applied to enhanced cation transport in biomembrane channels. Halle (1988), for example, questioned physical aspects of the model on the basis of classical mechanics, and indicated that fluid friction would preclude significant magnetic field effects on ion transport. The cyclotron resonance model also was criticized on the basis of predicting an inconsistently large ion radius of gyration and longer ionic collision damping times than predicted from physical principles (Sandweiss; 1990).

Weaver and Astumian (1990) developed physicochemical models to explain the coupling of weak periodic electric fields to cells. They modelled effects of applied electric fields on transmembrane potential under various assumptions, comparing the magnitude of induced alterations to thermally induced fluctuations. For large elongated cells with membranes having informational processing sensitivities limited to specific extrinsic low frequency EM field band widths of 10 or 100 Hz, minimal detectable electric fields (i.e. transmembrane induced signals at least as great as thermal fluctuations) of 8×10^{-4} and 3×10^{-3} V/m, respectively were predicted. Phenomena such as cell membrane signal averaging and electroconformational coupling of applied electric fields to membrane macromolecules, such as enzymes, were also considered with respect to cellular effects of LF EM fields (see also Astumian et al., 1990). Weaver and Astumian (1990) concluded that their estimates are consistent with experimental observations that low intensity EM fields affect living systems via non-thermal interaction mechanisms.

In summary, theoretical models have not adequately described LF EM field effects on ion binding or membrane transport, or other low intensity field effects. It may be anticipated that ever increasing knowledge of the unique nature of biological systems that has rendered them refractory to straight forward description by the application of physicochemical principles may advance theoretic understanding of phenomena such as LF EM effects on living systems. Obviously, a more extensive *in vitro* data base, including dose responses and thresholds, will facilitate the development of theoretical models of the interactions and effects of LF EM fields.

SUMMARY AND CONCLUSIONS

In vitro studies provide direct evidence that LF EM fields induce physiologically significant alterations in normal and transformed human and other mammalian cells. The weight of experimental and theoretical evidence indicates that the outer surface of the cell membrane is the primary locus for EM field induced cellular alterations.

In a general sense, the type and magnitude of EM field effects on cells *in vitro* are not inconsistent with purported effects on humans or experimental animals, principally effects on cancer incidence, behavior, and development. However, the limited extent and nature of *in vitro* data preclude drawing conclusions about the specific relevance to *in vivo* exposure effects. Although there are uncertainties in EM exposure levels in *in vitro* as well as *in vivo* systems, it may be concluded that EM field induced alterations in *in vitro* systems occur at approximately the same levels encountered in occupational settings.

In vitro data indicates that effects such as altered biosynthesis or proliferation occur from exposure to extrinsic LF EM fields that induce cellular level fields of the same approximate magnitude and frequency as endogenously generated fields (Cleary et al., 1988; Robinson, 1985). This suggest that instead of inducing unique physiological alterations, EM fields may perturb normal cell functions by mimicking endogenous fields. Mechanisms for EM field effects may thus be sought by contrasting endogenous and exogenous field characteristics, such as band width, wave form, etc.

Major impediments to utilization of extant *in vitro* data relate to: (a) the dearth of dose response relationships and/or effects thresholds; (b) dosimetric and densitometric uncertainties, especially in the case of magnetic field exposure, that result in imprecise knowledge of cellular level induced EM field magnitudes; and (c) the lack of an adequate theory to account for LF EM field effects characterized by: (1) extremely low interaction energies, (2) intensity and modulation windows, and (3) apparently complex temporal dependency.

In view of the unique and essential contributions of *in vitro* studies to defining and understanding occupational health effects of LF EM field exposure, future efforts must be directed toward removing these impediments.

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