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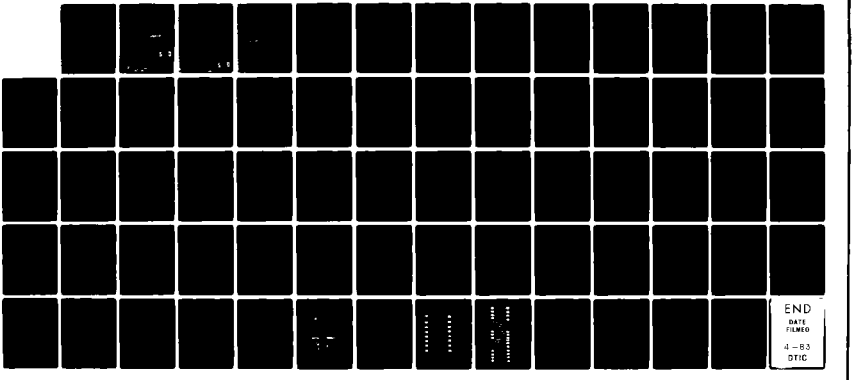

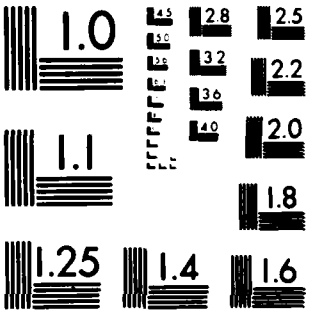

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A Theoretical Analysis of the Observed Calcium Anomalies Concomitant with The Interaction of Low Intensity Electric Fields with Cerebral Tissue

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Section I
INTRODUCTION

We have developed an analytical model that decribes certain aspects of the displacement of divalent calcium ions, $Ca^{(+2)}$, from the surface of biological tissue exposed to radiofrequency radiation. This model explicitly provides a means of quantifying certain ideas that have heretofore been suggested by others, but only in a qualitative sense, as providing a basis for $Ca^{(+2)}$ ion displacement.

A detailed description of the conceptual basis as well as the analytical framework and examples of how this model can be tested are discussed in Sections II and III of this final report. A review of the scientific literature devoted to developing theoretical concepts to explain the coupling of radiofrequency electromagnetic fields to biological membranes is presented as Section IV of this report.

As most theoreticians will readily admit, most models are relatively short-lived. Our approach in the development of the model construct in this effort was to develop an analytical tool, based upon as few assumptions as possible, that could serve as a means of testing some of the rather speculative hypotheses concerning Ca^{+2} displacement from radiofrequency irradiated tissue. In doing so, we have already discovered certain factors, for example the probable importance of intramembranous hydrogen bonding at the membrane-ambient electrolyte interface, which should be included in describing such effects. We are in the process of incorporating this effect into our work.

There still exists disagreement within the bioelectromagnetics community as to whether or not the reported "alterations in Ca^{+2} efflux" really exist. Based upon our own efforts in this area, coupled with our understanding of the work presented by others, for example, Fröhlich, there is a strong theoretical basis for such effects to occur. It would be advantageous if additional experiments in this area were designed and performed, with special consideration being given to the development of a systematic experimental protocol that lends itself to analysis; for example, as based on our discussion in Sections II and III. Although a number of experimental reports can

be found in the literature on radiofrequency induced Ca^{+2} efflux, the data is not presented in a form that lends itself for comparison with models developed to describe such phenomena.

Section II
*MODEL DESCRIPTION**

I. Prefatory Remarks

This section contains a description of the conceptual basis for our model description of a possible viable mechanism governing the alterations in divalent cationic calcium, Ca^{+2}, efflux from cerebral tissue exposed to low intensity electromagnetic fields in the radiofrequency, RF, range. RF is used here in the generic sense to reference all frequencies less than 300 GHz. Herein we develop the mathematical formalism that serves as the basis of our analysis.[1,2]

II. Introduction

Two independent groups of investigators have reported that certain types of cerebral tissue, upon exposure to either low-intensity extremely low frequency (ELF) electromagnetic fields or amplitude modulated very high frequency (VHF) fields and ultra high frequency (UHF), will respond via a reduction or augmentation, respectively, in the efflux of divalent calcium ions (Ca^{+2}) from the tissue preparation.[3,4]

Ostensibly, the plasma membrane of the cell surface serves as the loci from which the Ca^{+2} flux evolves. A priori we shall assume that the surface of the plasma membrane in apposition to the extracellular fluid is the specific segment of the membrane substrate which, when coupled with an external electromagnetic perturbation, results in the observed alterations in Ca^{+2} efflux. This does not preclude the possibility of similar events occurring on the cytoplasmic side of the plasmalemma. We are, however, not considering in this analysis any mode of coupling across the transverse dimension of the membrane or Ca^{+2} ion trasmembrane transport. We focus here solely on the translocation of Ca^{+2} ions from the outer membrane surface to the extracellular pool, or, in other words, interfacial transport between the outer cell surface and its ambient electrolyte.

*A portion of this study was completed while the authors were employed by JAYCOR. The authors are presently employed at Science Applications, Inc., 1710 Goodridge Drive, McLean, Virginia 22102.

The negative surface charge of the intramembraneous protein inclusions as well as that of the various lipid moieties (these two chemical species collectively coexist in such fashion so as to form the structural basis for the fluid mosaic arrangement now generally accepted as the fundamental membrane macromolecular architecture) provides a natural substrate with which divalent cations can react. It has been postulated that the probable binding site for the calcium ions in question is the polyanionic sheet formed by membraneous glycoprotein inclusions. However, this has not been conclusively demonstrated experimentally, and we must remain cognizant of the fact that membrane phospholipids have a very high affinity for divalent calcium ions. We must keep in mind that an intact biological membrane is a very complex macromolecular configuration, the composite nature of which we simply cannot ignore. Suffice it to say at this point that we do not know the specific binding sites for calcium ions at the membrane surface.

The importance of Ca^{+2} ions in influencing and regulating a variety of physiological functions, ranging from the Ca^{+2} dependence of synaptic release of acetylcholine to the obligatory requirement for Ca^{+2} in the hormonal release by endocrine and neuroendocrine cells, definitely warrants an understanding of how low intensity RF radiation affects the binding of this ion to cerebral tissue.

Our modeling efforts, a part of which is presented in this communication, are aimed at attempting to elucidate and understand the mechanisms whereby an external electromagnetic perturbation precipitates the release and/or uptake of membrane bound Ca^{+2} . We have adopted the view that the Ca^{+2} release and/or uptake is secondary to the membrane being perturbed by an external field. The basis for this viewpoint will be discussed in section IV. The mechanism whereby the impinging field actually couples to the membrane proper, that is the primary system response, is not explicitly obtainable in this analysis. However, we shall demonstrate how the membrane-field coupling mechanism can possibly be understood in a semi-analytic sense through what we shall refer to as a scaling transformation. This, too, will be presented in section IV.

III. Membrane Surface Charge

The role and significance of the negative surface charge associated with membrane macromolecules has been discussed extensively by Trauble.[5,6] The interaction between a biological membrane and its extracellular and cytoplasmic environment is quite sensitive to the charge state of the membrane surface. The structural stability of membranes has been shown to be a sensitive function of the charge state;[5] an example of how this is made manifest is observed in shifts in the thermal phase transition temperature of lipid bilayer membranes where the charge state was altered by varying the pH and/or ionic strength of the electrolytic environment. The charge state of the membrane surface could equally as well be altered as a result of changes caused by an external perturbation, for example a time varying electric field, $\vec{E}(\omega,t)$. Such a structural reconfiguration could be viewed as a phase transition precipitated by the cooperative or collective interaction of some or all of the macromolecular membrane components. Depending on the specific nature or direction (in the sense of whether there is an increase or decrease in surface charge density) the membrane could serve as a sink or source of cations bound to its surface. It has been suggested that the coupling of low intensity RF fields occurs via induced or transient electric dipoles that subsequently lead to conformational changes.[7,8] There currently exists no explicit experimental data to indicate that such a redistribution of membrane surface charge accompanies the interaction of a weak time-varying electric field with biological tissue. The Ca^{+2} efflux data, however, certainly implicitly suggests that such a redistribution of membrane surface charge could be the basis for the observed alterations. Release of divalent calcium ions in lipid bilayers at the ordered-fluid phase transition has been well documented.[5] If indeed the perturbing influence of $\vec{E}(\omega,t)$ can be viewed as initiating a cooperative or collective membrane response, as suggested by Adey, Fröhlich, and Tenforde, then it is conceivable that the aforementioned variations in membrane structure lead to variations in surface charge density and thus regulate the movement of Ca^{+2} to and from the membrane surface. In Section V we develop the basic thermodynamic formalism associated with foregoing assumptions.

IV. Membrane Structure and Structural Changes

Before developing the basic thermodynamic formalism upon which our model analysis is predicated, we should like to briefly review the structure of functional biological membranes. The evolution of the fluid mosaic model, which is currently in vogue, was via thermodynamic arguments applicable in general to a macromolecular system immersed in an aqueous environment.[10] The lowest free energy state achievable for an intact membrane in an aqueous environment is obtained by sequestering the nonpolar amino acid residues of the protein, along with the fatty acid chains of the phospholipids, from contact with water, and maximizing the aqueous contact of the ionic polar groups of protein and lipid. The ratio by weight of protein to lipid ranges from 1.5 to 4 for most functional membranes.

The phospholipid molecules can be viewed as forming the structural matrix within which certain protein molecules are intercalated. Those specific proteins are generically referred to as integral proteins (they comprise approximately 70% of the total membrane protein) to distinguish them from the more loosely bound peripheral protein.

The lipid moieties are either acidic or zwitterionic at physiological pH. The protein, both integral and peripheral, normally are characterized by acidic groups and can be charged or strongly polarized. As we shall discuss later in this section the characteristics of the electrolytic environment in apposition with the membrane strongly influence the electrostatic state of the membrane surface, and while under physiological conditions only small variations in pH or ionic strength might occur, effecting changes in these variables externally under controlled experimental conditions permit us to utilize them as effective probes to examine membrane structure-function relationships.

It is interesting to note that, historically, protein played the favorite role in attempts to understand the functional dynamics of cellular metabolism and membrane phenomenology. Once the functional importance of the lipid component of membranes was recognized, most of the initial experimental effort was aimed at hydrophobic effects. However, it is now recognized that surface charge effects

associated with membrane phospholipids have been shown to profoundly effect enzymatic activity and influence the action of various hormones, vitamins, and drugs.[11]

There exists considerable evidence for the occurrence of thermal phase transitions in pure phospholipid membranes and intact biological membranes. At the transition temperature the following changes have been observed for a transition from the ordered to fluid state:

a) the formation of rotational isomers within the hydrocarbon chains;

b) the onset of rapid lateral diffusion of the lipid molecules;

c) an expansion of the bilayer structure.

Biological systems, however, are for the most part reasonably constant in temperature. Another possible "trigger" to initiate a phase transition might reside in the form of an external electromagnetic field provided that the field can couple to the membrane via some direct or indirect mechanism. Whatever the coupling mechanism might be, if indeed there is a phase transition-like event analogous to a thermal phase transition with concomitant structural changes, then the electrostatic interactions at the membrane surface must be considered. Although electrostatic interactions between surface polar groups do not alter the basic bilayer structure, they can influence the conformation, stability, and binding affinity of the component macromolecules.

V. Thermodynamic Formalism

The following thermodynamic arguments are based on previous analyses developed to account for the influence of electrostatic effects on the phase transition temperature of lipid bilayers[6] *and also structural transitions of charged biopolymers.*[14]

Assume that a time-varying electric field, $\vec{E}(\omega,t)$, *interacts with a cellular membrane so as to initiate a structural reconfiguration; structural reconfiguration is defined here as any change in membrane structure which leads to a change in the area occupied per charged macromolecular component. We may think of the membrane residing in a given state A prior to interacting with* $\vec{E}(\omega,t)$ *and in state B after interaction. We define f as the area per charged macromolecule; then*

$$\Delta f = f_B - f_A \quad . \tag{1}$$

The electrostatic molar Gibbs free energy may be written as

$$G^{el} = Lf\phi \quad , \tag{2}$$

where $L \equiv$ *Avogadro's number and* ϕ *is the surface density of electrostatic free energy of the membrane. We are using the molar electrostatic free energy here to characterize a given thermodynamic state. Since we are assuming the membrane to undergo a reversible two-state transition, we need an expression for* ΔG^{el}, *the difference in* G^{el} *between states A and B. We can write*

$$\Delta G^{el} = L\left(\phi + f\frac{d\phi}{df}\right)\Delta f \quad . \tag{3}$$

According to Equation (3) we need to obtain an explicit analytic expression for ϕ. In order to do so, we invoke the use of the Gouy-Chapman theory of the electrical double layer.[15] *Because of the inherent assumptions associated with the Gouy-Chapman theory, i.e., the mobile ions regarded as point charges and the surface charge distribution assumed to be uniform, we cannot expect good quantitative agreement with experiments, especially for specific binding phenomena. We use it here, along with the assumption of a 1:1 electrolyte, to develop the basic formalism. We point out, however, that Gouy-Chapman has been applied quite successfully to membrane phenomena even when only a small fraction of the membrane surface is ionized.*[5] *According to the Gouy-Chapman theory for a 1:1 electrolyte*

$$\psi_0 = \left(2\frac{kT}{e}\right) \sinh^{-1}\left[\left(\frac{2\pi e}{\epsilon kT}\right)\frac{\sigma}{\kappa}\right] \quad , \tag{4}$$

where $\psi_0 \equiv$ *membrane surface potential,* $\sigma \equiv$ *membrane surface charge density,* $k \equiv$ *Boltzmann's constant,* $T \equiv$ *absolute temperature,* $e \equiv$ *electron charge,* $\epsilon \equiv$ *dielectric constant, and*

$$\kappa = \left[\left(\frac{8\pi}{\epsilon}\right)\left(\frac{e^2}{kT}\right)n\right]^{1/2} \tag{5}$$

with $n \equiv$ *bulk salt concentration in molecules/cm*^{3}. *The electrostatic free energy density is given by*

$$\phi = \int_0^{\sigma} \psi_0\,(\sigma')\,d\sigma' \tag{6}$$

Substituting Equation (4) into Equation (6) yields

$$\phi = \sigma\psi_0 - \left(\epsilon/\pi\right)\left(\frac{kT}{e}\right)^2 \kappa \left\{\cosh\left(\frac{e\psi_0}{2kT}\right) - 1\right\} \tag{7}$$

If we define

$$\sigma = \frac{e\alpha}{f} \tag{8}$$

where $\alpha \equiv$ *degree of dissociation of a membrane macromolecule, then we can write*

$$\Delta G^{e\ell} = -\left(\frac{\varepsilon}{\pi}\right)\left(\frac{kT}{e}\right)^2 L\kappa\left\{\cosh\left(\frac{e\psi_o}{2kT}\right) - 1\right\}\Delta f \quad . \qquad (9)$$

We shall consider in this discussion only those cases where the high potential approximation is valid, i.e., $\frac{e\psi_o}{2kT} \geq 2$ *. In this case Equation (4) reduces to*

$$\psi_o = \left(2\,\frac{kT}{e}\right)\ln\left[\left(\frac{4\,e}{\varepsilon kT}\right)\kappa\sigma\right] \quad , \qquad (10)$$

and Equation (9) becomes

$$\Delta G^{e\ell} = -2\left(\frac{kT}{e}\right) L\sigma\Delta f + \left(\frac{\varepsilon}{\pi}\right)\left(\frac{kT}{e}\right)^2 L\kappa\Delta f \quad . \qquad (11)$$

Equation (11) provides us with a simple analytic expression for changes in the molar Gibbs free energy. This particular equation was used quite successfully to predict shifts in the thermal phase transition temperature, ΔT_t *, as a function of surface charge density (first term) and salt concentration (second term via* κ*).*[5] *That is,*

$$\Delta T_t = \frac{\Delta G^{e\ell}}{\Delta S^*} \quad , \qquad (12)$$

where ΔS^* ≡ *entropy difference between the two states for a neutral membrane. Small changes in pH, manifested via* σ *in Equation (11), as well as variation in salt concentration manifested via* κ *for a given pH, have been shown to induce phase transition at constant temperature.*[16] *As has been pointed out by Trauble, any parameter affecting the electrostatic free energy of the membrane can be expected to alter the phase transition temperature.*

Now, in our discussion we are attempting to understand a phenomena that has been characterized as a nonthermal effect. As indicated earlier a basic assumption in our thinking is that the external perturbation, $\vec{E}(\omega,t)$*, precipitates a phase transition which results in a change in the molecular area occupied by those membrane molecules that bear ionizable polar groups. We might then elicit shifts in* $\vec{E}(\omega,t)$ *by*

variations in those same parameters that alter T_t, e.g., σ and/or κ. Shifts in $\vec{E}(\omega,t,)$ would then be manifested by shifts in those power densities thus far observed to cause changes in Ca^{+2} efflux.

Consider the following example. Let $W \equiv$ energy absorbed by the tissue coupling to $\vec{E}(\omega,t)$. Define an "effective" temperature T_{eff} as follows:

$$T_{eff} = \frac{W}{k} \quad . \tag{13}$$

Then, combining Equations (11), (12), and (13) yields

$$\frac{\Delta W}{k} = -2\left(\frac{kT}{e}\right)\frac{L\sigma}{\Delta S^*}\Delta f + \left(\frac{\epsilon}{\pi}\right)\left(\frac{kT}{e}\right)^2\frac{L\kappa\Delta f}{\Delta S^*} \quad . \tag{14}$$

Equation (14) suggests that a typical experimental protocol would be to attempt to reproduce the Ca^{+2} efflux experiments by systematically varying σ for a given ionic strength, thus determining if and what the pH dependence is, and varying the ionic strength at high pH where the surface charge density is likely to be relatively constant since most of the charged moieties would be fully deprotonated. Such experiments could be conducted utilizing synthetic lipid bilayers, lipid micelles, reconstituted membrane systems using lipid and protein, as well those systems that have been used to date. We are currently developing an experimental protocol to conduct such studies with a group at the University of Texas.

Specifically though how do we account for the changes in Ca^{+2} efflux; Equation (14) only tells us that, if indeed there is a phase transition occurring, we should see shifts in the observed power densities at which these alterations occur.

The binding of Ca^{+2} to the membrane is of course a function of ψ_0 . If K is the apparent binding constant, then

$$K = K_0 \exp\left(2\frac{e\psi_e}{kT}\right) \quad , \tag{15}$$

where $K_0 \equiv$ binding constant for $\psi_0 = 0$.

Thus by causing a change in ψ_0 , we alter K . As we suggested earlier, one means whereby this could occur would be through membrane structural changes.

We introduce the following equation, only slightly modified, that was derived by Träuble to account for the release of Ca^{+2} from charged lipid bilayers as a result of membrane structural changes.[5]

$$\frac{\Delta[Ca^{+2}]}{[Ca^{+2}]} = \frac{4[t]}{[Ca^{+2}]\frac{(5-4q)}{q(1-q)} + [t]} \left(\frac{\Delta f}{f}\right) , \quad (16)$$

where $[Ca^{+2}]$ ≡ concentration of free calcium ions, $[t]$ ≡ concentration of singly ionized membrane macromolecules, q ≡ ratio of free binding sites to total binding sites for Ca^{+2}. Note the appearance of Δf , the change in areas occupied per membrane macromolecule. For $\Delta f > 0$, i.e., an ordered to fluid transition, equation (16) predicts a release of Ca^{+2}. For $\Delta f < 0$, i.e., a fluid to ordered transition, equation (16) then predicts an uptake of Ca^{+2}. The specific electrostatic effects are "buried" in the parameter q.

Granted, we must be cautious in taking the analogy too far, because equation (16) was derived to account for changes in $[Ca^{+2}]$ assuming the binding substrate was a lipid with singly ionizable charge groups. Thus far in our analysis we have treated equation (16) as an empirical relationship that possesses certain qualitative features of the Ca^{+2} efflux data to date. For instance if one plots $\Delta[Ca^{+2}]/[Ca^{+2}]$ as a function of q, for given values of the other parameters, you will note that the curve has the same shape as would an envelope drawn around the data as presented in Figure 1. The symmetry observed in the frequency domain can also be accounted for depending whether $\Delta f > 0$ or $\Delta f < 0$. We are currently trying to ferret out the specific functional dependence of q on the electrostatic characteristics of the charged membrane surface, and the manner in which $\vec{E}(\omega, t)$ might couple with q to provide an explanation for the discreteness of the observed frequency dependence.

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*Section III**

EXAMPLE OF MODEL APPLICATION AND RELEVANCE TO EXPERIMENT

The objective of this communication is to suggest a mechanism whereby divalent cations, specifically Ca^{+2}, could be displaced to or from the surface of a biological membrane subjected to a weak external electromagnetic perturbation in either the extremely low frequency (ELF) range or in the very high frequency (VHF) and ultra-high frequency ranges (UHF) that are amplitude modulated with ELF frequencies. Extant data now exists which suggests that the mechanisms responsible for such alterations in Ca^{+2} efflux from cerebral tissue reside in changes that occur at the neuronal membrane surface in apposition with the extracellular fluid (Bawin, Kaczmarek, & Adey, 1975; Bawin & Adey, 1976; Blackman, Benane, Elder, House, Lampe, & Faulk, 1979). A theoretical analysis of changes in Ca^{+2} binding to specific anionic membrane sites indicates that the transduction mechanism is likely nonthermal (Tenforde, 1980). The observations to date suggest that the external field couples with the membrane proper via some cooperative or collective interaction among the macromolecular components that comprise the plasmalemma. This point is especially appreciated when recognition is given to the fact that the estimated electric field strengths at the tissue level vary from $1 Vm^{-1}$ for the VHF-UHF exposures to $1 \times 10^{-5} Vm^{-1}$ for the ELF exposures (Adey, 1981).

We focus explicitly on the apparently anomalous changes in Ca^{+2} displacement from the membrane per se and not on the specific mode of coupling between the external field and the membrane with which it interacts. Assume that an external, time-varying electric field, $\vec{E}$, interacts with a biological membrane so as to precipitate a cooperative or collective interaction among the constituent molecules, perhaps analogous to a phase transition. Various specific mechanisms whereby this can be accomplished have been suggested (Fröhlich, 1980). Biological membranes possess a net negative surface charge, and we assume that subsequent to the field-membrane interaction there is a redistribution of this surface charge as a result of membrane structural changes. The structural changes could be associated with the lipid or protein moieties or both. The binding constant for divalent cations is very sensitive to the membrane surface potential, ψ^{o}, which is a function of the membrane surface charge density, σ. The importance of membrane surface charge effects on the binding of protons and divalent cations has been previously demonstrated (Träuble, 1976; Träuble, Teubner, Woolley, & Eibl, 1976; Woolley & Teubner, 1979). It has also been

**This section has been submitted to J. Theor. Biol. for publication.*

demonstrated that alterations in the membrane surface charge density can lead to either a destabilization or stabilization of the membrane depending upon whether the surface charge density increases or decreases respectively (Träuble et al., 1976; Jähnig, 1976).

One means whereby these stabilization-destabilization effects manifest themselves is through shifts in the thermal phase transition temperature, (Träuble et al., 1976). Shifts in the thermal transition temperature of certain lipid bilayers can be effected by altering the surface charge density via changes in pH and ionic strength, and an analytic form to describe such effects derived by Träuble et al. (1976), is given in equation (1).

$$\Delta T_t = -2 \frac{kT}{e} \frac{L}{\Delta S^*} \sigma \Delta f + \frac{\epsilon}{\pi} \left(\frac{kT}{e}\right)^2 \frac{L}{\Delta S^*} \kappa \Delta f, \qquad (1)$$

where $\Delta T_t \equiv$ shift in thermal phase transition temperature $\Delta S^* \equiv$ entropy difference between fluid and ordered states for an uncharged membrane, $\Delta f \equiv$ change in area occupied per molecule, $\kappa^{-1} \equiv$ debye length, $L \equiv$ Avogadro's number, $\kappa \equiv$ Boltzmann's constant $e \equiv$ electronic charge, $T \equiv$ absolute temperature, and σ we have previously defined. Equation (1) is an approximation based on the assumption of high surface potentials which is valid in most cases of physiological interest. The pH dependence is reflected in σ and the ionic strength dependence in κ.

We adopt the view that the external perturbation, $\vec{E}$, serves to trigger a response resulting in an alteration in membrane surface density, σ. Qualitatively an increase in σ would result in a reduction in Ca^{+2} displaced from the membrane, and conversely a decrease in σ would yield an enhancement of Ca^{+2} released from the membranes.

We define an effective temperature, T_{eff}, such that

$$T_{eff} = \frac{W}{k} \ , \qquad (2)$$

where $W \equiv$ energy absorbed by the membrane coupling to $\vec{E}$. Combining equations (1) and (2) yields

$$\frac{\Delta W}{k} = -2 \frac{kT}{e} \frac{L\sigma}{\Delta S^*} \Delta f + \frac{\varepsilon}{\pi} \left(\frac{kT}{e}\right)^2 \frac{L\kappa\Delta f}{\Delta S^*} \qquad (3)$$

Equation (3) suggests that a typical experimental protocol would be to look for shifts in the reported power density and/or frequency windows, at which alterations in Ca^{+2} have been reported, as a function of pH and as a function of ionic strength. Such experiments would provide insight into whether the loci of reported alterations in Ca^{+2} displacement is indeed the membrane surface. Experiments could be conducted utilizing synthetic lipid bilayers, liposomes and synaptosomes; Ca^{+2} displacement via low frequency amplitude modulated microwave fields have been reported in synaptosomal preparations (Lin-Liu & Adey, 1982).

Realistic values for the parameters and variables appearing on the right hand side of equation (3) can be obtained from the extensive literature on thermal phase transitions in membrane systems. For example, in a lipid bilayer system at a pH where the polar groups are completely deprotonated, we can write $\sigma = e/f$, where $f \equiv$ surface area occupied per lipid molecule. For low salt concentrations, the last term in equation (3) is negligibly small. These conditions reflect the constraints that would yield a maximum response. A reasonable estimate of ΔS^* would be 25 cal/deg/mole (Träuble et al., 1976). Thus for $T = 37^oC$, we can write

$$\Delta W = -407 \frac{\Delta f}{f} \frac{\text{joule}}{\text{mole}} . \qquad (4)$$

Typical values of $(\Delta f/f) = 0.25$ for lipids suffering a transition from the ordered to fluid state (Chapman, 1975; Marsh, 1974). Substitution into equation (4) gives $\Delta W = -102 \frac{\text{joule}}{\text{mole}}$. If we assume a molar volume of $1x10^{-3}$ m^3/mole for a lipid bilayer configuration, then $\Delta W = -1.02x10^5 \frac{\text{joule}}{m^3}$ Tenforde (1980) calculated a $W = 1.37x10^7 \frac{\text{joule}}{m^3}$ for the total absorbed energy for a given experiment. Thus

$$\frac{\Delta W}{W} \simeq 1\% . \qquad (5)$$

We recognize of course that W was calculated for an intact tissue preparation. The comparison must be viewed with that in mind; however, it should reflect within an order of magnitude the range of possible observable shifts in absorbed electromagnetic energy as a consequence of altering the charge state of a membrane preparation.

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Section IV
LITERATURE REVIEW

NONIONIZING ELECTROMAGNETIC FIELD INTERACTIONS WITH BIOLOGICAL MEMBRANES: A REVIEW OF ATHERMAL MECHANISMS

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ABSTRACT

The literature discussing the response of biological membranes to low intensity, time varying electromagnetic fields is reviewed. This review is restricted to what have been characteristically referred to as athermal effects. A critical analysis of theoretical work related to electromagnetic field effects at the level of biomembrane organization is presented. A very brief description of biomembrane structure serves as background information along with a discussion of the existence and nature of intrinsic field effects in biological membranes and the role such intrinsic fields play in governing the response to external field coupling. The emphasis is on reviewing works that have attempted to define coupling mechanisms between a weak external electromagnetic perturbation and a composite macromolecular structure such as a membrane. Certain methodologies and approaches that have proved fruitful in other areas of membrane science, e.g., excitable membrane phenomena, are discussed in the context of their applicability to studies that could conceivably provide new insight into establishing a physical basis for athermal effects.

I. INTRODUCTION

The purpose of this review is to examine that portion of the scientific literature that attempts to provide an understanding of how low intensity, nonionizing, time-varying electromagnetic fields interact with a certain class of biological systems. Specifically, we focus on what has in recent years become generically known as the radiofrequency domain, i.e., that portion of the electromagnetic spectrum with frequencies less than 300 GHz, and even more specifically we restrict our review to athermal phenomena.

Particular emphasis is placed on those reports that either directly or indirectly implicate the cellular membrane as the coupling substrate for an external field perturbation. The rationale for limiting the scope of this effort to membranes and membrane related phenomena is that there exists a number of papers in the literature suggesting that the membrane serves as the coupling agent responsible for athermal effects. Herein we provide a critical analysis of some of these works within the context of well established, documented properties of biological membranes. A very careful attempt is made to distinguish speculation from what is considered accepted scientific fact.

This review is critical in nature; however the criticisms should be interpreted as constructive with the aim of attempting to create a better understanding of coupling mechanisms.

There exists a number of published reviews far broader in scope than what we shall present herein. These reviews appear in the work of Adey (1981), Fröhlich (1980), and Taylor (1981).

II. BACKGROUND

The fact that electric fields play an important role in governing a variety of physiological processes has been well established (Katz, 1966). In fact, Newton, during the seventeenth century, viewed nerve propagation as a phenomenon whose basis resided in optical vibrations within a solid, transparent medium (Newton, 1952). The correlation between the nature of light and electromagnetic radiation had not yet been established, however. It was during the middle of the nineteenth century that duBois-Reymond conclusively demonstrated the existence of electric currents in both nerve and muscle behavior (Harmon and Lewis, 1966). Not until the twentieth century, however, was an idea advanced that established a connection between biological membranes and electric field effects.

The membrane hypothesis of Bernstein (1902) was significant in that it established a functional correlation between the plasma membrane of a nerve cell and the permeabilities of the various ionic species known to contribute to the resting potential across the membrane; primarily monovalent potassium ions.

In the early 1950s, the membrane hypothesis of Bernstein was modified by Hodgkin and Huxley in an effort to explain certain experimental observations on nerve cell behavior (Hodgkin, 1964). Their most significant contribution during this time was to construct an empirical model that accurately described the time dependence of the nerve action potential and the time dependence of the potassium and sodium currents during excitation. They hypothesized that charged particles, possibly electric dipoles within the membrane, are redistributed when the electric field across the membrane is altered, and that these particles govern the movement of various ionic species across the membrane. The Hodgkin-Huxley theory was phenomenological and did not provide a physicochemical basis of membrane excitation.

Although there now exists considerably more knowledge concerning membrane structure, and correlations between membrane structure and ionic permeability changes under a host of different conditions have been documented, there still does not exist a molecular theory explaining excitable membrane phenomena in the usual

sense where the application of statistical mechanics yields empirically observed and derived thermodynamic relationships, e.g., the Hodgkin-Huxley equations.

The foregoing discussion serves to illustrate that electric field effects, and the response of biological membranes to changes in the electric fields to which they are exposed, can have important physiological consequences, and that the membrane serves as a viable substrate to which internal fields can couple to regulate physiological processes. The nature of the fields in excitable membrane phenomena are, however, many orders of magnitude larger than the external electric field strengths responsible for eliciting athermal biological responses. The small field magnitude is the argument often used as the basis to preclude the direct action of an external electromagnetic field, e.g., causing the rotation of a polar molecule within the membrane matrix, as being a viable athermal means of membrane-field coupling (Fröhlich, 1982).

There exists an extensive literature dealing with cooperative phenomena in biological membranes per se and/or with certain macromolecular components that reside within the membrane complex (Hill, 1958, 1967; Changeux et al, 1967; Chernitskii et al, 1969; Engelman, 1970; Kijima and Kijima, 1978). In view of the improbability of direct field effects, dipole reorientation being an example, it has been suggested, and is reasonable to assume, that perhaps it is via a cooperative or collective mechanism that membranes respond to certain weak, time-varying electromagnetic fields (Tenforde, 1980; Fröhlich, 1980; Adey, 1975). We discuss these concepts in greater detail below.

Much of the ensuing discussion is devoted to the examination of various models and model systems that have been developed in attempts to characterize, in terms of physical and chemical mechanisms, the response of a biomembrane to weak electromagnetic fields. The role and utility of modeling is often misunderstood and thus a brief philosophical comment is in order here. Models have proved to be an important component in methods that have been successfully employed, primarily by physical scientists, as "deductively manipulative constructs essential to the evolution of theory from observation" (Harmon and Lewis, 1966). As such, they should serve as

supplements to experimentation in the sense of suggesting new experiments that would test predictions that should be an inherent part of the model, as well as possessing the capability of explaining existing experimental observations.

This review focuses solely on electric field effects and only on those reported effects that are associated with power densities of 10 mW/cm^2 or less.

1. Biomembrane Structure

The fluid mosaic model provides a structural framework, consistent with basic thermodynamic arguments, for the organization of the primary components of cell membranes in general (Singer and Nicolson, 1972). These primary components are protein and phospholipid. The phospholipid comprises a viscous fluid bilayer (Figure 3) that serves as the solvent in which proteins are imbedded. The arrangement of proteins is such that their charged and polar groups maintain contact with the aqueous environment, whereas the nonpolar groups reside within the hydrophobic core provided by the lipid bilayer (Figures 3,4,5,7,8). There exist also peripheral protein molecules that are weakly bound to the membrane and not strongly associated with the lipid component. It is generally thought that, of the protein moieties, only the integral proteins are important from the standpoint of contributing to membrane structural integrity. A point that is often overlooked, although it was explicitly discussed by Singer and Nicolson, is that a significant fraction of membrane phospholipid may not be in bilayer form; as much as thirty per cent might exist in a configuration state physically distinct from the bilayer configuration (Singer and Nicolson, 1972).

Assuming the validity of the fluid mosaic model, it is evident that there should exist lipid-protein interactions in membrane systems. In fact, in some specific membrane systems as much as forty-five percent of the total lipid content is thought to be juxtaposed with protein to form a lipid-protein interface. Presently, it is thought that there exist a wide range of protein-lipid interactions ranging from weakly associated protein-lipid complexes to cases where lipids are specifically bound to proteins and effectively immobilized (Jost and Griffith, 1980).

The structural features of membrane lipid and protein probably most relevant to this review are the charged and polar characteristics of these membrane constituents. Both macromolecular species are amphipathic in nature. The electrical asymmetry manifested via this amphipathic character is due largely to ionic amino acid residues and nonpolar residues in the case of proteins and to a variety of acidic polar head groups, each attached to two hydrocarbon tails, in the case of phospholipids (Tanford, 1973). The protein moieties may be multiply charged or possess a large dipole moment, of the order of hundreds of Debye units at physiological pH. The phospholipid head groups, under similar conditions, bear either a net negative charge or possess a dipole moment of the order of twenty to thirty Debye units. (For reference, the dipole moment of a water molecule is 1.8 Debye units).

A general composite picture of a biological membrane with both surfaces in apposition to an aqueous environment is then a structure approximately two macromolecules thick with each surface bearing a net negative charge. There is usually an asymmetry in the charge distribution between the two membrane surfaces, i.e., between the cytoplasmic and extracellular surfaces. Distributed throughout this composite lipid-protein structure are protein molecules that can possess very large electric dipole moments, as much as several orders of magnitude larger than a water molecule. Certain lipid components, too, can possess permanent dipole moments approximately one order of magnitude larger than that of a water molecule.

The foregoing discussion presents only the basic morphological features of biomembranes in a very general way. There exists phenomena which cannot be accounted for in terms of such a mosaic arrangement, and, in all probability, the fluid mosaic model will need to be modified and refined before being regarded as an accepted unified theory of biological membranes (Tanford, 1973).

2. Intrinsic Electromagnetic Fields Within Biological Systems

Phenomena such as the generation and propagation of nerve impulses, the neurostimulated secretion of hormones, muscle contraction, and memory recording are but a few of the many cellular processes where internal electric field effects are vital

to physiological function. Magnetic effects are apparently of less importance, especially effects due to coupling with external fields (Schwan and Foster, 1980), and are not addressed in this review.

In attempts to delineate mechanisms of coupling of an external electric field to a biological system, e.g., a membrane and its electrolytic environment, and the subsequent or concomitant response, careful attention should be paid to the nature and role of intrinsic fields within the system of interest. Intrinsic fields refer here to chemical bonding, electrostatic interactions, as well those internal electric potential gradients established by the asymmetric distribution of various ionic species, e.g., across a nerve cell membrane. There still exists much uncertainty, however, regarding the nature of many of the interactions within composite macromolecular systems; the nature of lipid-protein interactions in biomembranes being a prime example. Cognizance of the magnitude of long range van der Waals' interactions, consisting of electrostatic, induction, and dispersion components, are of primary interest in examining the structural and functional integrity of systems subjected to external perturbations (Watts and McGee, 1976).

Of no less importance, especially for amphiphilic macromolecular systems such as membranes, are hydrophobic interactions. The origin of the hydrophobic effect does not reside in van der Waals' interactions; in fact van der Waals' interactions are of minor significance in the overall hydrophobic effect. Hydrophobic interactions stem from the strong attractive forces between water molecules which are disrupted by the addition of another molecular species (Tanford, 1973).

Hydrogen bonding is another important consideration in understanding the structure and properties of water in biological systems, in accounting for the stability of helixes formed by polypeptides, and in determining the physical state of various membrane systems. Intrafacial hydrogen bonds, e.g., along the plane of the membrane surface, can make a significant contribution to the overall surface free energy. Hydrogen bonds between macromolecular structures, e.g., within a cellular membrane, have often been neglected because they have either been deemed insignificant relative to hydrogen bonds to water molecules or they have been difficult to detect. Recently,

however, their importance has been demonstrated in their ability to influence the thermal phase transition temperature in certain membrane systems (Eibl and Woolley, 1979; Toko and Yamafuji, 1981).

3. *Cooperative Effects*

The magnitude of the electric field encountered in initiating athermal responses apparently dictates that the nature of the coupling with a given system be other than via a direct action of the field. In view of this fact, possible cooperative modes of interactions among the components of a macromolecular composite, such as a membrane, are likely candidates for consideration as coupling mechanisms.

Cooperative phenomena refers here to those molecular processes that result in physical changes in the structure of matter which are associated with a definite value of an intensive thermodynamic variable, for example the absolute temperature or electric field strength. The idea of cooperative behavior is related to the fact that transitions between different states of matter cannot be understood in terms of individual molecular components; interactions among the components must be taken into account.

The possibility of cooperative effects in membranes has been discussed extensively (Hill, 1967; Changeux et al, 1967; Blumenthal et al, 1970). These effects stem from changes in membrane structure triggered by changes in the concentration of bound ligands as well as cooperative responses triggered by an electric field.

III. *MEMBRANE - FIELD COUPLING MECHANISMS*

We examine in this section those works in which concepts have been proposed to explain how an external electromagnetic field could conceivably couple to a biological membrane in a nonthermal manner.

1. *Collective Phenomena*

The notion of collective phenomena refers to the fact that certain properties of an assembly of particles, however large or small an assembly, cannot be explained solely in terms of the properties of the individual component particles, but can only be explained in terms of properties of the system of particles as a whole. In a general sense, collective phenomena is synonymous with cooperative phenomena in that interactions among the constituent parts of a given system must be considered.

The most concerted effort to date that invokes the concept of collective phenomena as a vehicle whereby radiofrequency radiation can couple to a biological system, with specific reference to membranes, is that of H. Fröhlich (1980, 1982). The ideas concerning the coupling of external electromagnetic fields to biological systems, membranes in particular, stem from his earlier thoughts on metabolically induced coherent electric vibrations and also the existence of highly polarized metastable states within such systems (Fröhlich 1968 a, b, 1977).

Specific importance is given to the role of the large electric fields, of the order of $1x10^7$ V/m, encountered in cell membranes. The presence of such large field strengths could indeed lead to highly polarized states within the membrane. We should like to point out here that a variety of cells, not just nerve cells, as has been stated by Fröhlich (1977) and also by Taylor (1981), effectively utilize the plasma membrane potential as a means of controlling their physiological function. Examples are nerve and muscle membranes in regulating ionic permeabilities; the release of neurotransmitters by presynaptic membranes; the control of contractile processes in muscle membrane; the secretion of insulin by pancreatic cells; and possibly the secretion of adrenalin by adrenal medulla cells (Almers, 1978).

The general approach adopted by Fröhlich in one of his models, namely the vibrational model, is based upon the assumption of electric dipole oscillations of units or subunits of a biological system; e.g., the system might conceivably be a biomembrane, and the units correspond to specific polar segments of the membrane, capable of oscillating with a frequency, ω_o. Interactions among the oscillators via Coulombic forces can then produce a branch of longitudinal modes in a narrow frequency range such that

$$0 < \omega_1 \leq \omega \leq \omega_2 \qquad (1)$$

where ω might be significantly different from ω_o. These modes correspond to the establishment of longitudinal electric waves within the given systems.

The precise nature of such oscillating dipoles in biological membranes is purely speculative, as there exists no extant data to support such an hypothesis. However, it is conceivable that membrane segments consisting of integral protein could sustain such oscillations. In view of the dielectric properties and the hydrophobic character of the hydrocarbon core associated with the lipid matrix, it is not likely that such oscillations could be sustained by the lipid moieties, at least not in the sense suggested by Fröhlich (1968b).

Starting with an assumed form for a rate equation that governs the time rate of exchange of energy quanta with a heat bath in which the dipole oscillators are immersed, and considering only first and second order interactions with the heat bath, Fröhlich is able to show that the stationary state solutions for such a system can result in a phase transition analogous to Bose condensation. Such a transition is manifested when the rate of energy supplied to the longitudinal electric modes exceeds a critical value, and subsequently all energy is channelled into a single mode having the lowest frequency. Externally applied electromagnetic energy could serve to trigger such an event. Fröhlich proposes that such electric vibrations in the $10^{11} - 10^{12}$ sec^{-1} could be excited; these estimates are based upon a membrane of thickness $1x10^{-8}$ m capable of accommodating an acoustic velocity on the order of $10^3 - 10^4$ m/sec, all reasonable estimates consistent with membrane mechanical properties.

The prediction of the existence of a phenomenon analogous to a Bose-Einstein condensation, as in Fröhlich's vibrational model, has been confirmed via several other approaches; namely via a transport theory formalism by Kaiser (1979) and a molecular Hamiltonian approach by Bhaumik et al. (1976), and also by Wu and Austin (1977, 1978); the basic concept has been shown to be on firm theoretical ground. The difficulty in these ideas gaining wide acceptance is the lack of conclusive experimental evidence to provide confirmation that such effects occur in biological

membranes. In fact, as mentioned earlier, there exists no direct experimental evidence that biological membranes comprise a biosystem that can sustain such coherent oscillations. A number of experiments have been cited by Fröhlich as providing evidence for the existence of coherent modes in biological systems (Fröhlich, 1980); however, none implicate or reflect the involvement of biomembranes. It has been suggested that a possible explanation of the alterations in Ca^{+2} efflux precipitated by either extremely low frequency (ELF) or amplitude modulated very high frequency (VHF) and ultra high frequency (UHF) fields resides in the possibility to coherently excite enzymes imbedded within a membrane (Fröhlich, 1980). However, here again there is no experimental evidence to support such a supposition, and the theory presented thus far is not at the state in which it can be utilized in an analytical sense to explain those data thus far reported or be used in a predictive manner.

It would indeed be diffcult to incorporate detailed microstructural properties of membranes into Fröhlich's vibrational model, as he points out. However, examination of how variations of certain macroscopic characteristics of a given membrane system might modulate the proposed states of coherent excitation could be conducted. This might be accomplished empirically by systematically investigating the effects of ambient pH and ionic strength on metabolically intact membrane systems, since metabolic input is a necessary ingredient in this model. For example, modulation as a consequence of altering the membrane surface charge density could conceivably be manifested in a variation in the rate of energy supply or exposure time necessary to trigger an athermal response.

The general concept of the excitation of coherent oscillations in biological systems, with limited reference to membranes, has been nicely reviewed by Kaiser (Kaiser, 1978 a, b, 1981, 1982). A discussion of Fröhlich's model is presented in terms of how it possesses the requisite characteristics to undergo various types of phase transitions subsequent to external perturbations. Reference to the specific involvement of biological membranes in such phase transitions is only briefly mentioned. The emphasis in Kaiser's analysis of Fröhlich's basic hypothesis is on the idea that stable limit cycle behavior serves as an adequate description for coherent oscillations in biosystems, and that such limit cycles can be driven by external fields.

This concept provides a basis of explanation for the coupling of weak external fields to biological tissue. For example, the inherent limit cycle behavior can serve as a means of storing metabolic energy provided by the system, and weak external perturbations, e.g., an electromagnetic field, can ultimately result in limit cycle collapse.

Another model proposed by Fröhlich, and extended by Kaiser, is referred to as the high-polarization model (Fröhlich, 1980, 1977; Wu and Austin, 1977; Kaiser, 1978 b, 1977). This idea is predicated on the establishment of metastable strongly polarized states. The fact that biological membranes can sustain a high degree of electric polarization, for example, protein imbedded within the membrane matrix can conceivably reside in states of high polarization, allow for membranes to serve as a possible biological complex in which such a state could be realized. According to Fröhlich and Bhaumik et al., the strong excitation of polar modes results in the deformation of the given system, and thus the activation of elastic constraints. Such a response can lead to polarization mode softening and the establishment of a ferro-electric state (Fröhlich, 1980, 1969; Bhaumik et al, 1977). Bhaumik et al. have shown that the energy threshold required to initiate the Bose-condensation-like phenomenon discussed previsouly is actually lowered when elastic deformations are considered (Bhaumik et al, 1977). No discussion of an experimental protocol to look for such effects is provided.

The relevance of the models proposed by Fröhlich and extended by Kaiser, Bhaumik et al., and Wu and Austin, to biological membranes as being the coupling substrate for external radiofrequency fields resides in the fact that

(1) membranes can be considered as open systems typically found in non-equilibrium steady states;

(2) membranes possess the macromolecular components, as well as the appropriate ambient electrolytic environment, compatible and consistent with the basic tenets of both models;

(3) *membranes can accommodate the requirement of both models for energy input via the utilization of metabolic energy produced by the cell;*

(4) *membranes can be viewed as composite macromolecular systems capable of supporting collective or cooperative interactions that can be triggered via various stimuli; low intensity external radiofrequency being only one example.*

At the risk of overstatement, we should like to again mention the lack of experimental evidence to support Fröhlich's hypotheses as related to the specific role of membranes. There has been some attempt to correlate the reported alterations in divalent calcium ion efflux from cerebral tissue as being evidence to support Fröhlich's hypotheses as they might relate to influencing enzymatic activity of protein within the membrane; however, given the complexity of the system studied, the results reported do not provide definitive evidence of such a correlation (Fröhlich, 1980; Adey, 1981).

A model that invokes the concept of cooperative behavior and simultaneously incorporates to some extent membrane structure has been formulated by Grodsky (1975, 1976, 1977). This model is based upon a type of lattice statistics formulation in which the polar head groups of membrane lipid moieties occupy one set of lattice sites, and displaced normal to the plane of this lattice is another lattice configuration corresponding to cationic binding sites associated with membrane glycoprotein residues.

A Hamiltonian is developed describing the interaction among neighboring sites within a given lattice, interactions between the two lattices, and interactions of both lattices with an external field. The model represents an attempt to incorporate basic membrane physical and chemical characteristics, for example dipole-dipole coupling, charge-dipole coupling, and ligand binding into a framework that is not purely phenomenological. Conceptually, it is similar to earlier attempts to examine the role and importance of electric dipoles in governing transmembrane transport in excitable membrane phenomena (Ward and Bond, 1971; Almeida et al, 1974; Van Lamsweerde-Gallez and Meessen, 1975).

The formalism used by Grodsky is analogous to that developed to describe ferromagnetic and antiferromagnetic systems and phase transitions in such systems (Huang, 1963).

It has been pointed out that the constraint of spatial symmetry inherent in Grodsky's model is too severe to serve as a viable membrane model (Adey, 1981). This simplification was introduced, however, to provide a means of developing an analytical model based on certain structural characteristics and properties of biomembranes at the microscopic level, and the approach via lattice statistics is an acceptable one.

The assumption of the various configurations of the polar head groups of the phospholipid molecules is questionable in view of the stereochemical constraints associated with a given polar head group and the presence of intramembrane hydrogen bonding along the plane of the membrane between adjacent lipids. It is true that the polar head groups of the phospholipid molecules within the membrane are to some degree flexible; however, in view of the aforementioned constraints it is difficult to imagine, for instance, an antiparallel configuration as postulated by Grodsky. Attempts to employ similar lattice statistic arguments based on cooperative dipole-dipole coupling among phospholipid head groups to account for changes in membrane permeability in excitable membranes have not been successful (Van Lamsweerde-Gallez and Meessen, 1975). The general concept of a critical value of an externally applied electric field precipitating a phase transition-like event has been suggested as a possible mechanism to account for various other membrane phenomena and is certainly worthy of further consideration (Hill, 1967; Changeux et al, 1967; Ward and Bond, 1971; Almeida et al, 1974; Träuble, 1974, 1976).

One major conceptual difference between Grodsky's model and those proposed by Fröhlich is the role of metabolism; Grodsky's model does not require metabolic input. Grodsky postulates the existence of resonance behavior with the inherent frequencies of the coupling structures, and suggests, too, that the rectification of time-varying electromagnetic perturbations could be accommodated by the cationic lattice in his model behaving as a natural diode. These suggestions are highly speculative, and there is no experimental evidence to support them.

Perhaps it is appropriate to interject here that many of the attempts at modeling the effects of electromagnetic fields on biological systems have been, to put it mildly, highly conjectural. An attempt to justify such a conjectural approach has been discussed by Fröhlich (1980). Although we do not disagree entirely with Fröhlich's rationale, it is apparent that there should be a more conscious effort to correlate theory and experiment, especially in the area of membrane effects. Membranes are seemingly in vogue as vehicles to explain electromagnetic effects at the microscopic level. While membranes are certainly viable substrates for such interactions, a greater responsibility should be exercised in attempting to construct models of such phenomena.

Grodsky does suggest many interesting analogies between known processes in various physical systems; for example, the physics of solids, and how similar processes might exist in electromagnetic field-membrane interactions. However, these ideas have not been carefully developed within an analytical framework appropriate to correlate with membrane properties.

Adey, Bawin, and Sheppard, in a number of publications, have discussed the rationale behind invoking cooperativity as the means of explaining weak field coupling to membranes (Adey, 1975, 1977, 1979a, b, 1980; Bawin et al, 1978; Bawin and Adey, 1976a, b). There is no rigorous attempt in these works at constructing a specific model, at least in the sense of an analytical model, that would describe membrane-field coupling.

Lawrence and Adey have presented a model of membrane-field interactions based upon the possible establishment of solitary wave motion, solitons, within the membrane complex (Lawrence and Adey, 1982). While there exists no clear-cut evidence of soliton formation in cell membranes, it is conceivable that membranes could support the propagation of such solitary waves via nonlinear coupling among the constitutent molecules. Fröhlich has previously suggested the possibility of soliton excitation in biological systems in connection with his high polarization model (Fröhlich, 1980). The basis of the Lawrence-Adey model is Davydov's (1979) work on soliton formation in biological systems coupled with work by Vaccaro and Green and

Triffet and Green in which they model the current and conductance changes in excitable membranes in terms of nonlinear plasma oscillations (Vaccaro and Green, 1979; Triffet and Green, 1980). Here again many interesting concepts are discussed, and a number of analytical relationships are presented which rest heavily on the work of Vacarro, Green, and Triffet as well as Davydov, along with the aid of many simplifying assumptions. The approach, in our opinion, is too global. No comparison with experimental data was presented. It would be of real interest and a valuable contribution to the literature if some of the interesting notions presented by Lawrence and Adey were developed more completely with the aim of making meaningful comparisons with experimental data.

2. Phenomenological Models

Cain has presented a phenomenological description of the effects of radiofrequency fields on excitable membranes by examining the effects of a time varying potential, V(t), on the voltage-dependent rate constants, α and β as originially employed by Hodgkin and Huxley to account for ionic conductance changes in squid axon membranes (Cain, 1980). Assuming V(t) of the form

$$V(t) = V_o + V_m \cos \omega t, \tag{2}$$

where $V_o \equiv$ membrane resting potential, $V_m \equiv$ amplitude of applied potential, $\omega \equiv$ angular frequency, and, $t \equiv$ time, a derivation of the corresponding αs and βs is presented, and the sodium and potassium conductance calculated via the Hodgkin-Huxley formalism. Although such an approach does not explicitly yield information regarding molecular mechanisms at the membrane level, it is informative in the sense that it does predict how the ionic conductances of the Hodgkin-Huxley theory should vary according to such a time-varying perturbation, and thus could be tested experimentally. Indirectly such approuches can provide insight into possible mechanisms in the same manner in which numerous modifications of Hodgkin-Huxley phenomenology have provided insight into possible molecular mechanisms associated with membrane excitation. Cain hypothesizes that the presence of an oscillating field in an excitable biological membrane can lead to conductance changes through an

alteration of the state distribution of gating particles within the membrane (Cain, 1981a, b). Although there is no experimental evidence of such, it would be interesting to examine experimentally the effects of time varying fields on gating currents per se.

The suggestion by Cain that the effect occurs through an actual dipolar reorientation would require unusually large field strengths or extremely large dipole moments. The prediction of an inhibitory effect due to membrane hyperpolarization because of increased steady-state potassium conductance concomitant with decreased steady-state sodium conductance certainly should merit enough attention to design experiments to test the existence of such a response.

Barnes and Hu (1977, 1980) examined the effect of a time varying field on the concentration profile of ionic species adjacent to either surface of the plasmamembrane. The concentration profile was described by a Boltzmann distribution and the potential associated with the impressed field was described by an expression of the form of equation (2). Estimates of the field strength within the membrane as a function of the dielectric constants and resistivities of the membrane and its aqueous environment and calculated values for the field strength within the aqueous surroundings were obtained via an equivalent circuit analysis. Numerical estimates of the shift in the ionic concentration profile for an incident power density of 10 mW/cm^2, which yields a 9μV potential drop across the membrane, are of the order of one part in 10^6, and an associated transmembrane ionic current density of $6x10^{-11}$ amp/cm^2, according to the model. We do not discuss the possible specific biological implications of such effects here, other than to state that alterations in the distribution of the concentration of ions adjacent to either membrane surface could influence a number of surface-specific membrane processes.

The above model of Barnes and Hu does not address directly the question of how very low intensity electromagnetic fields can couple to a membrane. Their suggestion of the orientation of polar molecules due to the torque they experience in the presence of an external field would not be a viable mechanism for field strengths associated with nonthermal phenomena.

An approach somewhat analogous to that of Barnes and Hu was adopted by Pickard and Rosenbaum (1978). They examined the effects of an impressed RF field on the resting potential across the plasmamembrane and the effects of such fields with regard to ion transit time within an ion channel spanning the membrane. Of interest is their conclusion that the frequency range at which transit time effects are no longer important lies well below the microwave portion of the spectrum. This estimate was based upon data for the sodium ion current in the squid axon membrane.

Pickard and Rosenbaum also presented some rough calculations to determine the frequencies at which electromechanical resonance effects between an impinging field and the displacment of so-called gating particles within excitable membranes might be important. Their estimates fall within the microwave range. However, the models they used to represent the "ion gates" are, as they point out, extremely crude, and their results should at best be considered only as an order of magnitude estimate. The concept of gating particle resonance with an applied field is an interesting one, and in principal could be studied experimentally by looking for alterations in gating currents under various exposure conditions.

While the modeling efforts of Barnes and Hu, Cain, Pickard and Rosenbaum are not likely to reveal the precise nature of athermal coupling between field and membrane, they are useful in that correlations can conceivably be made, in the case of excitable membranes, between possible changes in membrane permeability to various ionic species, effects on gating currents, displacement of the resting potential, etc., to the frequency and intensity of the applied radiation as well as to the duration of exposure.

Nazarea (in Broucke et al, 1981) has adopted an interesting approach to studying electromagnetic field effects on ionic conductance through biological membranes by examining the noise spectra associated with transmembrane conductance. Analysis of electrical noise spectra in general has provided valuable insight into conductance mechanisms in the excitable membranes of nerve and muscle (Stevens, 1972; Chen, 1976; Verveen and DeFelice, 1974).

The details of Nazarea's calculations are not presented in the one brief report to which we had access (Broucke et al, 1981). The fluid-mosaic model serves as the structural basis for his analysis with the assumption that a conformational change in transport proteins alters the conductance properties of these proteins. Furthermore, it is assumed that this conformational change is initiated by a perturbation of the orientational order of the phospholipid matrix in which these proteins are imbedded, and that fluctuations in the conductance has an autocorrelation function proportional to the autocorrelation of the phospholipid order parameter. The time-varying field perturbation is assumed to couple only to first order to fluctuations of the dynamic parameter that characterizes the phospholipid orientational order. Nazarea is able to show that a field perturbation can indeed alter membrane properties such that variations in the conductance noise spectra can be expected. Unfortunately, in the one report known to us, no specific calculations or comparisons with experimental data were presented. This technique, however, could in principle be a very powerful tool for elucidating the coupling mechanisms between field and membrane.

Conclusion

Having conducted a comprehensive review of the open literature concerning the possible mechanisms of nonthermal interaction of nonionizing electromagnetic fields with biological membranes, it becomes readily apparent that this is an area in which there is a significant need for theory and/or modeling. As evidenced by many of the papers discussed in this review, there exists a great deal of speculation in a number of modeling efforts thus far developed. This is certainly not necessarily bad as long as it can provide insight for the development of new experimental protocols or serve as a basis for the development of more rigorous theoretical treatments that can be realistically compared to experiment. Conjectural treatments that provide new insight for new experimental and/or theoretical approaches and that are inconsistent with existing data quite obviously serve no useful purpose.

The ideas that have been advanced by Fröhlich are based on sound theoretical principles even though they, too, as Fröhlich himself states, are somewhat speculative. It would be of considerable value if his ideas were examined more closely in an effort to devise additional experiments that would serve to test these concepts.

There exists a tremendous literature on cooperative or collective phenomena in physics, chemistry, biophysics, and biochemistry. As discussed in the text of this review, this appears to be the vehicle by which many of the observations that implicate an athermal membrane response will be explained, and therefore more theoretical effort in this area would be desirable.

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Section V

PUBLICATIONS, PRESENTATIONS, INVITED PAPERS RELATED TO THIS CONTRACT EFFORT

1. *Bond, James D., Carol A. Jordan, Silverio P. Almeida, and Joseph F. Soukup, "An Analysis of Changes in Ca^{+2} Binding to Cerebral Tissue Concomitant with Exposure to Low Intensity RF Electromagnetic Fields," Bioelectromagnetics Abstracts, p. 31 (1982); presentation made at 4th Annual Scientific Session of the Bioelectromagnetics Society, Los Angeles, California (1982).*

2. *Bond, James D., and Carol A. Jordan, "Electrostatic Influences on Electromagnetically Induced Displacement of Divalent Calcium Ions from the Nerve Cell Surface," submitted to J. Theor. Biol., November 16, 1982.*

3. *Bond, James D., "A Quasi-Thermodynamic Model of Ca^{+2} Efflux from Cerebral Tissue Exposed to Low Intensity RF Electromagnetic Fields," a seminar presented at the USAF School of Aerospace Medicine, Radiation Sciences Division, Brooks, AFB, San Antonio, Texas, February 4, 1982.*

4. *Bond, James D., "A Physicochemical Basis for Alterations in Ca^{+2} Efflux from Cerebral Tissue Exposed to Low Intensity Electromagnetic Fields," presented as part of the Office of Naval Research Seminar Series on the Biological Effects of Electromagnetic Fields, March 2, 1982.*

5. *Bond, James D., and Carol A. Jordan, "Electrostatic Influences on Electromagnetically Induced Calcium Ion Displacement from the Cell Surface," to be presented at the International Symposium on Bioelectrochemistry, Stuttgart, FRG, July 18-22, 1983.*

6. *Bond, James D., "Electrostatic Modulation of Electromagnetically Induced Cation Displacement from Cell Membranes," an invited paper to be presented at the 1983 American Chemical Society symposium on "Bioelectrochemistry: Ions, Surfaces, Membranes," August 28-September 2, 1983.*

Section VI

OUTSIDE RECOGNITION

College of Physicians & Surgeons of Columbia University | *New York, N. Y. 10032*

DEPARTMENT OF PHYSIOLOGY

630 West 168th Street

December 13, 1982

Dr. James D. Bond
Science Applications, Inc.
1710 Goodridge Drive
P.O. Box 1303
McLean, Virginia 22102

Re: American Chemical Society Meeting
Washington, D.C. Aug 28-Sept 2, 1983

Dear Dr. Bond:

The Colloid and Surface Chemistry Division of the A.C.S. has regular symposia in the series "Surface Chemistry in Biology and Medicine", and in 1976 we had a successful meeting on Bioelectrochemistry. The papers were publisned in the Advances in Chemistry Series (Vol. 188), and the book had favorable reviews. There has been considerable progress in this area since the last meeting, and I am organizing a symposium under the title "Bioelectrochemistry: Ions, Surfaces, Membranes" for the ACS meeting next summer. There is a chance that we may publish the papers presented at the symposium like the last time, but we have to explore this possibility with the authors and the publisher.

As the title of the symposium implies, we shall cover a relatively broad range of phenomena involving electrochemical effects at biological surfaces, e.g. ion transport, redox processes, adsorption, effects of electric fields on structure. In view of your work in this area, I invite you to contribute to this symposium.

I hope that you will be able to participate, and that you will send me the title of your paper. I look forward to hearing from you within the next few weeks.

Sincerely yours,

Martin Blank

Martin Blank, Ph.D.
Associate Professor of Physiology
ACS Symposium Chairman

at
Enclosure

P.S. I am enclosing a copy of my Abstract for the Electrochemical Society Meeting this May.

THE MOUNT SINAI MEDICAL CENTER
ONE GUSTAVE L. LEVY PLACE • NEW YORK, N.Y. 10029

OF THE CITY UNIVERSITY OF NEW YORK

Mount Sinai School of Medicine • The Mount Sinai Hospital

Department of Orthopaedics

December 15, 1982

Dr. James D. Bond
Science Applications, Inc.
1710 Goodrich Drive
McLean, VA 22102

Dear Dr. Bond:

I am co-organizing a symposium for the Electrochemical Society entitled "Ions and the Electrochemical Control of Cell Functions" to be held in San Francisco, California, May 8-13, 1983. Your work is extremely pertinent to the multidisciplinary nature of this symposium, and I am honored to invite you to give a presentation in San Francisco.

The Electrochemical Society requires a short abstract which is published in the Journal (and is referenceable), and an extended abstract which is published separately and available approximately 6 weeks before the meeting. The enclosed material will provide more detailed information.

I sincerely hope that you will be able to accept this invitation.

Sincerely yours,

Arthur A. Pilla

Arthur A. Pilla
Professor
Director, Bioelectrochemistry
Laboratory

AAP/dv
enclosure

College of Physicians & Surgeons of Columbia University | New York, N.Y. 10032

DEPARTMENT OF ORTHOPEDIC SURGERY 630 West 168th Street

June 2, 1982

Dr. James D. Bond
JAYCOR
205 S. Whiting Street
Alexandria, Virginia 22304

Dear Dr. Bond:

Thank you very much for your letter of May 26, 1982 and for the preprint of the article entitled "A PHYSICOCHEMICAL BASIS FOR ALTERATION IN Ca^{+2} EFFLUX FROM CEREBRAL TISSUE EXPOSED TO LOW INTENSITY ELECTROMAGNETIC FIELDS". I've read this with considerable interest and have found a number of areas of intellectual "resonance". I am enclosing a recent reprint which you may find of some interest. The potential mechanisms of action of these varying types of time-varying electromagnetic fields continue to occupy a considerable amount of time and effort by our group and we are beginning to establish some of the principles which may account for selected biological responses in the laboratory and clinic. Certainly, calcium plays a central role in much of this work. We would be interested in maintaining very close contact with your group as it goes forward with what appears to be extremely interesting and potentially productive endeavors. Do you plan to publish the paper which was presented at the L and R Research Seminar? Is the material quoteable? If so, how should it be cited? With best wishes and many thanks.

Sincerely yours,

C. Andrew L. Bassett, M.D.
Professor of Orthopaedic Surgery
Director of Orthopaedic Research Laboratories

CALB:jk

THE MOUNT SINAI MEDICAL CENTER
ONE GUSTAVE L. LEVY PLACE • NEW YORK, N.Y. 10029

Mount Sinai School of Medicine • The Mount Sinai Hospital

Department of Orthopedics

December 15, 1982

Dr. Carol Jordan
Science Applications, Inc.
1710 Goodrich Drive
McLean, VA 22102

Dear Carol:

I am co-organizing a symposium for the Electrochemical Society entitled "Ions and the Electrochemical Control of Cell Functions" to be held in San Francisco, California, May 8-13, 1983. Your work is extremely pertinent to the multidisciplinary nature of this symposium, and I am honored to invite you to give a presentation in San Francisco.

The Electrochemical Society requires a short abstract which is published in the Journal (and is referenceable), and an extended abstract which is published separately and available approximately 6 weeks before the meeting. The enclosed material will provide more detailed information.

I sincerely hope that you will be able to accept this invitation.

Sincerely yours,

Arthur A. Pilla/dv

Arthur A. Pilla
Professor
Director, Bioelectrochemistry
Laboratory

AAP/dv
enclosure

P.S. Carol, you might want to use the text in the call for papers in my symposium for the BEMS Newsletter.

Paper Presented at the 4th Annual Scientific Session of the Bioelectromagnetics Society, June 28 - July 2, 1982, Los Angeles, California

AN ANALYSIS OF CHANGES IN Ca^{+2} BINDING TO CEREBRAL TISSUE CONCOMITANT WITH EXPOSURE TO LOW INTENSITY RF ELECTROMAGNETIC FIELDS. James DeHihns Bond, Carol A. Jordan, Silverio P. Almeida*, and Joseph F. Soukup. Health and Environment Division, JAYCOR, Alexandria, VA 22304.

We present a theoretical model based on classical electrochemistry that qualitatively accounts for certain features of the observations to date on the alterations of Ca^{+2} efflux from cerebral tissue exposed to low intensity radiofrequency electromagnetic radiation. This model is predicated on the existence of a cooperative structural reconfiguration at the membrane level, subsequent to which is an alteration in membrane surface charge density. An expression for the electric field intensity that precipitates the initial phase-transition-like event is presented along with an isotherm governing the concomitant changes in the amount of bound Ca^{+2} to the plasmalemma. The effects of pH and alterations of the ionic strength of the extracellular medium are explicitly represented, and it is predicted that the observed power density and frequency windows should experience shifts as a function of pH and/or ionic strength. Sponsored by the Office of Naval Research Contract N00014-81-C-0449.

Invitation from the National Academy of Sciences

On January 13, 1983, Dr. Bond was contacted by Dr. A. Lazen's Office (Executive Director, Commission of Life Sciences, National Academy of Sciences) to serve as resource person for the Environmental Protection Agency in the evaluation of the effects of microwaves on brain biochemistry.

Section VII
FIGURES

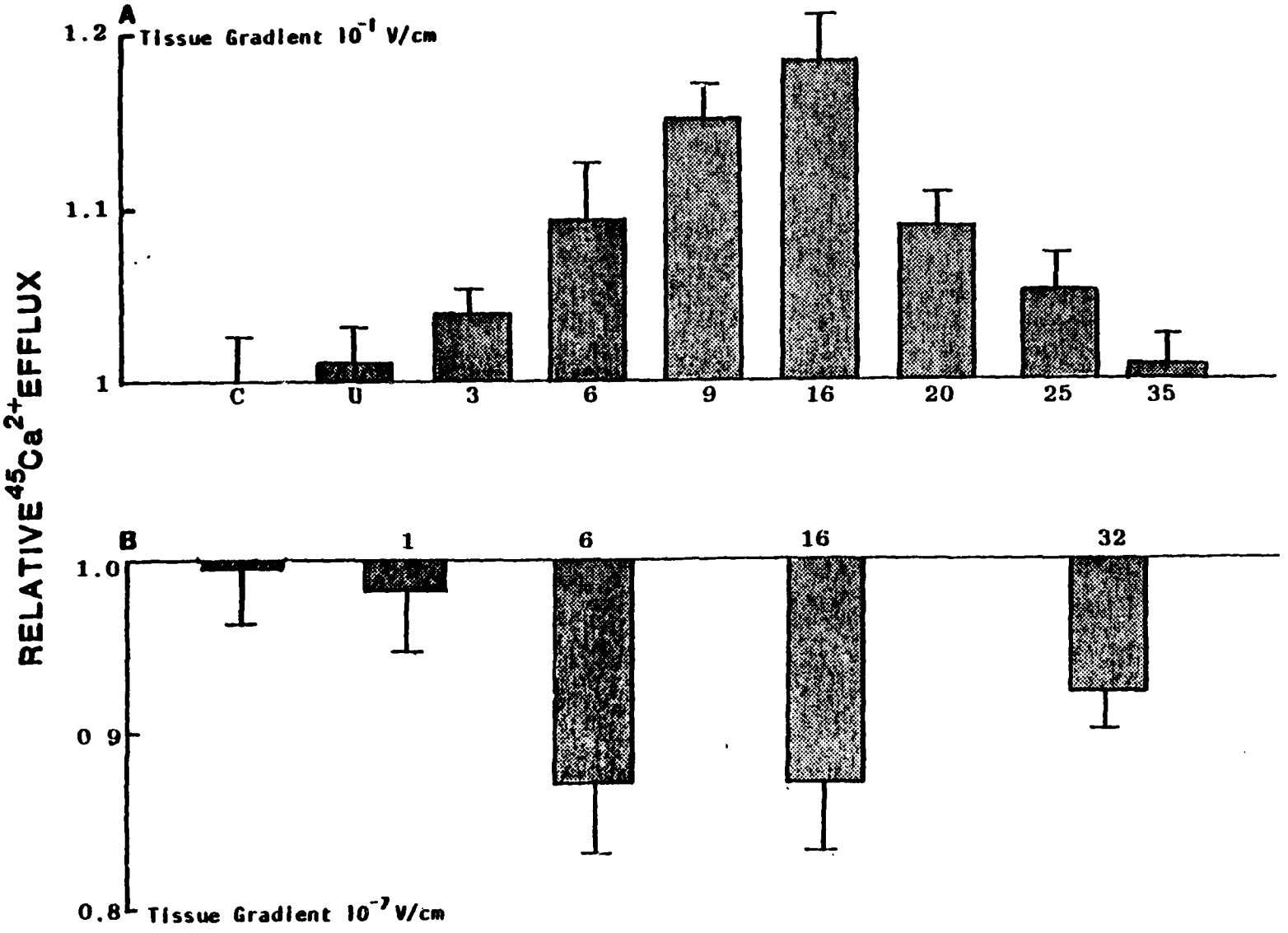


Figure 1 (Adey, 1981)

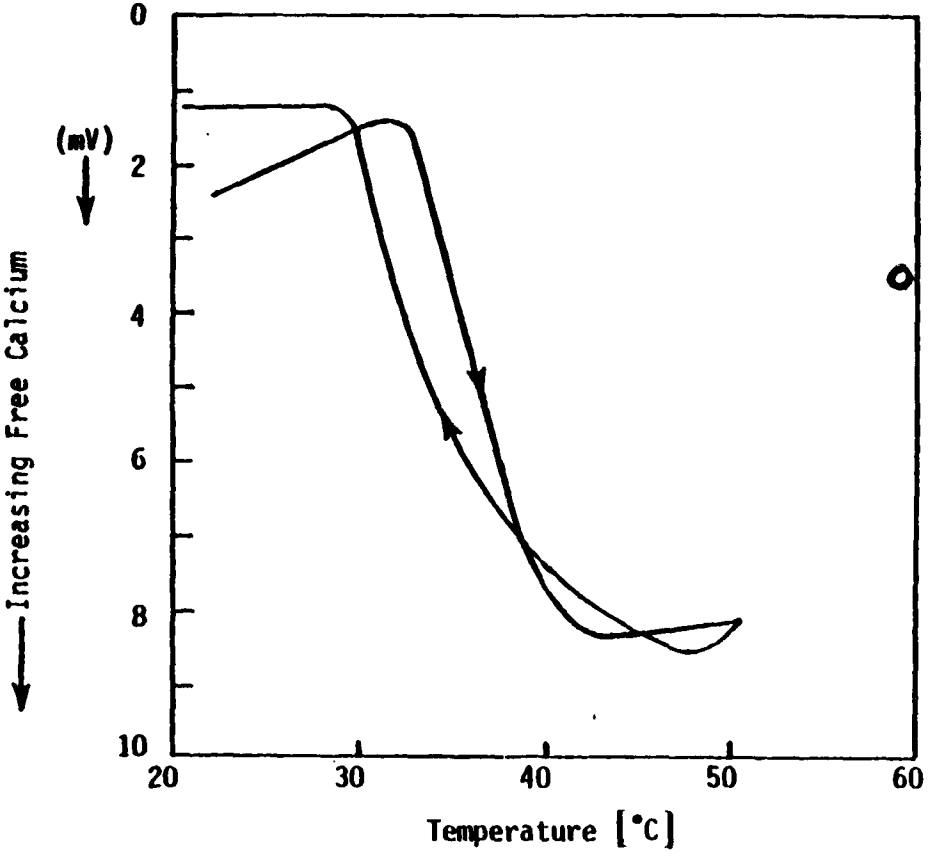
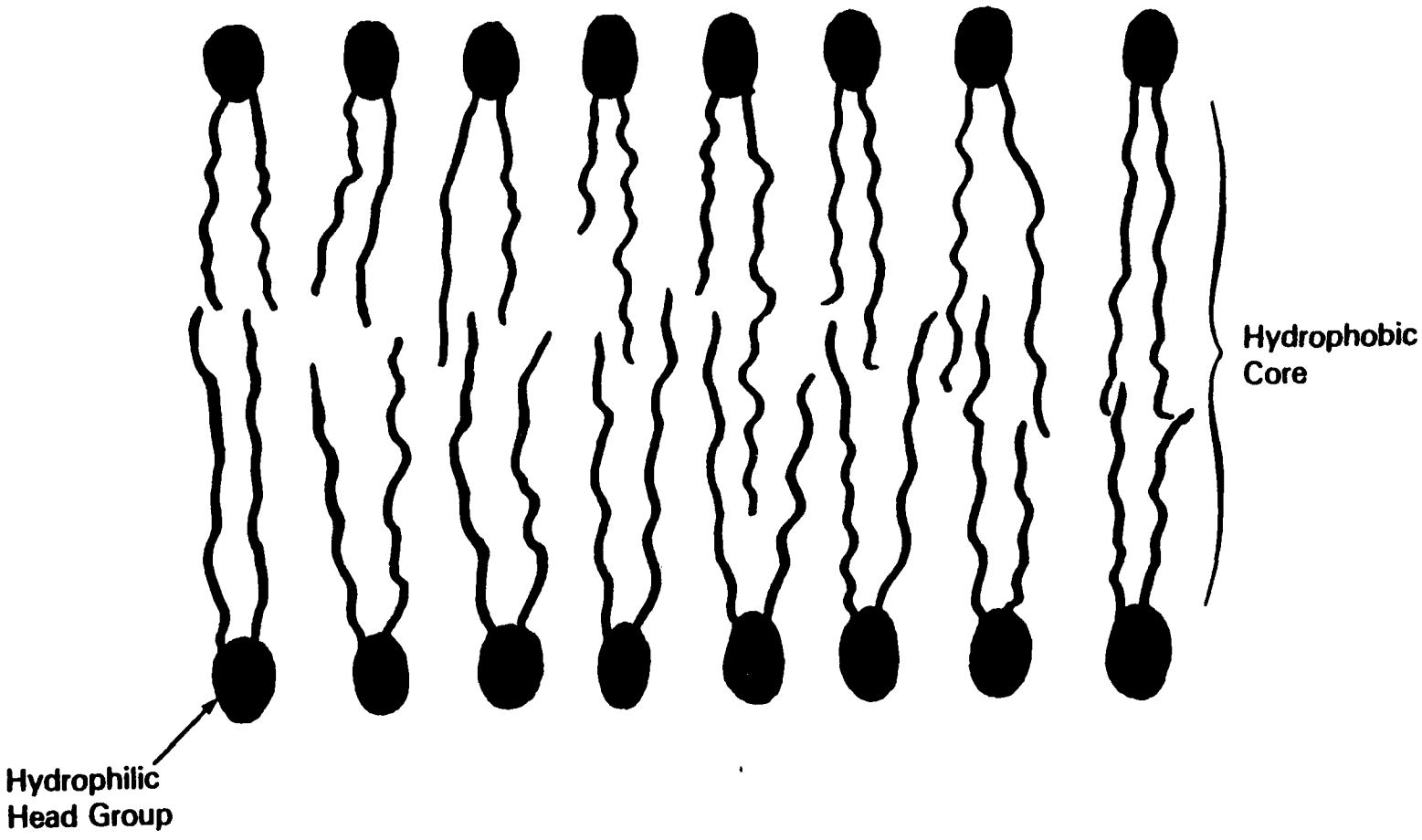
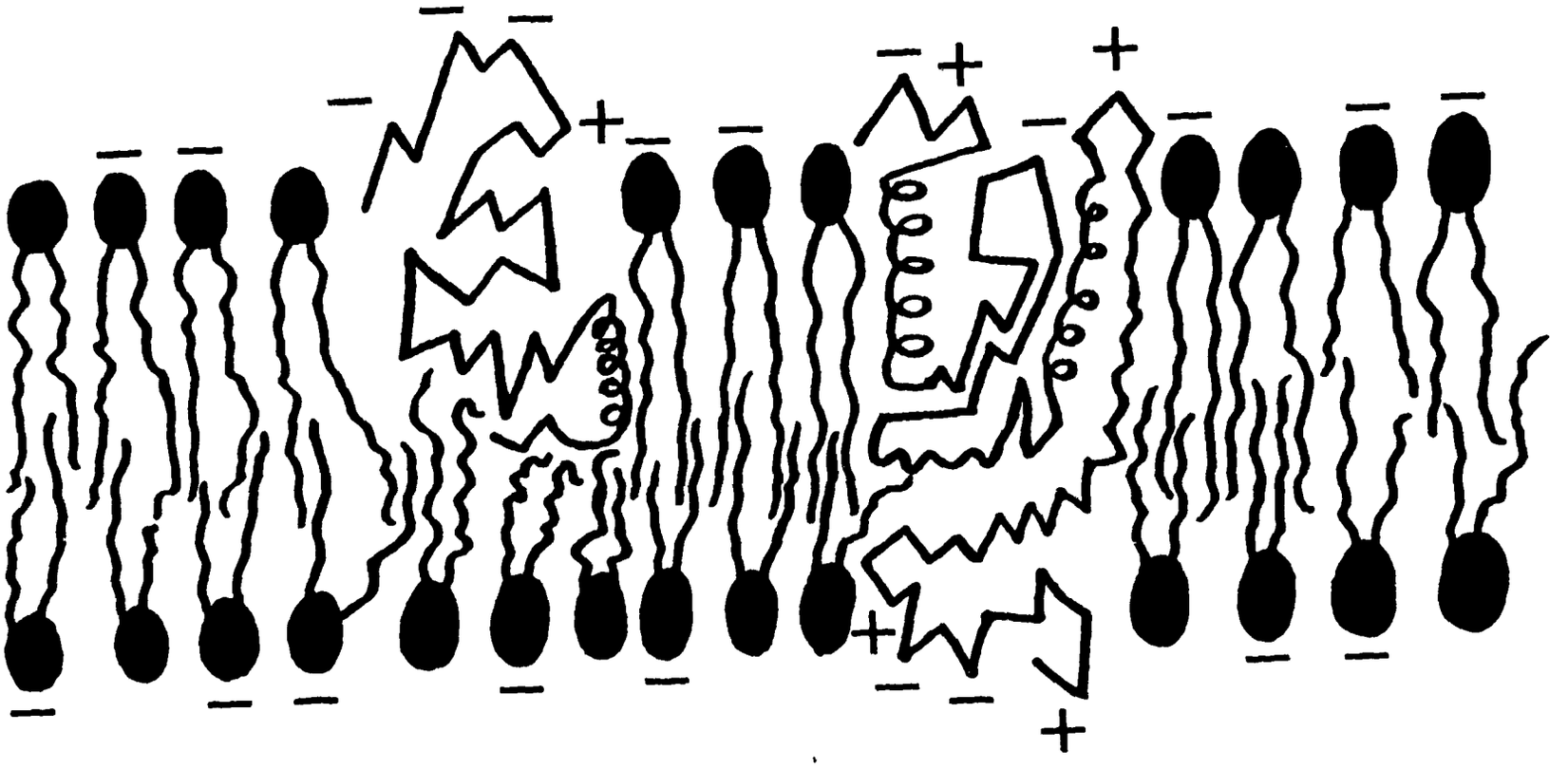


Figure 2 (Träuble, 1976)



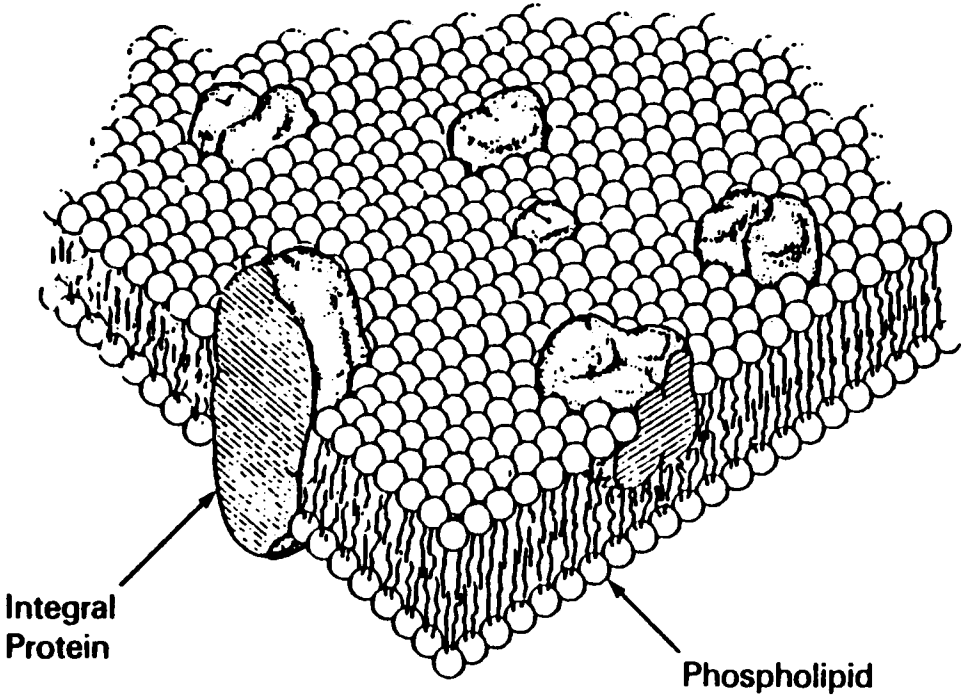
PHOSPHOLIPID BILAYER

Figure 3



LIPID BILAYER WITH
GLOBULAR PROTEIN INTERCALATED

Figure 4



FLUID MOSAIC MODEL

Figure 5

System: Charged Membrane in Apposition with an Electrolyte

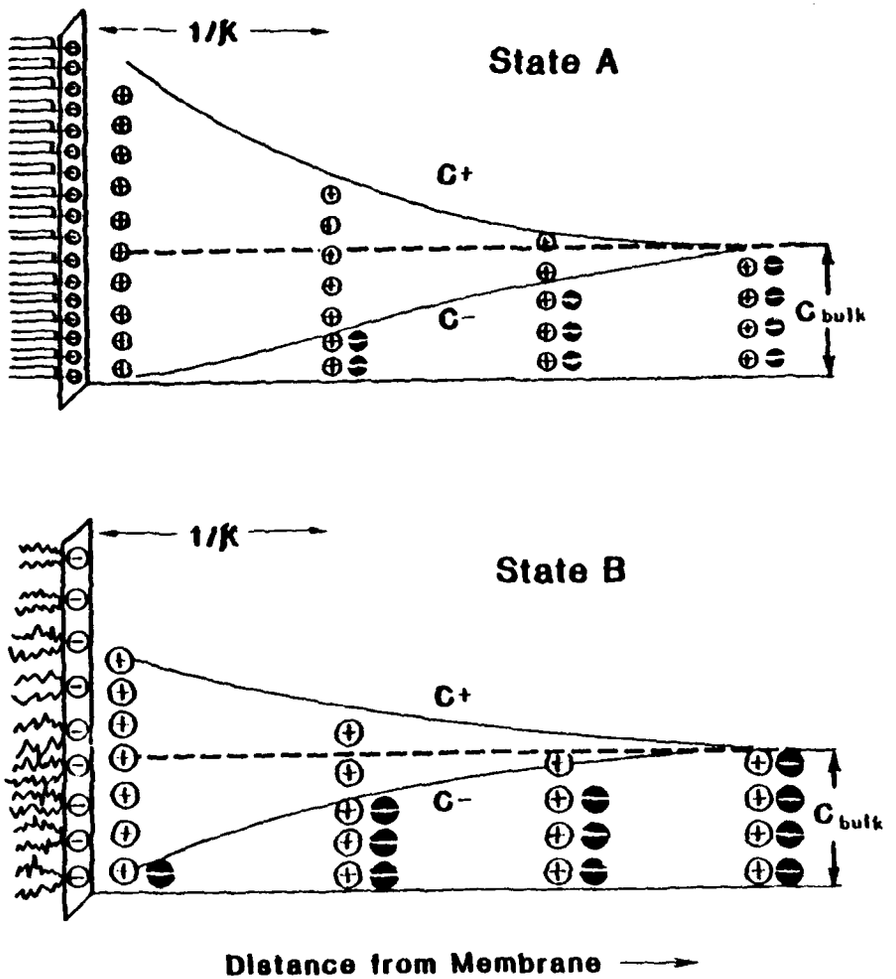
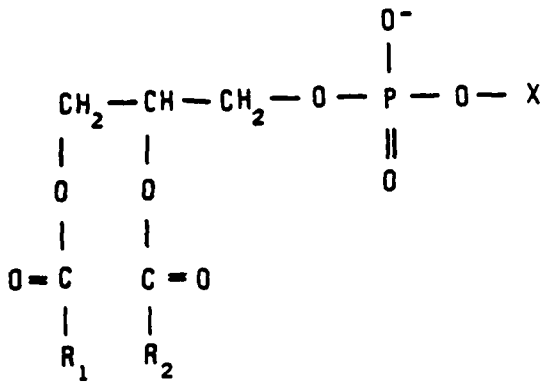


Figure 6 (Träuble, 1976)



PHOSPHATIDIC ACID	$-X = -H$
PHOSPHATIDYLSERINE	$-X = -CH_2CH(NH_3^+)COO^-$
PHOSPHATIDYLINOSITOL	$-X = -C_6H_6(OH)_5$
PHOSPHATIDYLGLYCEROL	$-X = -CH_2CHOHCH_2OH$
PHOSPHATIDYLETHANOLAMINE	$-X = -CH_2CH_2NH_3^+$
PHOSPHATIDYLCHOLINE	$-X = -CH_2CH_2N(CH_3)_3^+$

R_1 AND R_2 CORRESPOND TO HYDROCARBON CHAINS

Figure 7

GENERAL STRUCTURAL FORMULAS FOR SOME TYPICAL PHOSPHOLIPIDS PRESENT IN BIOLOGICAL MEMBRANES. (AFTER TANFORD C, 1973)

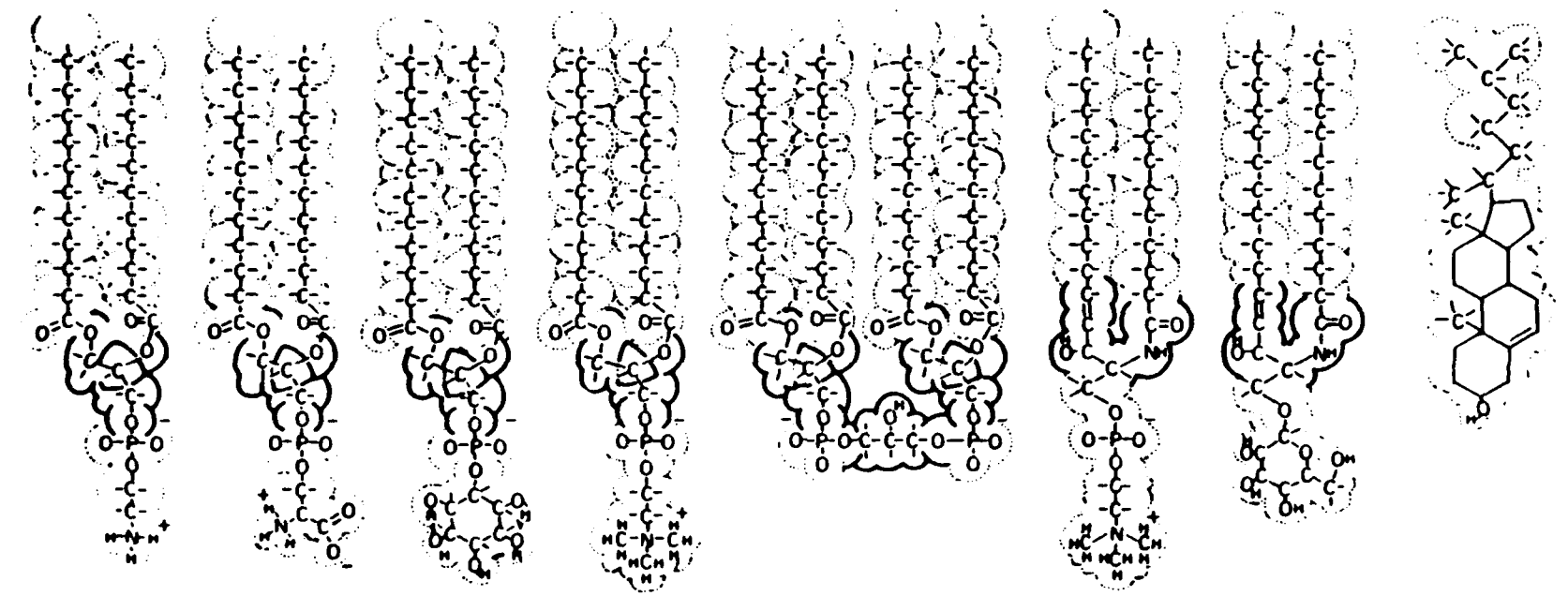

Phosphatidyl
-ethanolamine
-serine
(Cephalins)
-inositol
-choline
(Lecithin)
Cardiolipin
Sphingomyelin
Cerebroside
Cholesterol

Figure 8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