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DIELECTRIC SPECTROSCOPY AND MEMBRANE ORGANISATION

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ABSTRACT

The possible bases for field-mediated effects on cellular processes are reflected in the passive electrical properties of biological systems. The historical, present and prospective utility of dielectric spectroscopy in assessing the static and dynamic organisation of biological membranes is reviewed within this context. The basis for the view that the static capacitance of biomembranes is as great as 1 $\mu F/cm^2$ is doubted; contributions from the (partially restricted) motions of membrane components, and of double-layer ions, probably contribute to this apparent value in biomembrane vesicle suspensions. The importance of improving our knowledge of the static electrical capacitance of energy coupling membranes is stressed. Theoretical and experimental procedures for assessing the contribution of rotational and translational motions of membrane components, and of double-layer/membrane interactions, to dielectric spectra in the approximate frequency range 10 to 10^7 Hz are described. Finally, three outstanding and generally unsolved problems requiring further work are detailed.

INTRODUCTION

In this article, we wish to address ourselves to the following related questions: (1) can dielectric measurements tell us anything new or significant about the organization and dynamics of biological membranes, when the measurements are made with macroscopic, extravesicular electrodes in suspensions of closed vesicles; (2) can modern ideas concerning the structure and organization of such membranes contribute to the interpretation or reinterpretation of the dielectric spectra obtained in such systems? For reasons of space, and because fine discussions are available elsewhere, we

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will not include studies performed at frequencies exceeding 100 MHz, nor studies of neurophysiological systems, nor of other non-linear biological systems.

However, noting the need for a mechanistic explanation of the many exciting, thought-provoking and important studies concerning the alteration of physiological processes by rather weak electromagnetic (EM) fields (1-8), and noting further that to actually have an effect, EM fields must first be absorbed, we shall aim for a rather fundamental and molecular discussion of the possible means by which such effects may be mediated (at least in part) by membrane-located enzymes. We begin with a very brief discussion of the salient features of early historical contributions of dielectric spectroscopy to the study of membrane organisation.

HISTORICAL

"It is found that C_0 is independent of the frequency up to 4.5 million cycles, and it is also independent of the suspending liquid. These results furnish considerable evidence of the validity of the theory, that C_0 represents the static capacity of a corpuscle's membrane. On this assumption, and using a probable value for the dielectric constant of the membrane, the thickness of the membrane is calculated to be 3.3×10^{-7} cm."

With these words, Fricke (9) concluded his classic paper, in which it was indeed shown for the first time that the cell-delimiting lipoidal membrane required by the Overton (10) theory must be of molecular thickness. As discussed in a number of excellent texts and monographs, subsequent work on the frequency-dependence of the dielectric properties of a great number of cells and vesicles has indicated that they all possess a pronounced (β-) dispersion which reflects, or may be ascribed to, the presence of a cell membrane with a (static) capacitance of 1.2 ± 0.7 $\mu F/cm^2$ (11-35). The fact that this finding still stands after sixty years may be regarded as a

testament to Fricke's insight and to the power of dielectric spectroscopy in assessing the structure of biological membranes. Since the concept of the static membrane capacitance forms the background to detailed discussion of the frequency-dependent dielectric properties of membrane vesicle suspensions, we must therefore describe the so-called suspension equations that are pertinent to the simplest type of biomembrane-bounded vesicle, the spherical shell membrane (Figure 1).

THE SUSPENSION EQUATIONS FOR SPHERICAL MEMBRANE VESICLES

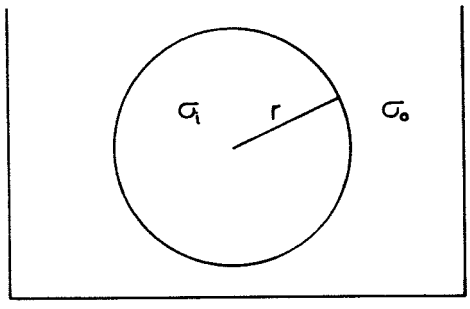
As the frequency of an alternating field is increased through the radiofrequency range, the permittivity and conductivity of a typical (microbial or other) cell suspension are respectively decreased and increased (Figure 1B). This can be interpreted as a progressive short-circuiting of the cytoplasmic membrane capacitance, via a Maxwell-Wagner type of mechanism (Figure 1). If we model the cells as identical spheres present at a total volume fraction (P) of $P < 0.2$, with a bulk, internal conductivity of σ_i, surrounded by a membrane with a static capacitance of C_m (farads/unit area), and immersed in a medium of conductivity σ_o (Figure 1A), the following equations express the high- and low-frequency permittivity (ε_∞', ε_1') and conductivity (σ_∞', σ_1') and the relaxation time τ (11):

$$\varepsilon_1' = \varepsilon_\infty' + (9P_r C_m)/4\varepsilon_r \tag{1}$$

$$\sigma_1' = \sigma_o[1 - (3P/2)] \tag{2}$$

$$\sigma_\infty' = \sigma_o[1 + 3P(\sigma_i + \sigma_o)/(\sigma_i + 2\sigma_o)] \tag{3}$$

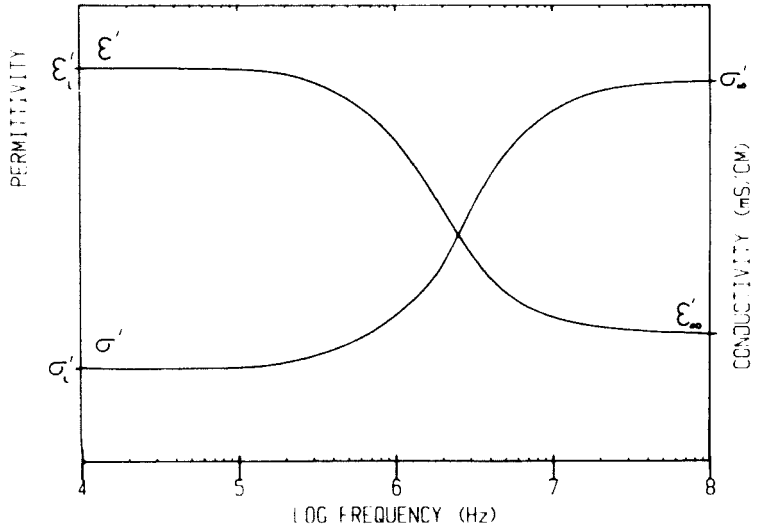
$$\tau = rC_m[(1/\sigma_i) + (1/2\sigma_o)] \tag{4}$$



(a)

FIGURE 1A

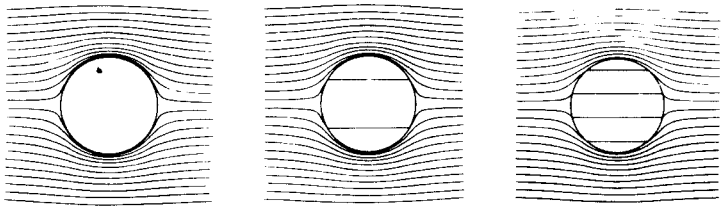
Spherical model of a biomembrane-bounded cell suspension: r, radius; σ_o, σ_i, bulk external and internal conductivities respectively.



(b)

FIGURE 1B

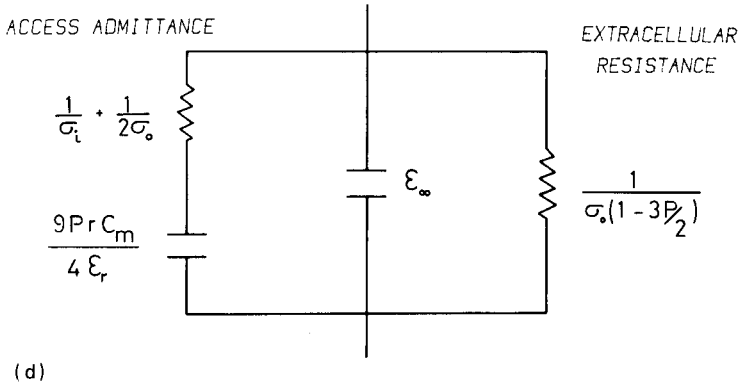
Frequency dependence of the dielectric properties of the model system.



(c)

FIGURE 1C

Interpretation of the dielectric data; (from left to right) there is a progressive short-circuiting of the membrane capacitance by the applied field.



(d)

FIGURE 1D

The equivalent circuit of the system.

where ε_r is the permittivity of free space (8.854×10^{-14} F/cm). These equations assume that the membrane conductivity (G_m) is zero. Since the conductivity of the majority of bacterial membranes does not exceed 10^{-4} S/cm^2 (36), and since G_m must exceed 1 S/cm^2 to be detected by means of measurements on suspensions with macroscopic electrodes (32), this assumption is acceptable. It is particularly instructive to examine equations 1 to 4 in some detail, and we note first that we accept, for the analysis of this type

of system, the superposition principle, which states, among other things, that the contribution to a dielectric dispersion of any individual vesicle is independent of that of any other vesicles which may be present.

The contribution of any (size class of) vesicle to the overall dielectric properties will be a sum of Debye-like dispersions, with a dielectric increment and relaxation time appropriate to the radius and internal and external conductivities in question, given that the volume fraction is just a function of the cell radius and number. Thus, one may simulate the behaviour expected on the basis of a distribution in the cell size (18) from which it is found (Figure 2) that only a small difference exists between the dispersions simulated on the basis of (a) a superposition of size classes of vesicles with different radii, (b) a suspension of equivalent volume fraction but of mean fourth power of the radius $\bar{r}$, ($\bar{r} = ((\sum nr^4)/N)^{1/4}$), where n is the number of cells in each size fraction, and N the total number of cells), and (c) the same, but using the mean cube radius ($r = ((\sum nr^3)/N)^{1/3}$). Note also the predominant tendency for ϵ'_1 (but not f_c) to favor cell size fractions with a somewhat greater than average radius, due to the dependence of ϵ'_1 upon volume fraction and radius (i.e. it increases as a function of r^4). Thus, the conclusion from this type of simulation is that a substantial value for the Cole/Cole α cannot be ascribed in general to the size distribution of (say microbial) cells, and hence, for ellipsoids of a reasonably low axial ratio, to any realistic shape-distribution. Similarly, the electrical anisotropy that one may invoke in the case of condensed mitochondria (18) is also inapplicable to microbial cells.

In this type of simulation, one assumes that the volume fraction is known (from microscopic observation) or may be determined (for the sum) by methods such as isotope dilution or from measurements based upon conductimetry according to equation 2. Thus, the membrane capacitance, which is a free variable, may be calculated directly from the measured dielectric increment (equation 1). It is this type of measurement (37) that has led to the view

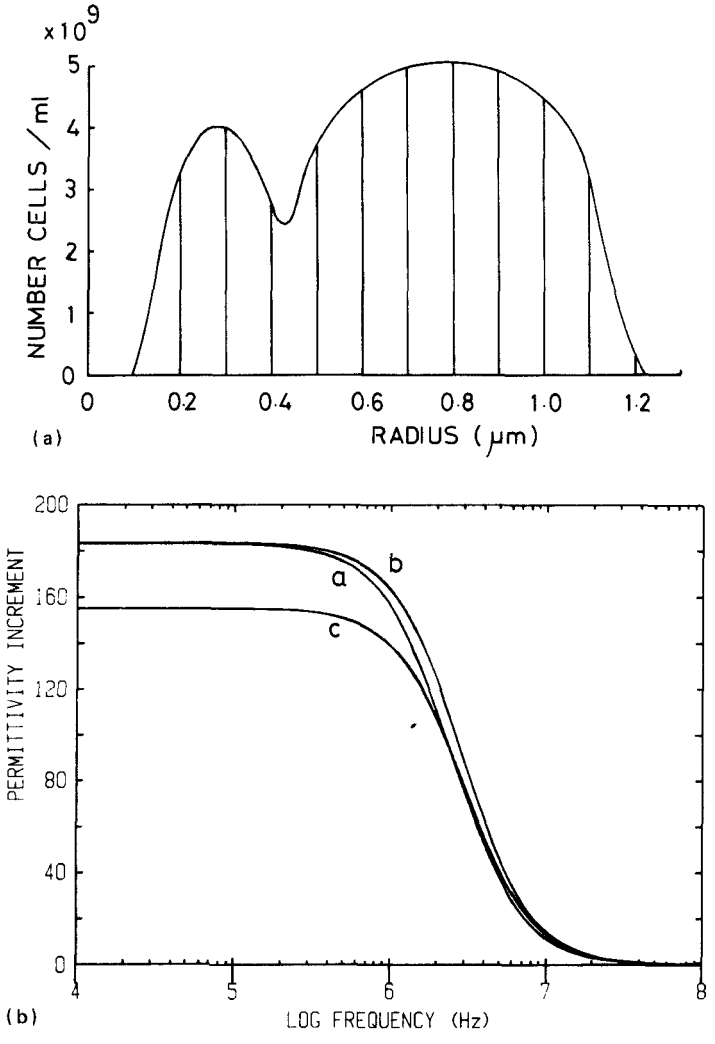


FIGURE 2

Simulation of the effect of a heterogeneous size distribution on the dielectric properties of spherical shell vesicles of the type described in Figure 1. (A) The size distribution considered, which, although arbitrary, may be construed as representative of a typical bacterial population. To obtain a numerical solution (in terms of equations 1 and 4), the 12 size classes indicated are used. (B) Effect of size heterogeneity on β-dispersion. The Debye-like dielectric behaviour of the system, simulated according to equations 1 and 4, (a) by assuming the 12 size classes, (b) by assuming the same total number of cells but assuming the mean fourth root of the radius (see text) and (c) by assuming the same total number of cells but using the mean cube of the radius (see text). It is evident that (i) the breadth of the dispersions are almost exactly Debye-like, and (ii) quite a significant size-distribution has relatively little effect upon either the mean relaxation time or its distribution.

that biomembranes have a static electrical capacitance of roughly 1 $\pm$ 0.5 $\mu F/cm^2$. Similarly, estimations of the internal conductivity of typical cell suspensions by means of equation 3 have given realistic values, which are nevertheless lower by a factor of 2-3 than those to be expected on the basis of the ionic content of the cells (21,38,39). Although new methods are becoming available, by which an independent measurement of the long-range diffusivity of bulk cytoplasmic ions may be obtained (40-43), we do not as yet know the precision with which the value of σ_i, calculated from equations 2 or 3 actually reflects the true, bulk, internal conductivity of the cells. In other words, although there is a reasonable, and at least semi-quantitative, agreement between experimental results and the predictions of equations 1 to 4 (18,28,29,27,35), the available data do not permit us to dispose of the view that other processes, with characteristic frequencies ($f_c = 1/2\pi\tau$) in the range of 0.5-5 MHz might make a significant contribution to the RF observed radiofrequency dispersions. (Note also that because of the cell radius-dependence of both relaxation time and dielectric increment for this type of relaxation, the ease of observation of other processes will itself depend on cell size.) We wish to stress in particular that the breadth of the β-dispersions observed in bacterial suspensions (26,32,33,44-46), as judged by the Cole/Cole plot (47) seems to require that one invoke a heterogeneity in r, C_m or σ_i far greater than is realistic if one were to claim that the dielectric dispersions observable in this frequency range may be ascribed solely to a Maxwell-Wagner type of mechanism. In other words, the fact that C_m and σ_i are free variables in calculations based upon equations 1 to 4 allows us to invoke contributions to the β-dispersion due to other properties of cellular, and in particular of energy coupling, membranes additional to their possession of a static electrical capacitance.

According to the now widely accepted fluid-mosaic model of biologial membranes (48)(Figure 3), biomembranes consist of phospholipid bilayers in, on and through which are located proteins and protein complexes, and both lipids

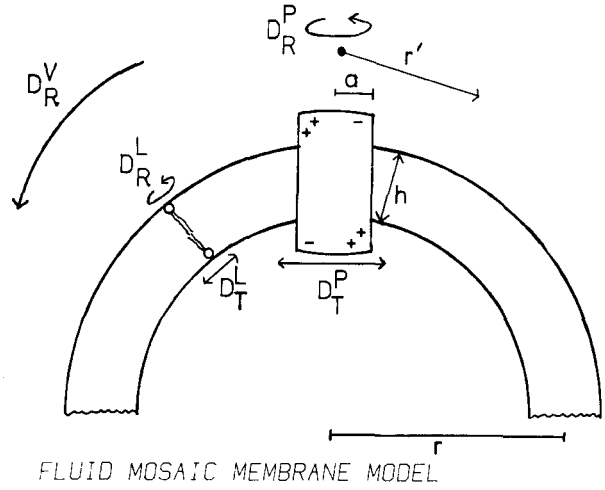


FIGURE 3

The salient features of a fluid mosaic membrane model. The model consists of an array of polytopic, integral protein complexes, which are free to rotate (with a rotational diffusion coefficient D^P_R) and to move laterally and independently (with a translational diffusion coefficient D^P_T) in a "sea" of phospholipids. Free rotation and translation of the phospholipids is also permitted, with the diffusion coefficients indicated. The membrane is arranged as a vesicle of radius r, vesicular rotation (orientation) being possible with a rotational diffusion coefficient D^V_R. If protein translational motion is restricted in some way, the average distance moved before a "barrier" is encountered is given by r' (see 88). The "bulk" viscosities of the membranous and aqueous phases are given by η and η' respectively.

and proteins can display thermal (and metabolically) induced rotational and translational diffusional motions in the plane of the membrane (49). Undoubtedly the static capacitance measured in planar phospholipid bilayer "black" lipid membranes which lack protein, or that measured at high frequencies with transmembrane electrodes, is smaller by as much as a factor of 2 than the widely quoted value of 1 $\mu F/cm^2$ calculated for biomembrane vesicle suspensions (20,50-64). Certainly the fact that biomembranes contain proteins which possibly possess a higher static dielectric permittivity than do pure phospholipid bilayers, may contribute to the explanation of this fact

(though we are not aware of any direct indication of this under physiologically meaningful conditions). However, for reasons given above, it is equally plausible in many cases that other factors contribute significantly to the frequency-dependent dielectric properties of membrane vesicle suspensions, and we must broaden our discussion to enquire more closely into what is known of the structure, and more particularly of the dynamics, of charged biomembranes and their adjacent electrical double-layers. We begin by discussing exactly why it is so important to improve the precision of our knowledge of the static capacitance of biological membranes, especially as it relates to so-called energy coupling membranes.

WHY SHOULD WE WISH ACCURATELY TO KNOW THE "STATIC" ELECTRICAL CAPACITANCE OF BIOLOGICAL MEMBRANES?

Modelling the membrane as a static electrical capacitor of thickness d, containing a slab of dielectric of a uniform permittivity ε_m (see Figure 5), we have:

$$C_m = \varepsilon_m \varepsilon_r / d \qquad (5)$$

where C_m is the capacitance per unit area. Thus, as did Fricke, we may obtain an estimate of d from estimations of C_m and assumptions concerning ε_m, or vice versa. Further, since in most cases the membrane capacitance is dominated by that due to the hydrophobic phospholipid core (55a,65,66, but cf. 67), and since in any case the value of d is reasonably well established from X-ray and neutron diffraction measurement, or from molecular models, a value for ε_m of 2.0-2.2 has become accepted for the bilayer membrane (BLM)(50,55,65,67,68). Similarly, since it is necessary to postulate that only a relatively small fraction of the surface of a phospholipid bilayer membrane is penetrated by aqueous pores with a dielectric permittivity of between 10 (bound water) and 80 (free water) to obtain values of C_m typical of biomembranes, there is a general satisfation with the present theory. At least with respect to energy

coupling membranes in general, and the bacterial cytoplasmic membrane in particular, we submit that, for three reasons, this is not a satisfactory state of affairs. These reasons pertain to our ignorance of (a) the mechanism of action of the ionophoric types of uncoupler, (b) the rate and extent of the lateral mobility of the free energy-conserving protein complexes of such membranes, and (c) the degree of electrogenicity of redox-linked proton pumping to the bulk phase. However, it is first necessary briefly to summarize, for the benefit of readers who are not involved in these issues, the outstanding area of controversy in membrane bioenergetics.

THE PROBLEM OF ENERGY COUPLING IN ELECTRON TRANSPORT PHOSPHORYLATION

As is now axiomatic (69), the exergonic reactions of biological electron transport may be coupled to the otherwise endergonic synthesis of ATP, the two sets of reactions being catalyzed by spatially separate enzymes, embedded in an ion-impermeable phospholipid membrane (Figure 4). The coupling may be decreased, ultimately to zero, by the addition of so-called uncoupler molecules, many of which have the ability to transport protons across both artificial BLM and natural biomembranes (68,70)(Figure 5), a fact consistent with, and widely interpreted as strongly supportive of, the delocalised chemiosmotic coupling theory elaborated by Mitchell (71). However, this general set of ideas (69) has some shortcomings (72-75), and one would like to know to what extent there is quantitative agreement between the protonophoric activity induced by uncouplers in BLM and that calculated as necessary to account for their uncoupling activity.

As discussed by McLaughlin and Dilger (68), while there is an excellent correlation between the protonophoric activity of different uncoupler molecules in BLM and their uncoupling effectiveness in rat liver mitochondria, a paradox exists in that it is necessary to assume that the molecules are 100 times more potent as protonophores in the latter case, to account for their

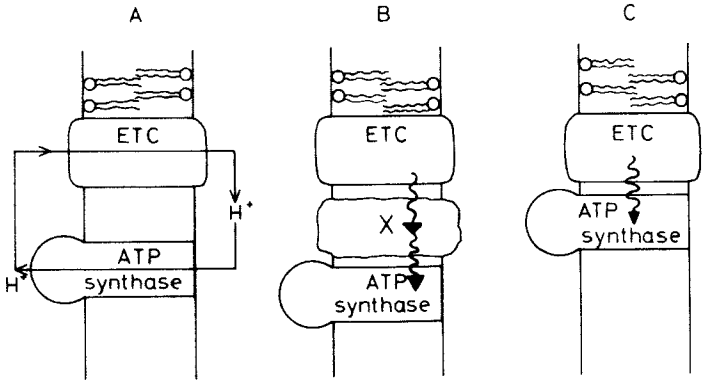


FIGURE 4

The problem of energy coupling in electron transport phosphorylation. In such systems, a membrane (vesicle) contains proteinaceous electron transport (ETC) and ATP synthase complexes, whose mutual geometric relationship is presently unspecified, and the problem relates to the means by which free energy is transferred between the exergonic reactions of electron transport and the otherwise endergonic process of ATP synthesis. (A) In a chemiosmotic model, free energy transfer is affected by the production and consumption of a proton electrochemical potential difference ("protonmotive force") across the energy-coupling membrane; in this case, no special topological relationship is required between ETC and ATP synthase complexes, and their mobilities and dispositions are thus in this sense unimportant. Alternatively, (B,C), free energy transfer might be affected by an intra-membranal route, either by a direct (C) or more long-range (B) interaction between the complexes. In the case of a long-range interaction, additional proteins (X) may be involved. Although the free-energy-transferring step in B and C only occurs when an appropriate geometrical relationship exists between the relevant (and mobile) complexes, the arrangement in C suggests a much greater degree of random, long-range mobility than that in B.

ability to uncouple oxidative phosphorylation in chemiosmotic terms by means of a purely passive protonophoric activity alone. These workers pointed out (59,68,76) that the Born charging energy required for the transfer of a nonpolarisable, spherical, monovalent ion from an aqueous phase into the center of the bilayer is actually an extremely sensitive function of the membrane permittivity, such that increasing that of a BLM by a factor of two, by using 1-chlorodecane ($\varepsilon_m \approx 4.5$) in the membrane-forming mixture, led to an increase of 2-3 orders of magnitude in the ion permeability of the BLM. It

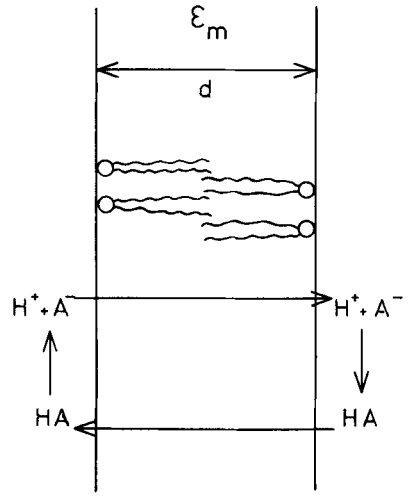


FIGURE 5

The protonophoric activity of a lipophilic weak acid in a black lipid membrane. The protonophore acts by virtue of its ability to cross the membrane in both charged (A^-) and uncharged (HA) forms. As discussed in the text, the rate of transmembrane movement of A^- is dependent on the value of ε_m, and it is therefore important to know this value in assessing whether a protonmotive-force-driven activity of the present type adequately explains the ability of protonophores to uncouple electron transport phosphorylation.

was taken, therefore, that since mitochondria contain proteins, which might be expected to have a much greater permittivity than that of phospholipids, an explanation for the aforementioned paradox based on ε_m values might be possible. Thus, although the fact that mitochondria are not slab dielectrics with a permittivity twice that of pure phospholipid BLM, a fact that serves rather to strengthen the opposite conclusion to the following (74), the conclusion was drawn by Dilger, McLaughlin and colleagues (59,68,76) that the above observations were fully consistent with a chemiosmotic view that uncoupler action could be explained solely in terms of the dissipation of the protonmotive force, by protonophoric action in bilayer areas of the mitochondrial membrane via a mechanism of the type diagrammed in Figure 5. It

is thus obvious that a much better knowledge of the static permittivity of biomembranes than we have at present will be required to give any type of quantitative account of uncoupler action in vivo. Similarly, we would mention that a knowledge of the static membrane capacitance is of importance in deciding between alternative explanations of the non-appearance of appropriate numbers of protons in the outer aqueous phase in suspensions of microorganisms in "O_2-pulse" experiments in the absence of added 'permeant' ions (77-79).

In this type of measurement (77-80) a small volume of air-saturated KCl is added to an anoxic suspension of respiratory microorganisms, any resultant pH changes in the extravesicular phase being converted to numbers of protons appearing, such numbers usually being related to the amount of oxygen consumed, and expressed as the $\rightarrow H^+/O$ ratio. (We consider here only vectorial pH changes, scalar ones in any event being absent under the conditions described, as may be confirmed by findings obtained in the presence of uncoupler.) In practice, it is found that certain treatments, such as the addition of rather high concentrations of KSCN, greatly enhance the $\rightarrow H^+/O$ ratio measured. The chemiosmotic explanation for the low $\rightarrow H^+/O$ ratio observed in the absence of KSCN relates to the electrogenicity of H^+ pumping and the relatively low static electrical capacitance of the cellular membrane. In this view, the low $\rightarrow H^+/O$ ratio is caused by the build-up of a large, delocalised membrane potential, caused by the electrogenic transfer of H^+ from the inner to the outer phase.

Using the simple electrostatic equation ($Q = CV$) that relates the voltage (V volts) across a capacitor of C farads when it is charged by the passage of Q coulombs of charge, we have, for cells or vesicles, $\Delta\psi_{max} = en/C$, where e is the elementary electrical charge (1.6×10^{-19}C), n is the number of H^+ translocated (electrogenically) across a single cell of capacitance C, and $\Delta\psi_{max}$ is the maximum membrane potential that such protonmotive activity can create. If we treat the cells as spherical shell capacitors (no membrane invaginations) of capacitance 1 $\mu F/cm^2$, a typical bacterium of radius 500 nm

has a capacitance of 3×10^{-14} F (77,79,81). If we measure the total number of H^+ translocated, and assume that all are electrogenic, then n may be calculated from a knowledge of the cell numbers. By using small oxygen pulses and large cell concentrations, the maximum bulk-to-bulk phase transmembrane potential which may theoretically be built up, $\Delta\psi_{max}$, may thus be made arbitrarily small, so that, according to chemiosmotic considerations, the H^+/O ratio should now be as great in the absence of KSCN as in its presence (KSCN being taken to act by dissipating a chemiosmotic membrane potential). In practice, however, such behaviour is not observed (77,79,81), suggesting that the non-appearance of most of the protons in the outer bulk phase in the absence of KSCN is not due to the build-up of a chemiosmotic membrane potential but to the fact that the protons never entered this phase electrogenically. Since this conclusion would not necessarily be correct if the static membrane capacitance were only, say, 0.02 $\mu F/cm^2$, it is important to know its actual magnitude. However, data available concerning the β-dispersion of Paracoccus denitrificans strongly suggest that the static membrane capacitance is at least 0.5 $\mu F/cm^2$ (32,33).

Thus, we have seen that a knowledge of the values of even the static electrical capacitance of energy coupling biomembranes is of crucial importance in assessing the veracity of the chemiosmotic and other coupling hypotheses in at least three areas: the mode of action of uncouplers, the electrogenicity of bulk-to-bulk phase proton pumping catalyzed by redox-linked proton pumps, and their lateral mobility relative to that of ATP synthase enzymes (Figure 4).

DIELECTRIC SPECTROSCOPY AND THE DYNAMIC ORGANIZATION OF BIOMEMBRANES

In the foregoing, we have concentrated mainly upon the static electrical capacitance of the membrane. However, as depicted in Figures 3 and 4, current

thinking conceives of the long-range, hydrodynamically-constrained, lateral mobility of lipid and protein complexes in the plane of the membrane, according to the fluid-mosaic picture. It was mentioned by Singer and Nicolson (48), stressed by Jaffe (82,83) and confirmed (84-86), that the application of a steady electric field to a biomembrane-bounded cell or vesicle suspension should result in the lateral redistribution ("lateral electrophoresis") of charged, integral membrane protein complexes. Similar effects should follow from the application of any sinusoidally varying field whose frequency is less than the characteristic frequency of the Maxwell-Wagner-type dispersion of the vesicles in question. As first pointed out, to our knowledge, by Kell (87), such field-dependent motions should necessarily be accompanied by a frequency-dependence of the dielectric properties of such a system. They should be reflected as a dielectric dispersion, the dielectric increment and relaxation time of which may in principle be used to gain important information concerning the rate and extent (randomness) of such lipid and protein motions.

Because of the properties of the Langevin function (24,30,83,88), the relationship between the visual and dielectric observability of such motions is dependent upon the cell radius (if such motions are restricted by hydrodynamic forces alone). Further, there are good reasons to believe that there is a strong coupling between the motions of proteins and lipids in the membrane, on the one hand, and of the ions and solvent molecules in the adjacent double layers, on the other (32,88-90). Thus, the situation is complicated, and our purpose in the remainder of this section is to outline the possible contributions of such motions to dielectric spectra generally. A fuller discussion of these and related matters is given elsewhere (88) together with a rather extensive set of measurements carried out on a number of microbial cells, protoplasts and membrane vesicles (32).

With reference to Figure 3, the characteristic frequency ($f_c = 1/2\pi\tau$, where τ is the relaxation time in seconds) for the rotational diffusion of a

membrane protein may be derived from the Stokes-Einstein relation, and is given by

$$f_c = kT/8\pi^2 a^2 \eta h \quad (6)$$

where η is the membrane viscosity, k is Boltzmann's constant, T the absolute temperature and the other symbols are as in Figure 3. Using typical values of η (1-10 P), a (4 nm) and h (5 nm), it is found that typically f_c is 1-10 KHz (88). The advantage of the dielectric method is that the characteristic frequency is readily obtained, and the values of f_c as calculated from equation (6) might be expected to equal the values obtained dielectrically.

For calculating the translational diffusion coefficients of membrane proteins, it has become conventional to use an equation first derived by Saffman and Delbruck (91), which for our purpose may be modified (88) to give the characteristic frequency for translational motional relaxation in a closed membrane vesicle:

$$f_c = (kTK/4\pi^2\eta r^2 h)[\ln(\eta h/\eta' a) - \gamma] \quad (7)$$

where γ is Euler's constant (0.5772), and we consider either random (throughout the vesicle) or restricted 2-dimensional diffusion. In the former case, K = 1 and r = r, the vesicle radius (Figure 3), while in the latter case, K = 2 and r = r' (Figure 3), the average distance moved before a barrier, of whatever nature, is encountered. Plots of equation (7) indicate (88) that values of f_c for this type of relaxation depend critically upon the extent of translational randomness (i.e. degree of long-range mobility) of the protein(s), given the relative (order of magnitude) constancy of biomembrane viscosities. Some of the values obtained, however (32,92) give cause to doubt the applicability of the simplest type of fluid mosaic model to the bacterial cytoplasmic membrane (74,93,94). Problems of this type raised by experiments of a more biochemical nature in animal cells (95) may at least partially be explicable in terms of membrane protein-cytoskeleton interactions; however, bacteria are not thought to possess a cytoskeleton of the type found in

eukaryotes, and the exact mechanistic explanation for the apparently non-random distribution of membrane proteins in many microorganisms remains a matter for present concern and future research. These more general considerations lie outside our present scope, but have recently been discussed (74,88,93,94).

A dielectric relaxation is characterized not only by its relaxation time but also by its magnitude. This in turn depends upon the concentration of effective dipolar particles, their effective molecular dipole moments and the degree of restriction on their motions. It is usual to relate the observable dielectric increment $\Delta\varepsilon'$ due to the rotation of a globular protein in an aqueous solution to its molecular dipole moment μ according to the relation:

$$\mu = (9000kt\Delta\varepsilon'/4\pi NHc)^{1/2} \qquad (8)$$

(22,96), where c is the molar protein concentration, N Avogadro's number and H is an empirical constant which is usually taken (from a comparison between calculation and experimental data for glycine) to have the value 5.8. Note that in equation (8) μ is in Debyes, but since two unit charges of opposite sign separated by 10^{-10}m possess a dipole moment of 4.8 D, we may express the effective dipole moment of any potentially mobile distribution of charged particles in units of charge-Angstrom by dividing the value from equation (8) by 4.8. Plots of equation (8) indicate that exceptionally large dielectric increments may indeed be exhibited by systems in which the extent of diffusion of the charged particle in question is large (88).

The classical explanation of the α-dispersion is that it reflects (at least partially) the relaxation, tangential to the charged membrane surface, of the ions constituting the diffuse double-layer (97-100). However, in the case of typical biomembranes, the organization of the proteins in the phospholipid matrix is such that their surfaces extend substantially beyond the surface delineated by the phospholipid head-groups (Figure 6)(101). Thus, the relaxation times for the tangential motions of double layer ions will

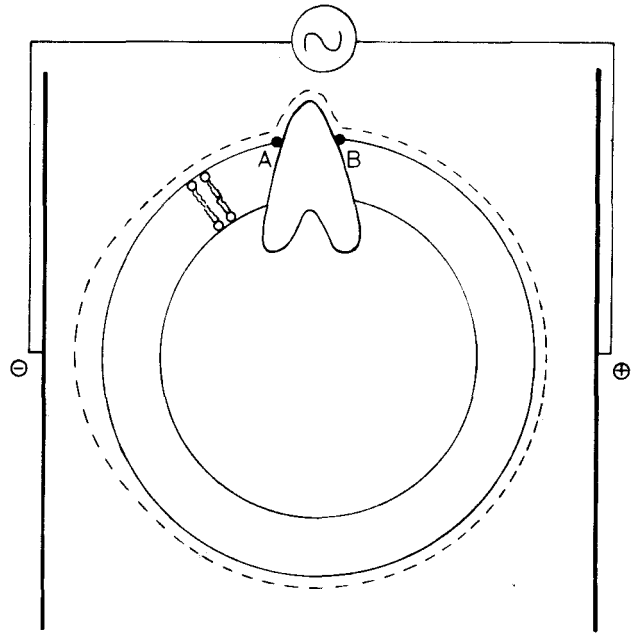


FIGURE 6

The molecular roughness of the surface of a protein-containing biomembrane vesicle can lead to a substantial broadening of the relaxation time(s) of double-layer ions moving "tangentially" to the charged membrane surfaces. In particular, "free, tangential" ionic relaxation will be stopped at the points marked "A" and "B" in systems of the present type. For further discussion, see text.

depend not only upon the vesicular radii but also upon the molecular roughness of the surface of individual vesicles, the effect being to cause dielectric relaxations at frequencies greater than those otherwise to be expected, in a fashion which will be unaffected by the presence of intermolecular cross-linking reagents (32,88). Further, any electroosmotic interactions between the diffuse double layer and the motions of the proteins of the membrane (89,90) will also serve to broaden such dispersions, by an uncertain amount.

The foregoing discussion applies equally, for rotation, translation and electroosmotic behaviour, to the charged lipids of the membranes, while even

neutral (zwitterionic) lipids may be expected to exhibit a dielectric dispersion due to their rotational relaxation (102).

Finally, a dielectric dispersion due to the rotation (i.e. orientation) of the entire vesicle might also be anticipated, its characteristic frequency being given from the Stokes-Einstein relation as:

$$f_c = kT/8\pi^2\eta' r^3 \qquad (9)$$

For typical values of aqueous viscosities (0.01-0.015 P) and cell (vesicle) radii, calculations based on equation (9) indicate that dielectric dispersions due to cellular rotation are not to be observed in the frequency range above 10 Hz, except for exceptionally small particles.

Thus, the conclusion from these largely theoretical considerations is: any motional characteristics of the lipids and proteins of biomembranes, and of the adjacent double layers, can underlie or give rise to a dielectric dispersion. Since the significance of this statement to dielectric studies of biomembrane organization has only recently become appreciated, we next discuss three particular experimental manipulations that may be or have been used to gain information about the contributions of such motions to dielectric spectra.

EXPERIMENTAL MANIPULATIONS OF RELEVANCE TO DIELECTRIC STUDIES OF MEMBRANE ORGANIZATION AND DYNAMICS

The more classical explanations of the α- and β-dispersions in charged membrane vesicle suspensions depend essentially only upon the radius, closed nature, surface charge density and surface potential of the vesicles, and on the mobility and concentration of the ions of the diffuse double layer (α-dispersion) and bulk phases (β-dispersion). Thus, as stressed elsewhere (32,87,88,92,103), they should be unchanged, other circumstances being equal, by the addition of chemical cross-linking reagents to the membrane vesicle suspension. In other words, any change in the dielectric properties caused by

the addition of chemical cross-linking reagents such as glutaraldehyde or dimethyl suberimidate, which do not change the surface charge density (104) or bulk phase conductivities, should be diagnostic of a contribution of the lateral (translational and rotational) motions of charged membrane components to the observed dielectric spectra, although care should be taken to exclude (or differentiate) inter-vesicular cross-linking, a diagnostic of a contribution from vesicle orientation. Similarly, the availability of photopolymerizable phospholipids (105,106) presents a favourable experimental opportunity of this type (87,88).

Since the viscosity of biomembranes exceeds that of the typical aqueous solutions to which they are adjacent by 2-3 orders of magnitude (107), increasing the aqueous viscosity by say a factor of 10, for instance with glycerol, will have an essentially 10-fold effect upon the rate of vesicle orientation, or on relaxations due to double layer ionic motions, but a negligible effect on relaxations due to the motions of membrane components, provided that the membrane:water partition coefficient of glycerol does not significantly exceed 1. Thus, studies of the dependence of dielectric spectra on both the viscosity and the temperature may shed light on the relative contributions of intra- and extra-membranal dipole relaxations, as occurred in studies of aqueous globular proteins (22,108,109).

The time constant and magnitude of dielectric relaxations due to the mechanisms of the classical α- and β-dispersions have a different dependence upon the vesicle radius in the two cases; similarly, relaxations due to mechanisms of the type presently under consideration exhibit definite and different vesicle radius-dependencies, and indeed it was the shifting of the α- and β-dispersions to high frequencies in bacterial chromatophores (87) which first drew attention to these possibilities. Thus, at least three types of experimental manipulations (cross-linking, viscosity changes, and sonication to change the vesicle radius) may be used to aid the experimenter

who seeks to unravel the various contributions of the type under discussion to dielectric spectra.

If we accept that field-membrane-protein/lipid interactions can explain some of the dielectric properties of membrane vesicles, is it possible that they may also underlie some of the observed effects of low-level electrical fields upon cellular physiology?

MODULATION BY ELECTRICAL FIELDS OF REACTIONS CATALYSED BY MEMBRANE-BOUND ENZYMES

The imposition of a macroscopic, sinusoidally alternating, electric field with a field strength (peak-to-peak) of E_o (V/cm) and a frequency of ω radians/s across a suspension of spherical membrane vesicles of radius r, induces a transmembrane potential of a magnitude given by:

$$V_m = (1.5\ E_o r\ \cos\theta\ )/(\ 1 + (\omega\tau)^2)^{1/2} \qquad (10)$$

(30), where θ is the angle between a portion of the bilayer and the field direction, and τ is the relaxation time for the classical Maxwell-Wagner dispersion. Thus, with cells of a moderately large size, quite modest exciting fields can induce physiologically significant membrane potentials. Such potentials can have both thermodynamic effects on putatively (quasi-) reversible reactions such as ion uptake (7,110-112) and ATP synthesis (113), and even if they are of insufficient magnitude to act as a free energy term in driving enzymatically catalysed reactions, the large dipole moments (114) and flexibility (115,116) of such proteins can lead to field-induced modulation of the kinetic properties of such membrane-bound enzymes by altering their conformational status or their dynamics (116a). Thus, field-protein interactions in a plane normal to the bilayer membrane might also cause small but significant dielectric dispersion under certain circumstances (88).

Another case in which a clear mechanism of field-protein interaction leading to a modulation of membrane enzyme activity has been elicited comes from the observations of Young and Poo (117), who showed that lateral electrophoresis may lead to protein aggregation and in turn to an alteration of the kinetics of ion channels (118). Thus, these (and other) studies show quite clearly that simple, direct field-enzyme interactions can lead to a modulation of enzymatic activity, and hence to biological effects. In the spirit of this article, therefore, we would argue in harmony, for instance, with Pilla (7,111,119), that dielectric studies provide a potent means of determining the mechanisms of such processes, provided that the latter are linear (see next section).

PROBLEMS AND PROSPECTS

The recent commercial availability of rapid, digital, impedimetric apparatus has been for us, and will no doubt continue generally to be, a potent means and motivation for advancing the theory and practice of membrane (and other) dielectric spectroscopy. However, we should like to end by outlining what we perceive to constitute the three major general problems, both theoretical and experimental, slowing the rate at which our collective understanding of the electrical organization of biomembranes may be improved by dielectrc spectroscopy using modern apparatus in the range 10^1-10^7 Hz. (1) The development of computer programs for the automated correction of the contribution of electrode polarization to the observed dielectric spectra would be a great boon, and would greatly extend the range of frequencies and conductivities available; (2) more objective and flexible means of fitting dielectric data to sums of individual (mechanisms of) dielectric dispersion, i.e. of deconvoluting dielectric spectra, need to be developed -- we have tended to assume that dielectric dispersions are best modelled by a Cole/Cole-type distribution function, an assumption that is at best unjustified and most

likely incorrect (120); (3) we need more explicitly to attack the problem of deciding exactly what a method which is by definition measuring linear properties is actually telling us about the properties and behaviour of systems which may be expected to be, and in some cases demonstrably are (6,74), strongly non-linear and far from equilibrium in vivo. The scientific, and bioanalytical (121) possibilities embodied in this area in particular would seem to us to constitute some of the most exciting lines of progress to be expected in the ensuing years.

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