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ELECTROELASTIC EFFECTS IN CELL MEMBRANES

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Biological cells are surrounded by a thin membrane consisting primarily of insulating lipids supporting an electric field which usually exceeds 25 MV/m, and may become much larger under some conditions. At these high field strengths, the electric force can cause appreciable deformation of the relatively compliant membrane, and may lead to rupture. An elastic model of the membrane has been used to study such instabilities, and has predicted breakdown voltages which agree quite well with those reported in experiments on biological cells and rapidly stressed artificial membranes.

INTRODUCTION

The membrane which surrounds a biological cell is one of its most important constituents. All chemicals which enter or leave the cell must pass through the membrane, which exercises a great deal of selectivity in this gate-keeping function, whether dealing with large protein molecules or small ions. Even the very similar Na^+ and K^+ ions are maintained at different concentrations within the cell, often by orders of magnitude. As a result of this ionic selection, the membrane is always subject to an electric potential difference.

The electric potential across a membrane is not just an unimportant byproduct of cell biochemistry; indeed, it plays a key roll in many cell functions. The best known of these is the propagation of nerve impulses along the axon, which has long been considered as an electrochemical wave. Conversely, the external application of a voltage to the membrane will affect the cell function or even its structural integrity. For these reasons, basic biophysical studies of cells and their membranes often involve electrical stimulation.

Many biomedical instruments also involve the application of voltage to a cell. Typical of these is the Coulter counter, which measures the concentration of red blood and other cells through the decrease in current flow between electrodes caused by the presence of a cell. Most of the voltage drop associated with the presence of the cell appears across the cell membrane, so passage through a Coulter counter can often affect the cell function. In fact, one of the major limitations of this device is the rupture of the membrane which occurs if the voltage drop is much greater than one volt (Zimmermann et al. 1974).

Failure of the cell membrane under electrical stress is not always a liability if the voltage is applied as a very short pulse. The membrane breaks down (or at least becomes more permeable) for a time, but then heals itself (Kinoshita and Tsong 1977, Benz et al. 1979). This temporary breakdown, called electroporation, furnishes an opportunity to open a cell, insert some chemicals which would normally be rejected by the membrane, and then return the cell to its original milieu. The most exciting application of this technique is the transfer of DNA into a foreign cell in genetic engineering (Auer 1976, Neumann et al. 1982).

Some time ago it was suggested that the electrical breakdown of a membrane could be explained as the failure of an elastic material under compression caused by electrostatic forces (Crowley 1973). This model assumed that the membrane could in fact be treated as elastic, and that it would be soft enough to deform under the electric pressures associated with potential differences on the order of one volt. Since then, the model has been put to the test for a variety of natural and artificial membranes. The object of the present paper is to review this model, and to assess its validity in the light of these tests.

ELECTROELASTIC MODELS OF CELL MEMBRANES

Electrically, the membrane consists of an electrically insulating layer surrounded by good conductors as shown in Figure 1.

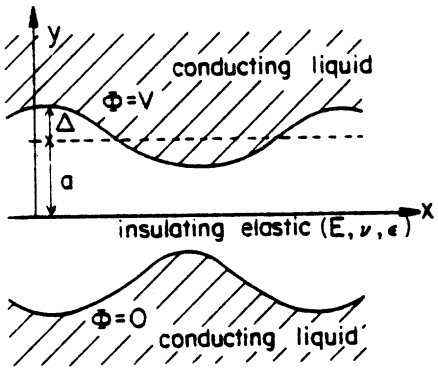

Figure 1. Elastic model of a membrane

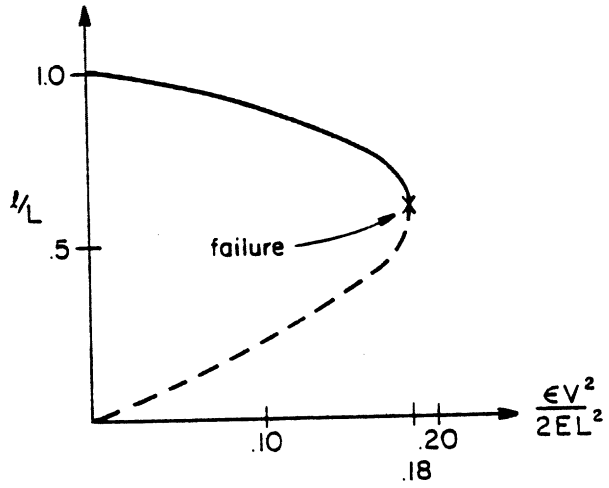

Figure 2. Membrane compression due to voltage

There is a large imbalance between the ions inside and outside a living cell membrane which leads to an appreciable voltage, in the range 0.1 to 1.0 V. The entire voltage drop appears across the 4 nm thickness of the membrane, so the electric field there,

$$V/\ell \simeq 25 \text{ to } 250 \text{ MV/m} \tag{1}$$

is enormous by macroscopic standards. (Sparks form in air at 3 MV/m.)

The elastic properties are represented by a Young's modulus, Y. The compression arising from the electric field across the membrane is given by (Crowley 1973)

$$(\ell/L)^2 \ln(\ell/L) = -\frac{\varepsilon V^2}{2YL^2} \tag{2}$$

which for small deflections

$$\delta\ell = \ell - L \ll L \tag{3}$$

takes the form

$$\frac{\delta\ell}{L} \simeq \frac{\varepsilon V^2}{2YL^2} \tag{4}$$

A plot of membrane thickness against electric pressure is shown in Figure 2.

An interesting aspect of the voltage dependence is the infinite compression (or failure) which occurs at a finite value of voltage, given by

$$\frac{\varepsilon V^2}{2YL^2} \simeq 0.18 \qquad (5)$$

This is a manifestation of an electro-elastic instability, since no stable equilibrium is possible at higher voltages. This instability occurs because the voltage is held constant while the opposite sides of the membrane approach each other, which causes the electric field and electric pressure to increase, further compressing the membrane. Above the critical voltage the membrane is no longer able to resist the additional compression, and it fails. Similar breakdown mechanisms also occur in soft solids such as plastics (O'Dwyer 1973).

The one-dimensional elastic model based on Hooke's Law offers a clear picture of the nature of the electroelastic failure of the membrane, but it seems a poor choice to describe the breakdown of a membrane with significant extension in the directions perpendicular to the compression. To meet this objection, the electroelastic instability was re-examined using a three-dimensional isotropic elastic model (Crowley 1973). The stability condition for the three-dimensional model is

$$\frac{\varepsilon V^2}{2YL^2} \simeq .17 \text{ to } .18 \qquad (6)$$

depending on the value of ν. This is essentially the same condition derived for the one-dimensional model.

EXPERIMENTAL CONFIRMATION

There are two principal predictions of the electroelastic model: The membrane should exhibit compression initially proportional to the square of the voltage, and it should fail in compression above a critical voltage. Because of the exceedingly small thickness of a biological membrane, it is extremely difficult to measure changes in thickness directly, so early confirmation of the model was sought in measurements of the electric capacitance of the membrane. For a thin insulating membrane, the capacitance per unit area,

$$C = \frac{\varepsilon}{\ell} \qquad (7)$$

is fairly large because ℓ is so small. Changes in thickness should be easily measured as the membrane is compressed, and these changes are, in fact, proportional to V^2 for low voltages, as shown in Figure 3 which is taken from Alvarez and Latorre (1978).

This figure gives results for a particular synthetic bimolecular lipid membrane (BLM) composed of the lipid glycerol monooleate, but it is typical of the voltage dependence obtained from measurements on most membranes. Since all of the properties of the membranes in equation 6 are known with the exception of the Young's modulus, results like Figure 3 can be used as an experimental determination of the Young's modulus of the membrane. For the membrane shown in that figure, as an example, these measurements correspond to a relatively high value of $Y \simeq 140$ MPa. Similar voltage dependence has been obtained by many others (Wobschall 1982, White 1974, Carius 1976). Thus it seems clear that the membrane can be treated as an elastic material compressed by electric forces.

The second major prediction of the model is failue of the membrane at a critical voltage which depends on the elastic properties of the membrane. At the time the model was proposed, data for elastic properties and breakdown voltages for BLM's were not available for the same preparations, but estimates based on individual reported values were in approximate agreement with the theory. Since then, experiments have been carried out on a number of natural and artificial membranes. A selection of results from these experiments is shown in Figure 4.

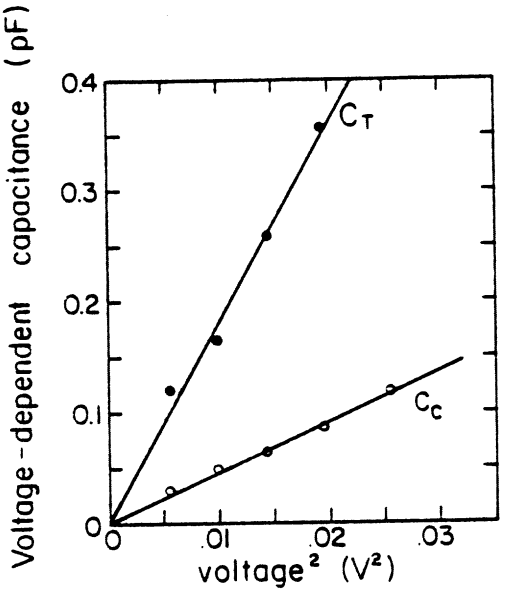

Figure 3. Capacitance variation with voltage for a stiff BLM

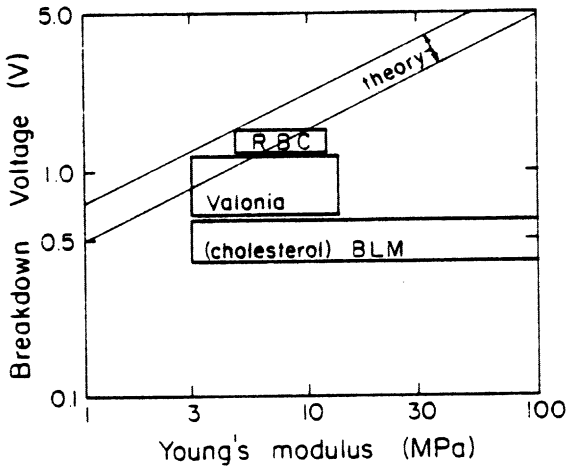

Figure 4. Breakdown predictions based on Young's modulus

From the range of measured values, represented by the boxes, it is clear that neither the Young's modulus nor the breakdown voltage can be predicted with great accuracy. Even so, the correlation between the two seems to justify the electroelastic model, especially for the biological cells (red blood cells and the alga Valonia). The synthetic membranes appear not to satisfy the electroelastic breakdown, especially for the high values of Young's modulus which have been obtained with very pure preparations.

DIFFICULTIES AND ALTERNATE MODELS

A closer look at the experimental evidence described above led to the suspicion that the homogeneous, istropic elastic model might be inconsistant with some of the phenomena observed in membranes. The first objections (Requena et al. 1975, Benz and Janko 1976) concerned the determination of Young's modulus by capacitance measurements. This method assumed that only compression of the lipid molecules contributed to capacitance changes. An alternate mechanism, however, relies on the observation that the synthetic membranes (BLM's) are not uniform across their surface. Rather, they contain numerous small microlenses of excess solvent, which are considerably thicker than the membrane itself. When a voltage is applied across the membrane, these microlenses grow as more solvent is squeezed from the bilayer structure. In this picture, the membrane thins not by elastic compression but by fluid flow through the membrane interior. The flow continues until the pressures in the lenses and the bilayer are equalized.

This flow is very slow because the membrane is so thin, and it may take seconds or minutes until it reaches equilibrium. When the voltage is applied for a shorter time (or at a higher frequency), these flows do not occur, and the membrane responds according to the elastic model. Measurements of the capacitance change for short times (Benz 1976, Carius 1976) suggest that the Young's modulus should be > 10 MPa, instead of < 1 MPa which is inferred from the DC measurements. According to the failure criterion, this would raise the breakdown voltage by a factor of exceeding $\sqrt{10}$. Similar conclusions can be drawn from measurements on BLM's made without solvent (Alvarez and Latorre 1978). Unlike the solvent BLM's, these preparations exhibit a capacitance much less dependent on applied voltage, indicating a stiffer membrane. As in the high frequency measurements on solvent BLM's, the apparent Young's modulus is much larger than initial estimates.

A second objection concerned the simplicity of the elastic model for the membrane. Even if a continuum model based on elasticity is valid for a material which is two molecules thick, it seems unlikely that a single elastic constant such as Young's modulus will be sufficient to describe the deformation and stability. In particular, the anisotropy of the bilayer structure seems to require an anisotropic elastic model. Such models have been proposed (Evans and Simon 1975a, b, Crowley 1976), but they have not proved particularly useful to date. The main problem with the use of more complex elastic models is the difficulty in determining the appropriate elastic constants for a small, inaccessible structure like a cell membrane.

An alternate approach to the anisotropy of the membrane is a microscopic model of the molecular arrangement (Crowley 1977, Abidor 1978, Sugár 1978, 1979). The most elaborate of these models (Sugár 1979) is based on the statistical mechanical description of the interactions between various parts of the modecules, such as the head groups and hydrocarbon tails, as well as the effects of elastic deformation and electric compression. This model also allows for phase transistions which alter the modecular arrangement. Since this is a thermodynamic model, it can express the electromechanical properties in terms of other thermodynamic coefficients related to thermal expansion or heats of reaction, quantities which are more easily measured. This model predicts large variations in Young's modulus with temperature (.5 to 9000 MPa), which seem to correlate with observed changes in membrane behavior. It also predicts the electrocompressive failure of the bilayer at voltages on the order of those observed.

VALIDITY OF THE ELECTROELASTIC MODEL

The preceding sections summarized a great deal of partially conflicting evidence on the usefulness of the electroelastic model of membranes. It is now time to take stock of the current situation, and to discuss where the model may justifiably be used. It will be helfpul to keep in mind that there are two types of membranes under consideration--the synthetic laboratory model membrane (BLM) and the membrane of a biological cell. Each has its own peculiar sources of variation. The BLM, for instance, is made from known chemical constituents, but it usually contains numerous microlenses of solvent scattered across the molecular bilayer. These films are inhomogeneous even on a microscopic scale, and the solvent is free to move between bilayer and lens. This process is much softer and much slower than elastic deformation of the membrane, since it involves fluid flow through small spaces. This picture of the membrane leads to thinning under applied voltage, but not to immediate breakdown, because after all the solvent is expelled the stiff lipid structures will support the electrocompression. Electroelastic breakdown can still occur, but the Young's modulus will be much larger than the apparent Young's modulus derived from low frequency measurements. When using high frequencies ($>$ 10kHz) or short pulses ($<$ 100μs), this problem does not arise, since the solvent does not have time to flow into the microlenses. Under these conditions, the breakdown voltage agrees with the apparent Young's modulus, confirming the model for use in the BLMs.

A similar conclusion can be drawn for explanations of breakdown as statistical defects in the membrane. Estimates of the time required for random pores to grow in response to an applied electric field suggest a delay of approximately 0.1 ms (Shchipunov and Drachev 1982). Such mechanisms may be important at low frequencies, but for fast pulses and high frequencies, they can have no effect. Thus the electroelastic effect is always present in BLM's, but it may compete with the microlens effect or statistical pore formation at low frequencies. For high frequencies or short pulses, it appears to be the only possible breakdown mechanism.

The second type of membrane is that surrounding a living cell. Unlike the BLM, this membrane may be composed of a large number of complex molecules, although the primary constituent is also a lipid. It is simpler in one respect, however;

it does not contain microlenses of excess solvent. Thus the principal source of difficulty with the synthetic BLM's is removed. As a result, there have been no reported experiments on biological cells which contradict the electroelastic model. Of course, experiments on living cells do not yield the precision possible with BLM's, so this conclusion may be modified as experimental techniques improve. Nonetheless, the electroelastic model is clearly the best available at the prsent time to explain the interrelations among compression, transport and breakdown under applied voltages.

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