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Electromechanical Stresses Produced in the Plasma Membranes of Suspended Cells by Applied Electric Fields

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Summary. We analyze the electrical and mechanical stress in the bounding membrane of a cell (or vesicle) in suspension which is deformed by an external applied field. The membrane is treated as a thin, elastic, initially spherical, dielectric shell and the analysis is valid for frequencies less than the reciprocal of the charging time (i.e. less than MHz), or for constant fields. A complete analytic solution is obtained, and expressions are given which relate the deformation, the surface tension and the transmembrane potential difference to the applied field. We show that mechanical tensions in the range which lyse membranes are induced at values of the external field which are of the same order as those which are reported to lyse the plasma membranes of cells in suspension.

Key Words membranes · electrical breakdown · lysis · electromechanical deformation · dielectrophoresis

Introduction

The rupture of the plasma membrane of an isolated single cell has important consequences for that cell. Permanent physical rupture of a cell's plasma membrane (lysis) may be caused by mechanical stresses in the plane of the membrane, or by electric fields across the membrane. Lysis due to isotropic surface tension may occur, for example, if a cell experiences a decrease in the osmotic pressure of the supporting medium. The surface tensions necessary to produce lysis are typically 5 mN/m (Evans & Skalak, 1980; Kwok & Evans, 1981; Wolfe & Steponkus, 1981; Gruen & Wolfe, 1982) but depend on the duration of application (Rand, 1964; Wolfe, Dowgert & Steponkus, 1985). Electrical breakdown of plasma membranes has been studied by several investigators (e.g. Neumann & Rosenheck, 1972; Knight & Baker, 1982). It is less common in physiological examples, although it has been suggested that some of the cells which are damaged in rapidly frozen solutions are lysed by the large transient electric fields which are produced at moving ice interfaces (Steponkus et al., 1984). Local, transient rupture of plasma membranes in the presence of deliberately applied sublethal electric fields is also interesting because it allows the artificial fusion of cells, and also allows the introduction into cells of such materials as drugs or DNA. The properties of cells in electric fields are also important in the consideration of sublethal fields. It is known that such fields cause cells to become transiently permeable, or "leaky" (Knight & Baker, 1982). Further, sublethal permeability allows for the loading of cells with drugs for specific delivery (Zimmermann, Pilwat & Vienken, 1980).

Membranes will rupture in the absence of mechanical stresses if the transmembrane potential is about one-half to one volt (the value depends on the duration of application). It is interesting to note that the membrane capacitance is typically 10 mF/m^2 (within a factor of two; reviewed by Smith, 1977), so the area energy density of electrical energy for lysis is typically 5 mJ/m^2, which is very similar to the mechanical energy density (5 mN/m), necessary for rupture. It is important, however, to distinguish between lysis produced by an electrical field applied directly across the membrane, and electromechanical lysis caused by the combined electrical and mechanical stresses exerted on the membrane of a cell exposed to an external field. In this paper we shall argue that, under specific conditions, lysis of a cell in the presence of an electric field is largely due to isotropic mechanical surface tension produced in deforming the cell rather than due to the electric field produced across the membrane. The analysis may be applied to both constant (d-c) and sufficiently low frequency alternating (a-c) fields.

The electrostatics of a thin, spherical, dielectric shell in an electric field which is uniform at infinity have been applied to cells in suspension since the work of Fricke and Morse (1925) and Cole (1928). Since then all published work on lysis of cells in suspension has implicitly assumed that lysis in an

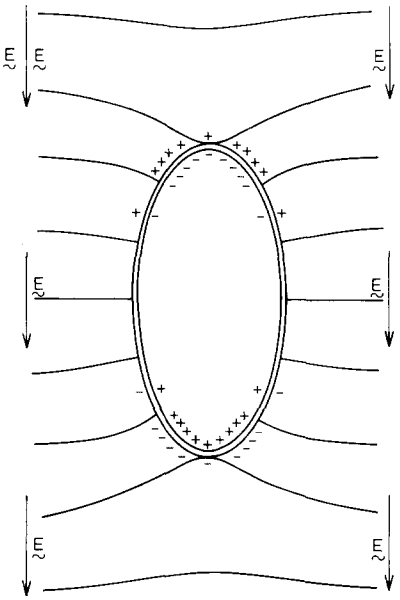

Fig. 1. This sketch shows the equipotentials near a thin spheroidal dielectric shell in a field which is uniform at infinity. The thickness of the shell has been very much exaggerated. The electric field acting on the induced charge at the interior face is greater than that at the exterior face, and thus a net force is produced which acts to elongate the shell in the direction of the field. In terms of the generalized co-latitude v we refer to the points on the ellipsoid at $v = 0$ and $v = \pi$ as the "poles" and the circle at $v = \frac{\pi}{2}$ as the "equator"

electric field is due solely to the direct electrical effects on the membrane. The observation by Steponkus (*personal communication*) that isolated protoplasts are deformed in the presence of an a-c electric field indicates the presence of mechanical stresses in the membrane. These stresses are produced when the charges induced by an applied electric field interact with that field. To our knowledge a detailed analysis of the electromechanical stresses and strains produced in the plasma membrane of a cell in a suspension upon which is imposed an electric field has never been published. This paper presents an analysis of the electrostatics, mechanical stresses and deformation of a cell in a uniform electric field.

Theory

We shall assume that the plasma membrane may be described as a thin, initially uniform dielectric shell separating two solutions whose conductivity is much greater than that of the membrane. (This is a fairly accurate model for describing the macroscopic properties of a large number of cell types.) A uniform electric field is applied to the suspending solution, inducing charge distributions on the membrane surfaces. The membrane (here treated as a capacitive element) and the solution (here a resistive element) have a charging time or time constant T which is of the order of $D\rho C$ where D is a typical dimension of the system (e.g. the radius of the cell), ρ is the resistivity of the conducting medium and C is the capacitance per unit area of the membrane. For frequencies much less than $1/T$, the impedance of the capacitor (the membrane) is much greater than the resistance of the solutions through which it is being charged, the charge distribution is in phase with the field, and the charge distribution at any time is that of the steady-state (d-c) calculation. Taking $D = 10\ \mu\text{m}$, $\rho = 10\ \Omega\text{m}$, and $C = 10\ \text{mF} \cdot \text{m}^{-2}$ gives $1/T = 1$ MHz. At frequencies higher than this, the field in the membrane and that in the external solution are no longer in phase, and the electric field in the external solution cannot be neglected in comparison with that in the membrane. A discussion of the electrical behavior of the membrane-solution system at high frequencies is given by Sackmann et al. (1984).

At low fields, no appreciable current flows across the membrane, and the cell interior is an equipotential volume. (At high fields, the membrane may become leaky and different boundary conditions apply. We shall discuss this possibility later.) The interactions of the electric field with the induced charges on the membrane deform the cell and impose mechanical stresses in the membrane. It will be assumed that the cell deforms to the spheroidal shape shown in Fig. 1. The assumption that a cell will deform to a spheroid is in empirical agreement with microscopic observations of deformed cells (Steponkus and Barnaby, *unpublished observations*). It might be argued, however, that an approach using the calculus of variations could be used, given the boundary conditions, to determine the shape *ab initio*. The difficulties associated with solving Laplace's equation with these boundary conditions (a complicated problem even in spheroidal coordinates), would make this calculation tedious, if indeed possible.

Briefly, the elements of our analysis are as follows: first, the electrostatic solution yields the forces acting on both faces of the membrane. These do not quite cancel, and the resultant force tends to elongate the cell in the direction of the electric field. To deform from a spherical shape, the cell both decreases its volume (this is opposed by an internal pressure excess) and increases its area (this is opposed by membrane surface tensions due to its elasticity). The internal pressure excess, the net electric force per unit area and the surface tensions are related by the generalized Young-Laplace law. The membrane area is increased not only by the surface tension produced in it as it deforms, but also by the action of the normal electric forces acting on both

surfaces. Analysis of all the above effects together produces a set of simultaneous equations which is solved to give the deformation produced by any applied field.

Explicitly, the system must conform to the following constraints:

1. ELECTROSTATICS. Except at interfaces, the potential Φ must satisfy Laplace's equation (in spheroidal coordinates) subject to the following boundary conditions:

a. The gradient of Φ must be uniform at ∞.

b. The gradient of Φ in the normal direction vanishes on the solution side of membrane-solution interfaces.

c. Φ = constant at the membrane's inner surface.

2. ELECTROSTRICTION. The field acting normal to the surface acts on the induced charge to compress the membrane along the normal. The inhomogeneous field in the membrane is also responsible for a tangential variation in membrane stress. The thickness and area of the membrane may vary. It is assumed in the main text that the membrane has a constant volume, i.e. that Poisson's ratio is 1/2, but the general case is treated in Appendix 1. Change in area is thus dependent to first order on variations in membrane thickness, as is the electric field in the membrane. All other quantities vary as higher orders of the proportional change in thickness and such variations are ignored because of the small size of the changes in thickness that can be withstood by the membrane.

3. ELASTICITY. The membrane is treated as a "fluid mosaic." Thus lateral diffusion is permitted, and only isotropic surface tensions are allowed; these obey an elastic law.

4. VOLUMETRIC COMPRESSIBILITY. An effective bulk modulus is attributed to the cellular interior. The real bulk modulus of the cellular contents is so much larger (~2 GPa) than the pressures considered here that the extent of compression of the contents is negligible. The membrane is, however, semipermeable, and so a positive pressure inside the cell causes water to leave the cell. A time τ characteristic of the approach to osmotic equilibrium depends on the cell size and the hydraulic conductivity of the membrane, and may be seconds to minutes (e.g. Wolfe, Dowgert & Steponkus, 1986). For times very much less than τ, the volume may be regarded as constant, and this condition may be obtained from the analysis by setting the bulk modulus infinite. For an ideal osmometer in osmotic equilibrium, it is readily shown that the bulk modulus equals the osmotic pressure of the suspending solution. Setting the bulk modulus equal to the osmotic pressure yields the behavior which is predicted for osmotic equilibrium.

5. TENSION AND PRESSURE. We treat the membrane as a thin shell, thus, at any point in the membrane, the net normal force per unit area, the membrane bifacial surface tension and the local curvature are related by the Young-Laplace equation. (We thus neglect curvature stresses in the membrane in comparison with stresses in the plane of the membrane. For small vesicles or organelles whose radii are comparable with the membrane thickness, this approximation would be invalid.)

Analysis

We use the prolate spheroidal coordinates u, v and ϕ which are related to Cartesian coordinates by

$$\begin{aligned} x &= f \sinh u \sin v \cos \phi \qquad u \geq 0 \\ y &= f \sinh u \sin v \sin \phi \qquad 0 < v < \pi \\ z &= f \cosh u \cos v \qquad -\pi < \phi < \pi \end{aligned} \tag{1}$$

and we use the convenient substitution

$$\mu = \cosh u, \text{ and } \nu = \cos v.$$

In the general case, the inner and outer surfaces are spheroids of differing eccentricities. We give the inner and outer surfaces eccentricities $1/\mu_i$ and $1/\mu_o$ where $f\mu_o - f\mu_i = h$. (In practice, the membrane thickness h is very much less than the dimensions of the cell, and can vary by only a few percent without rupture. Further, the variation in thickness occurs only in second- and higher-order terms in the analysis.)

ELECTROSTATICS

Provided that the conductivity of the membrane is very much smaller than those of the cell interior and the suspending medium, the current flowing through the membrane is negligible in comparison with that in the external medium. It follows that the interior face of the plasma membrane ($f\mu = f\mu_i$) is an equipotential (which we choose to be zero[1]) and that the field is perpendicular to the membrane at

[1] In a living cell in the absence of an imposed electric field, the potential difference across the membrane is usually uniform and small (less than about 0.1 V), with the cytoplasm negative. Because of the finite time constant of the membrane, the sudden application of an external field cannot produce an abrupt change in the total charge on the inner membrane surface. In this calculation we set the cytoplasmic potential equal to zero to retain symmetry in the equations. The original transmembrane potential perturbs this symmetry and imposes a larger field across the membrane at the high Φ pole than at the low Φ pole.

the outer surface ($f\mu = f\mu_o > f\mu_i$). Remote from the cell, the field is in the $v = 0$ direction and has the value E_∞ everywhere. We require a solution to Laplace's equation

$$\nabla^2\Phi = 0. \tag{2}$$

In the spheroidal geometry of Eq. (1), Laplace's equation becomes

$$\frac{(\mu^2 - 1)(1 - \nu^2)}{(\mu^2 - \nu^2)}\left\{\frac{\partial}{\partial\mu}\left[(\mu^2 - 1)\frac{\partial\Phi}{\partial\mu}\right] + \frac{\partial}{\partial\nu}\left[(1 - \nu^2)\frac{\partial\Phi}{\partial\nu}\right]\right\} + \frac{\partial^2\Phi}{\partial\phi^2} = 0. \tag{3}$$

The solution is separable, and may be expressed as sums of Legendre functions of the first and second kind. It may be shown that, subject to the above boundary conditions and to radial symmetry about $v = 0$, the general solution is

$$\Phi = 0 \qquad \mu < \mu_i$$

$$\Phi = E_\infty f C_2\left[\frac{\mu Q_1(\mu_i)}{\mu_i} - Q_1(\mu)\right]\cos v \qquad \mu_i < \mu < \mu_o$$

$$\Phi = E_\infty f[\mu - C_1 Q_1(\mu)]\cos v \qquad \mu_o < \mu$$

where

$$Q_1 = \frac{\mu}{2}\ln\left(\frac{1 + \mu}{\mu - 1}\right) - 1$$

$$C_1 = \left[\frac{\mu_o}{1 - \mu_o^2} + \frac{1}{2}\ln\left(\frac{\mu + 1}{\mu - 1}\right)\right]^{-1}$$

$$C_2 = \frac{1 - \frac{C_1}{\mu_o}Q_1(\mu_o)}{Q_1(\mu_i)/\mu_i - Q_1(\mu_o)/\mu_o}. \tag{4}$$

That this is a solution may be verified by substitution; the analysis is given in greater detail by Bryant (1985).

The potential drop across the membrane V as a function of the generalized co-latitude v is obtained from Eqs. (4). These equations may also be manipulated to give expressions for the electric field and charge distributions at the membrane surfaces (Bryant, 1985). These expressions are not explicitly required for the current analysis, however, because the electric forces can be expressed in terms of the transmembrane potential difference and the membrane specific capacitance (derivation in Appendix 1).

We use the expressions thus obtained for all the results reported in this paper. We have also solved the problem using the mechanical constraints for a small finite eccentricity but using the solution of Laplace's equation for spherical geometry, which is algebraically much simpler (Maxwell, 1892). We have used this case as a limit to check the general calculations, but it has other uses. Because the membrane lyses at small eccentricities, the results are not very different from the general solutions, and are rather easier to compute. This analysis is reported by Bryant (1985).

The Net Local Electric Force on the Membrane

An applied electric field induces surface charges of opposite sign on each face of a membrane as shown in Fig. 1. The electric field acts on each of these surface charges (and on the displacement charges at the dielectric boundary) and the normal components of the two forces[2] are in opposite directions. As we shall show, the normal components do not cancel exactly.

The pressure p exerted by electric fields acting on an incompressible fluid satisfies

$$\nabla p = -\tfrac{1}{2}E^2\nabla\varepsilon + \rho\underset{\sim}{E}$$

(Durand 1953; Landau & Lifschitz, 1960). The total electric force per unit area (P_n) exerted on each face of the membrane is readily calculated by integration across the dielectric boundary and the region containing the induced charge. This integration (Appendix 1) gives

$$P_n = \varepsilon E_n^2/2 \tag{5}$$

where E_n is the normal component of the field in the membrane at the interface and where ε is the permittivity of the membrane. The normal component of the electric force per unit area P_n acts towards the interior of the membrane in all cases. The net force per unit area P_E acting on both faces is thus

$$P_E = \varepsilon(E_i^2 - E_o^2)/2 \tag{6}$$

[2] The tangential component of the field at the interior membrane face is zero, but the tangential component of E at the exterior face is zero only at the poles. In the external solution, ions (including those providing the surface charge) move in response to the applied field with a velocity which is constant at any point in space. The equal and opposite force is provided by the drag in the solution. Nonetheless, because the membrane thickness is much smaller than the cell radius, the tangential component of the field is negligible in comparison with the normal component everywhere except at the equator, where the latter is zero.

where E_i and E_o are the normal fields at the inner and outer surfaces, respectively. P_E acts outwards at both poles and is zero at the equator.

$(E_i - E_o)$ is very much less than E_o, so P_E is very much less than P_n. Noting that $E_i \approx E_o \approx V/h$ and substituting $C = \varepsilon/h$ (where C is the capacitance per unit area) in Eq. (5) gives the convenient expression:

$$P_n = CV^2/2h. \tag{7}$$

Elasticity and Electric Deformation of the Membrane

Electric fields and mechanical tensions both act to increase membrane area. Biological membranes studied to date deform elastically over short periods and have low ($\lesssim$1 mN/m) or zero bifacial tensions in normal physiological conditions.

As mentioned above, electrical forces acting on the two charged faces of the membrane in each case act towards the membrane interior. These act to decrease the membrane thickness h. We assume that the membrane has a negligible volume compressibility. (The volumetric moduli of bulk alkanes, which are similar to the interior of the lipid bilayer region, are of the order GPa). The area A must therefore increase in the presence of a field. We impose the constraint of constant membrane volume by setting $hA = h_oA_o$ where the subscript refers to the undeformed membrane.

The area A of a given quantity of membrane in the absence of an electric field is determined by a mechanical equilibrium between attractive and repulsive intermolecular forces. This is achieved at an area $A = A_o$ at which the total energy U is a minimum (defined as zero). $U(A)$ may be approximated near the minimum by a Taylor expansion. The assumption of an elastic law corresponds to neglecting the third and higher order terms. Thus

$$U = k_A(A - A_o)^2/2A_o$$

and taking derivatives the bifacial surface tension is

$$\gamma = k_A(A - A_o)/A_o. \tag{8}$$

Membranes are anisotropic and their response to combinations of stresses in different directions is, in principle, quite complicated (*see* Evans & Skalak, 1980). To the knowledge of the authors, however, the components of the stress tensor of biological membranes are not yet known. Assuming isotropy in the normal direction, the general expression for area deformation in response to stresses P_n and γ is

$$(A - A_o)/A_o = (\gamma + \sigma hP_n/(1 - \sigma))/k_A \tag{9}$$

where σ = Poisson's ratio.

For an incompressible solid ($\sigma = 1/2$) or for an incompressible fluid interior bound by surfaces governed by a surface elasticity law (Eq. 8), Eq. (9) becomes

$$(A - A_o)/A_o = (\gamma + hP_n)/k_A = (\gamma + CV^2/2)/k_A \tag{10}$$

(*see* Appendix 1).

From Eq. (9), we may write

$$\frac{dA}{dA_o} = \frac{\gamma + \sigma CV^2/2(1 - \sigma)}{k_A} + 1 \tag{11}$$

where differential elements of area dA have been used in place of A because in the current case γ and V vary continuously over the surface. The total area A of the cell membrane (whose area is A_o under negligible electrical and mechanical stress) is then

$$A = \int_{\text{over area}} dA = \int dA_o[(\gamma + \sigma CV^2/2(1 - \sigma))/k_A + 1] = g4\pi R^2 \tag{12}$$

where R is the radius of the undeformed spherical cell and g is a numerical factor. The solution to the general case is outlined in Appendix 2. In the main body of text only the case $\sigma = 1/2$ will be considered.

Distribution of Tension in the Membrane

Mechanical equilibrium of any element of the membrane requires (Appendix 1) that the normal pressure $P_n = (CV^2/2h)$ and the tension γ are related by

$$d\gamma = -h \cdot dP_n$$

so over the whole surface

$$\gamma + CV^2/2 = \text{constant}.$$

To obtain the set of independent equations for this problem, one need only apply the constraints to two points on the surface. The easiest cases are the pole and the equator (subscripts p and e, respectively). Noting that $V = 0$ at the equator, the following equation results:

$$\gamma_p = \gamma_e - CV^2/2. \tag{13}$$

Geometry and Mechanical Constraints

Consider the prolate spheroid shown in Fig. 1. The standard mensuration formulae for volume $\mathcal{V}$ and area A are:

$$\mathcal{V} = \tfrac{4}{3}\pi a b^2 \tag{14}$$

$$A = 2\pi[b^2 + (ab \cdot \sin^{-1} e)/e] \tag{15}$$

where $e = (1 - b^2/a^2)^{1/2}$ is the eccentricity, and a and b are the semi-major and semi-minor axes, respectively.

Equating the expressions for A from Eqs. (12) and (15) gives one of the constraints. Elongation of the cell in response to the electric forces must increase the area and/or decrease the volume. Although the electric field causes an increase in area itself, larger area increases are opposed by the elastic response of the membrane (Eq. 8). Reduction in the interior volume of the cell is opposed in the short term by the very large bulk modulus of aqueous solutions, and in osmotic equilibrium by the changes in water activity produced by water efflux. As explained above, both these cases can be regained from the analysis if an effective bulk modulus β is assumed for the cell, where the change in volume $\Delta\mathcal{V}$ produced by a cytoplasmic (internal) hydrostatic pressure P and the volume $\mathcal{V}_o$ when $P = 0$ are related by

$$\Delta\mathcal{V}/\mathcal{V}_o = -P/\beta. \tag{16}$$

In order to apply the Young-Laplace equation to this problem we also need to know the curvature of the ellipsoid. Although curvature and tension vary continuously over the surface, only two independent relations among the parameters can be obtained from the Young-Laplace equation. Therefore one need only determine the curvature ζ_p at the pole ($v = 0$) and the curvature ζ_e at the equator ($v = \pi/2$). The curvature at any point is the sum of the reciprocals of the principal radii of curvature. It may easily be shown that:

$$\zeta_p = -2a/b^2 \tag{17}$$

$$\zeta_e = -1/b(1 + b^2/a^2). \tag{18}$$

The Young-Laplace relation states that the normal force per unit area acting at an interface is equal to the surface tension times the curvature in the interface. We apply it to the whole (very thin) membrane taking the bifacial surface tension and the curvatures from Eqs. (17) and (18). At the equator, the hydrostatic pressure P in the cell interior is the only normal force acting, but at the poles this is augmented by the normal component of the net electrical force per unit area P_E. Thus

$$P = -\zeta_e \cdot \gamma_e \tag{19}$$

and

$$P + P_E = -\zeta_p \cdot \gamma_p. \tag{20}$$

To explain the simultaneous solution of the preceding equations we shall combine them in summarized form.

(i) $V_p = V_p(E_\infty, \mu, f)$ at $v = 0$

(ii) $\gamma_e = \gamma_e(E_\infty, \mu)$

(iii) $\gamma_p = \gamma_e - CV_p^2/2$

(iv) $P_n = P_n(E_\infty, \mu, P)$ at $v = 0$; $P_n = 0$ at $v = \pi/2$

(v) $P + P_E = -\zeta\gamma$ at $v = 0$ and at $v = \pi/2$

(vi) $\int dA = g4\pi R^2$

(vii) Geometrical equations relating f, μ, a, b, h, A and R including:

$$a = f\mu, \qquad b = f\sqrt{\mu^2 - 1}$$

$$f\mu_o = f\mu_i - h, \quad f = f(\mu, R).$$

The solution may be found by using various equations (vii) to eliminate f from Eq. (i). Equations (iv) and (v) may be combined to yield an equation relating E_∞, μ, P, γ_e, γ_p. Using (i), (ii) and (iii) then leads to equations of the form

$$P = P(E_\infty, \mu) \quad \gamma_e = \gamma_e(E_\infty, \mu).$$

For convenience we introduce the substitution $\omega = E_\infty^2/K_2$, where $K_2 = \beta/(RCF_2)$ and $F_2 = F_2(\mu)$ where the explicit function is given in Appendix 3.

We can now substitute into Eq. (vi), and equate with the standard mensuration for a prolate spheroid. After this and some algebra the following solution results:

$$K_4\omega^3 - \omega^2 - (2 + K_4)\omega + (K_3 - 1) = 0 \tag{21}$$

where

$$K_4 = K_1K_2/k_A, \; K_1 = K_1(C, R, \mu)$$

$$K_2 = K_2(C, R, \mu), \; K_3 = K_3(\mu)$$

and where the explicit forms of the constants K_1, K_2 and K_3 are given in Appendix 3.

Thus the problem has an analytic solution, which for algebraic convenience is expressed with the deformation parameter μ as the independent

variable. The γ and all other variables are readily found if the physical parameters β, R, k_A, and C are given, by finding a solution for the cubic Eq. (21).

Discussion and Conclusions

The use of the laws of electrostatics in these calculations limits the application of the results to either d-c fields or to a-c fields with a frequency much less than the reciprocal of the charging time T of the membrane—typically about 1 MHz (Sackmann et al., 1984). The analysis is also restricted to uniform fields, i.e. to fields whose proportional variation is small over a scale of one cellular diameter. In a uniform field, the electric forces acting on each end of the cell are equal in magnitude but opposite in direction; they thus give rise to an elongation of the cell but, as the total electric force on the cell is zero, the cell does not move. In nonuniform fields, the force acting outwards is greater at the end of the cell in the more intense field, and so the net force acts to move the cell towards the region of most intense field. This phenomenon is (one type of) dielectrophoresis, which is discussed by Pohl (1978). In this paper we limit our discussion to the deformation and lysis of cells in d-c or low-frequency fields which are uniform or near uniform.

The Dependence of the Deformation of the External Electric Field

The most convenient variable for describing the deformation is the ratio a/b of the major and minor axes. The product $E_\infty R$ will be used to represent the electric field (where R is the initial radius) since, to a good approximation, several other variables scale to R.

Figure 2 shows a plot of the deformation of the cell as a function of the applied electric field. From the graph, it can be seen that for low electric fields, the axis ratio is approximately proportional to the field (up to $E_\infty R$ about 300 mV). For greater $E_\infty R$, the slope increases rapidly as the applied electric field is increased, provided of course that the cell survives such a deformation. (The large deformations (a/b) caused by relatively modest fields are in part a result of the very weak dependence on the eccentricity of the area/volume ratio of a spheroid. For constant volume, an increase in area of 1% results when (a/b) is 1.28.)

Figure 2 also shows the effect of differing bulk moduli and cell size. As we have argued above, the volumetric modulus of the fluid itself is very large, but in osmotic equilibrium, the effective bulk modulus of the cell volume is the osmotic pressure. For a cell with $R = 10$ μm and $\Pi = 500$ kPa (typical physiological values), there is little difference between the two results. This is because the hydrostatic pressure which can be supported by the membrane of a large cell is much smaller than physiological values of Π (Wolfe & Steponkus, 1983). Small cells, however, have larger curvatures than large cells, so, from the Young-Laplace equation, small cells are capable of supporting larger hydrostatic pressures, and thus will reduce their internal volumes by larger fractions as they approach osmotic equilibrium. This allows somewhat larger deformations at the same value of $E_\infty R$ (Fig. 2). It can nevertheless be seen that, even at osmotic equilibrium, the relation of deformation to the scaled field is similar for cells of a range of sizes and osmotic pressures. This is a useful result: a single relation can be used for calculations for cells of differing sizes, and will remain fairly accurate for all but the smallest cells, ($R \ll 5$ μm)[3], under electric fields large enough to produce lysis. The rest of the calculations use the following values for the parameters: $R = 10$ μm, $k_A = 200$ mN/m, $C = 8$ mF/m^2 and $\beta = 10^{10}$ Pa. (For

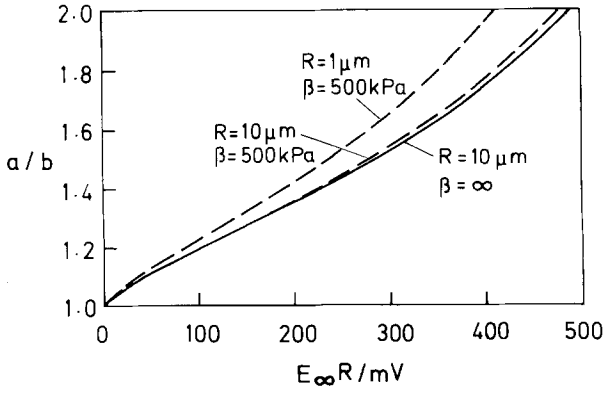

Fig. 2. The ratio of semi-major to semi-minor axes (a/b) is plotted against the scaled field $E_\infty R$ (the external field times the original radius). If the effective bulk modulus β is infinite, the curve is not strongly dependent on the size of the cell. For cells with $R \gtrsim 10$ μm, the effect of changing from 500 kPa to infinity is small. The effect is greater for smaller cells

[3] Although large deformations involve lethal tensions for cells with elastic membranes, vesicles may be produced in solutions of very low osmotic pressure and may thus suffer greater deformations. For large electric fields the gradient of the deformation increases quickly with field. Indeed, this analysis predicts critical behavior at large deformation, i.e. an infinite slope in a/b *vs.* $E_\infty R$. If the field were increased to this point and subsequently decreased, the deformation would continue to increase. After this point it is not certain what would happen, as the first-order approximations begin to break down. Details are given by Bryant (1985).

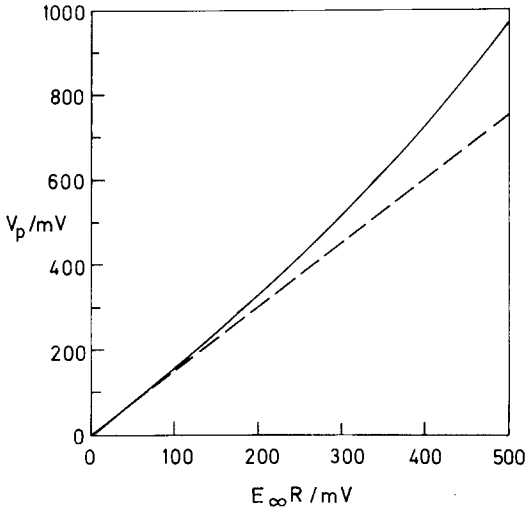

Fig. 3. The potential difference across the membrane at the poles (V_p) is plotted as a function of the scaled field $E_\infty R$. The dashed line shows the result for an undeformable sphere, i.e. $V_p = 3E_\infty R/2$

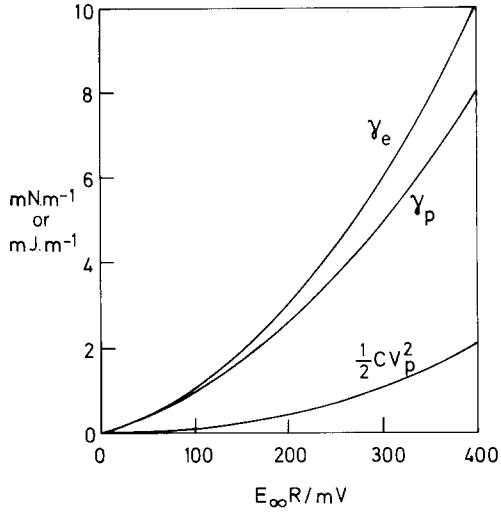

Fig. 4. The bifacial surface tension (or mechanical energy per unit area) at the poles (γ_p) and at the equator (γ_e) are plotted as a function of the scaled field $E_\infty R$. The electrical energy per unit area of membrane ($CV^2/2$) at the poles is shown on the same scale for comparison

all practical purposes, this gives the same results as β = infinity.)

Membrane Potential Difference and Lysis

In any discussion of purely electric breakdown, the most important physical parameter is the transmembrane voltage V which is maximal at the poles and zero at the equator. Figure 3 shows the maximum potential difference (that is the polar voltage V_p) plotted against the field. (The mechanical stress is proportional to the square of the field so, for a-c fields, the root mean square value of the potential must be used.) For comparison, the calculated voltage for a spherical membrane is also given (that is $V_p = 3E_\infty R/2$). For any particular electric field the voltage across the membrane is higher than that calculated for a sphere. This can be qualitatively explained by the elongation of the cell as V_p must increase with the product $E_\infty R$. For low field, the electrostatics of the sphere give a reasonably good approximation.

Figure 4 shows the bifacial surface tension at the poles (its minimum value) and at the equator as a function of the electric field. Note that potentially lethal membrane tensions ($\gtrsim$4 mN/m) are produced at $E_\infty R \gtrsim 240$ mV. At this field, the transmembrane potential at the poles is about 350 mV and less elsewhere. The potentials reported to cause dielectric breakdown in membranes are in the range $\gtrsim$500 mV (e.g. Coster & Zimmermann, 1975; Dressler et al., 1983). It is thus quite possible that the mechanical tension produced by the fields necessary to produce these potentials contributes significantly to the membrane breakdown.

In one theory of membrane lysis (Abidor et al., 1979 and Petrov, Mitov & Derzhanski, 1980; after Derjaguin & Gutop, 1962), lysis depends on the sum of electrical and mechanical energy densities in the membrane; i.e. on $\gamma + CV^2/2$. The maximum (polar) electrical energy density ($CV_p^2/2$) is shown on the same scale in Fig. 4: it is substantially less than the mechanical energy density which takes values between γ_p and γ_e. This theory suggests that the chance of lysis per unit area has a functional dependence on tension which is stronger than linear. If area-weighted average values are taken using such strong dependences, the relative importance of tension is even greater (Bryant, 1985).

At this point we call attention again to the assumption that the membrane has a conductivity which is much lower than that of the solutions on either side. As the tension approaches the value necessary to lyse the membrane, it is possible (and indeed likely) that the membrane becomes leaky and that a nonnegligible current flows through it, causing a finite potential difference across the cytoplasm. In such a case, the electric deforming force would be rather less than that calculated here, and the transmembrane potential would be likewise reduced. It is therefore possible that the application of modest electric fields cause transient increases in membrane permeability and the possible loss of internal solutes. Such effects, like rupture, would be

due in large part to the mechanical stress and not simply a result of the electric field.

This caveat does not change the qualitative conclusion of this study: that mechanical stress induced in the membrane of a spherical cell in an applied electric field is at least as important in determining membrane lysis as is electric stress *per se*. We therefore conclude that measurements of the field necessary to lyse cells in suspension are not sufficient to calculate the transmembrane voltage which alone would cause membranes to break down. It is possible that estimates of breakdown potential gained from application of Laplace's equation to suspensions of cells in electric fields may need to be considered in the light of these findings.

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Appendix 1: Stresses and Strains in a Membrane in an Electric Field

The force $d\underset{\sim}{F}$ due to the presence of an electric field $\underset{\sim}{E}$ which acts on a volume element $d\mathcal{V}$ with permittivity ε and charge density ρ is

$$d\underset{\sim}{F} = d\mathcal{V}(\rho\underset{\sim}{E} - \tfrac{1}{2}\underset{\sim}{E}^2\nabla\varepsilon) \tag{A1}$$

where the two terms are due to $\underset{\sim}{E}$ acting on, respectively, the net charge and the induced dipoles in the media (Durand, 1953; Landau & Lifshitz, 1960). We shall consider the normal component of the force per unit area p_n due to a normal electric field $\underset{\sim}{E}$ acting across an interface in the $y - z$ plane, so Eq. (A1) gives

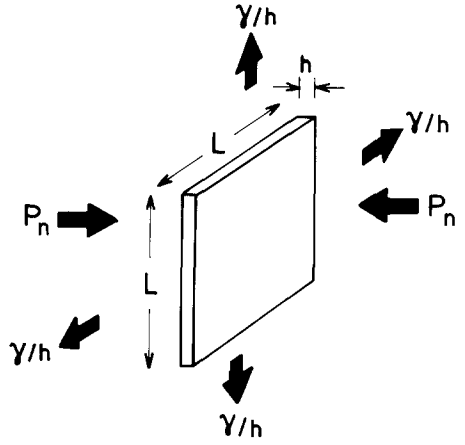

Fig. A1. The stresses acting on a small element of membrane: a square with side L and thickness h. γ is the total surface tension (i.e. the force per unit length acting to stretch the membrane) and P_n is the normal stress

$$dp_n = E_n \rho dx - \tfrac{1}{2} E^2 d\varepsilon. \tag{A2}$$

Medium 1 occupies $x < 0$ and is an insulator with $\rho = 0$ for $x < 0$. Medium 2 ($x > 0$) is an ionic solution, and contains a charge distribution near $x = 0$ which is considered to be contained within $\delta < x < l$ where l is several Debye lengths. The dielectric permittivity ε is assumed to vary from ε_1 (the bulk value of the insulator) to ε_2 (the bulk value for the solution) over a range $-\delta < x < +\delta$ which is very much smaller than the Debye length and so contains negligible charge. (The effects of Born energies and a Stern layer prohibit substantial charge density near the dielectric boundary.) The total normal force per unit area Δp_n across the interface is

$$\Delta p_n = -\int_{x=-\delta}^{\delta} \frac{1}{2} E^2 d\varepsilon + \int_{x=\delta}^{l} E \rho dx.$$

In the region $-\delta < x < \delta$, the electric displacement $D = \varepsilon E$ is constant so $d\varepsilon = -D dE/E^2$. For $x > \delta$, $\varepsilon dE = \rho dx$ so

$$\Delta p_n = \int_{x=-\delta}^{\delta} \frac{D}{2} dE + \int_{\delta}^{l} \varepsilon E dE = \frac{1}{2} D[E(\delta) - E(-\delta)] + \frac{\varepsilon(\delta)}{2} [E^2(l) - E^2(\delta)].$$

Now if $\varepsilon(-\delta) = \varepsilon_1$ and $\varepsilon(\delta) = \varepsilon_2$, and using $\varepsilon(-\delta)E(-\delta) = \varepsilon(\delta)E(\delta)$, this becomes

$$\Delta p_n = -\frac{\varepsilon_1 E^2(-\delta)}{2} + \frac{\varepsilon_2 E^2(l)}{2}. \tag{A3}$$

The membrane is an insulator so the normal component of the current density near the interface is zero so $E(l) \to 0$ for $l \ll R$, and so the last term in Eq. (A3) is neglected. In the application to the spherical, thin, dielectric shell, the radius R is several thousand times greater than the thickness h. The ratio of the normal field to the tangential field is infinite on the inner surface, and is of order R/h on the outer surface everywhere away from the equator, where the normal component of E is zero. Thus the normal component of the force per unit area exerted by the field on each face of the membrane is

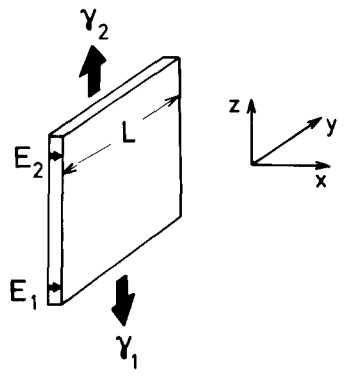

Fig. A2. Variation in the transmembrane electric field E and the tension γ

$$P_n = \frac{\varepsilon E_n^2}{2} \tag{A4}$$

where ε is the permittivity of the membrane and E_n is the normal component of $\underset{\sim}{E}$ at that face. Equation (A4) applies everywhere except near the equator where P_n is negligible.

Changes in Area

Figure A1 shows a thin square prism of side L and thickness h subject to a normal stress P_n to the square faces and normal stresses $-\gamma/h$ to the outer four faces where γ is the tensile force per unit length applied to these sides. If the material comprising the prism is isotropic with Young's modulus Y and Poisson's ratio σ, then the strain produced in L is

$$\frac{\Delta L}{L} = (1 - \sigma) \frac{\gamma}{Yh} + \frac{\sigma P_n}{Y}. \tag{A5}$$

For small deformations, the strain in the area ($A_o = L^2$) is $\Delta A/A_o = 2\Delta L/L$ and putting $k_A = Yh/2(1 - \sigma)$ gives

$$\frac{\Delta A}{A_o} = \frac{1}{k_A} \left[\gamma + \frac{P_n h \sigma}{(1 - \sigma)} \right].$$

Noting that $P_n h = CV^2/2$ (*see* text), this gives the electromechanical stretching of the membrane

$$\frac{\Delta A}{A_o} = \frac{1}{k_A} \left[\gamma + \frac{1}{2} CV^2 \frac{\sigma}{(1 - \sigma)} \right]. \tag{A6}$$

For a material which is volumetrically incompressible, $\sigma = 1/2$ and so Eq. (A6) becomes Eq. (10). [If the membrane interior is considered as an isotropic incompressible fluid then Eq. (10) is also obtained.]

Variation in γ

The induced dipoles in a dielectric medium are attracted towards the high-field regions of an inhomogeneous field. Thus the electric field draws material in the membrane towards the poles. This tendency is opposed by a variation in membrane tension—γ is greatest away from the poles. Figure A2 shows an element of

membrane subject to variation with z of transmembrane potential V and membrane tension γ. Suppose it moves a distance dz. The work done by the tensions γ_2 and γ_1 on opposite edges is $(\gamma_2 - \gamma_1)ldz$. The increase in electrical potential energy is $(\frac{1}{2}\varepsilon E_2^2 - \frac{1}{2}\varepsilon E_1^2)$ $hldz$ where $E_2 = V_2/h$ and $E_1 = V_1/h$ are the transmembrane fields. In mechanical equilibrium with no other forces acting

$$\gamma_2 - \gamma_1 + \tfrac{1}{2}\varepsilon h(E_2^2 - E_1^2) = 0$$
$$\therefore \gamma_2 - \gamma_1 = \tfrac{1}{2}C(V_1^2 - V_2^2) \tag{A7}$$

or

$$\gamma + \tfrac{1}{2}CV^2 = \text{constant} = \gamma_e.$$

[If the membrane interior is considered as an isotropic fluid subject to a pressure P_n then mechanical equilibrium requires that $h(P_{n_2} - P_{n_1}) = \gamma_2 - \gamma_1$, and Eq. (A7) follows directly.]

Appendix 2: The Area Change due to Electric Compression

From Appendix 1,

$$\frac{\Delta A}{A_o} = \frac{\gamma + \frac{1}{2}CV^2\left(\frac{\sigma}{1-\sigma}\right)}{k_A}. \tag{A6}$$

Using Eq. (A7) this may be rearranged to give:

$$A_o = \int_{\text{surface}} \frac{dA}{1 + \frac{\gamma_e + \frac{1}{2}CV^2\left(\frac{\sigma}{1-\sigma} - 1\right)}{k_A}}. \tag{A8}$$

For a membrane which is not lysing the tensions and electrical energies must be much less than the elastic modulus, so Eq. (A8) can be approximated

$$A_o \simeq \int_{\text{surface}} dA\left[1 - \frac{\gamma_e}{k_A} - \frac{CV^2}{2k_A}\left(\frac{\sigma}{1-\sigma} - 1\right)\right].$$

Only the voltage term is a function of latitude, as $V = V_p \cos v$

$$\therefore A_o = \left(1 - \frac{\gamma_e}{k_A}\right)A - \frac{CV_p^2}{2k_A}\left(\frac{\sigma}{1-\sigma} - 1\right)\int_{v=o}^{\pi} \cos^2 v dA.$$

The second term is awkward, but may be evaluated analytically. After some algebra we have

$$A_o = 4\pi R^2\left(\frac{W+a}{3W+a}\right)\left[\left(1 - \frac{\gamma_e}{k_A}\right)k_3 - \frac{W}{8}\left(\frac{W+a}{3W+a}\right)\left(\frac{\sigma}{1-\sigma} - 1\right)I_2\right]$$

where

$$k_3 = \left[\frac{\mu^2 - 1}{\mu} + \sqrt{\mu^2 - 1}\,\sin^{-1}\left(\frac{1}{\mu}\right)\right]D^2/2$$
$$I_2 = \left[\frac{\mu^2 - 1}{\mu} - \frac{\mu^2 - 1}{2} + \frac{\mu^2\sqrt{\mu^2 - 1}}{2}\sin^{-1}\left(\frac{1}{\mu}\right)\right]D^4C_6^2$$
$$W = \frac{CE^2R^2}{k_A},\ a = \frac{\beta R}{k_A F_2},\ \text{and}\ D = \left(\frac{\mu^2}{\mu^2 - 1}\right)^{1/3}.$$

Appendix 3: Expressions for the Coefficients of Equation (21)

$$K_1 = 3CR^2D^4C_6^2F_1$$
$$K_2 = \frac{\beta}{BCF_2}$$
$$K_3 = \frac{D^2}{2}\left[\frac{\mu^2 - 1}{\mu^2} + \sqrt{\mu^2 - 1}\,\sin^{-1}\left(\frac{1}{\mu}\right)\right]$$
$$K_4 = \frac{K_1K_2}{k_A}$$
$$F_1 = \left(2D^2 - \frac{(2\mu^2 - 1)}{\mu^2}D^{1/2}\right)^{-1}$$
$$F_2 = F_1D^{9/2}C_6^2\left(\frac{2\mu^2 - 1}{\mu^2}\right)$$
$$D = \left(\frac{\mu^2}{\mu^2 - 1}\right)^{1/3}$$
$$C_6 = \left(1 - \frac{C_1Q_1(\mu)}{\mu}\right)$$
$$C_3 = \frac{1}{\mu^{5/3}(\mu^2 - 1)^{2/3}}$$