MECHANICAL EQUILIBRIUM OF BIOLOGICAL MEMBRANES

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ABSTRACT The nature of mechanical and electrical forces on biological membranes in relation to mechanical equilibrium is examined. The presence of a double layer of electric charge is shown to give rise to an effective pressure drop across a curved membrane of finite thickness. For certain geometric shapes of a membrane, the magnitude of the pressure drop due to electrostatic forces may set a limit on the hydrostatic pressure drop that the membrane can support without buckling. The results are applied to the equilibrium shape of the red blood cell.

INTRODUCTION

The importance of electrostatic forces on biological membranes has been noted by Lopez et al. (1968) with reference to the biconcave shape of the red blood cell. They attributed the biconcave shape to a balance between hydrostatic pressure drop, electrostatic forces, and membrane tensions. In order to explain the observed equilibrium shape, they proposed that the external hydrostatic pressure on the cell membrane was greater than the internal hydrostatic pressure and included in the model a distributed surface charge on the cell membrane of only one sign. Both of these assumptions are doubtful when applied to the red blood cell or to biological membranes in general.

More recently, the effect of membrane potential on the mechanical equilibrium of membranes has been considered by Lew (1970). Lew considered the electrostatic forces produced on a membrane surface between two oppositely charged fluid layers of finite thickness with equal and uniform volume charge densities. This mathematical model is capable of explaining biconcave shapes under certain conditions, but is formulated in such a way that the magnitude of the electrostatic forces cannot be estimated and the effect of a finite membrane wall thickness cannot be determined.

The purposes of this report are to describe a simplified model of the effect of electrostatic forces as related to mechanical equilibrium, and to estimate the magnitude of these forces and possible limitations which may be placed on the hydrostatic pressure drops which membranes can sustain without buckling.

ELECTROSTATIC FORCES AND MECHANICAL EQUILIBRIUM

To account for the effect of electrostatic forces, the cell membrane is modeled as a layer of insulating material having surface charge distributions of univalent cations exterior to the cell and univalent anions interior to the cell. The resulting charge distribution is then characterized as a dipole layer of average local surface charge density $\bar{\sigma}$ where the dipole separation is taken as the membrane wall thickness. The value of $\bar{\sigma}$ may vary from point to point on the membrane surface because of variations in membrane thickness, since the dipole moment per unit area is constant over the membrane surface according to Woodbury (1965). The dipole moment per unit area is the product of $\bar{\sigma}$ and the wall thickness. In addition, the possible presence of local charge clustering on the membrane surfaces does not produce similar variations in $\bar{\sigma}$ because clustered charges move the charges in the conducting fluid media surrounding the membrane in such a way as to distribute the charges over the membrane surface (Woodbury, 1965).

The average local surface charge density $\bar{\sigma}$ is assumed to be equal to the charge densities on the outer and inner surfaces of a plane membrane. For a curved membrane, this condition is no longer satisfied and the outer and inner surface charge densities are not equal if charge neutrality is maintained over macroscopic regions including the membrane and surfaces. The effect of membrane curvature on the surface charge densities may be taken into account by considering a small area element on the midplane of a membrane with principal radii of curvature R_1 and R_2. For an axisymmetric membrane the area of a surface element is given as

$$\mathrm{d}A_m = R_1R_2 \sin \phi \, \mathrm{d}\phi \, \mathrm{d}\theta, \tag{1}$$

where ϕ is the meridional angle and θ is the polar angle (Fung, 1966). The projection of the midplane element $\mathrm{d}A_m$ on the outer surface is

$$\mathrm{d}A_o = \left(R_1 + \frac{t}{2}\right)\left(R_2 + \frac{t}{2}\right) \sin \phi \, \mathrm{d}\phi \, \mathrm{d}\theta, \tag{2}$$

and on the inner surface is

$$\mathrm{d}A_i = \left(R_1 - \frac{t}{2}\right)\left(R_2 - \frac{t}{2}\right) \sin \phi \, \mathrm{d}\phi \, \mathrm{d}\theta, \tag{3}$$

where t is the membrane thickness. Charge neutrality between the inner and outer surfaces requires that

$$\sigma_i \, \mathrm{d}A_i = \sigma_o \, \mathrm{d}A_o, \tag{4}$$

where σ_i and σ_o are the charge densities on the inner and outer surfaces. By substitution of equations 2 and 3 into equation 4, the ratio of charge densities may be written as

$$\frac{\sigma_o}{\sigma_i} = \frac{\left(1 - \frac{t}{2R_1}\right)\left(1 - \frac{t}{2R_2}\right)}{\left(1 + \frac{t}{2R_1}\right)\left(1 + \frac{t}{2R_2}\right)}. \tag{5}$$

If the average density is $\bar{\sigma}$ given by

$$\bar{\sigma} = \frac{\sigma_o + \sigma_i}{2}, \tag{6}$$

equations 5 and 6 may be combined to give σ_o and σ_i in terms of $\bar{\sigma}$:

$$\sigma_o = \bar{\sigma}\frac{\left(1 - \frac{t}{2R_1}\right)\left(1 - \frac{t}{2R_2}\right)}{\left(1 + \frac{t^2}{4R_1R_2}\right)}, \tag{7}$$

$$\sigma_i = \bar{\sigma}\frac{\left(1 + \frac{t}{2R_1}\right)\left(1 + \frac{t}{2R_2}\right)}{\left(1 + \frac{t^2}{4R_1R_2}\right)}. \tag{8}$$

In addition, if the membrane thickness is much smaller than R_1 and R_2, equations 7 and 8 may be approximated as

$$\sigma_o \cong \bar{\sigma}\left(1 - \frac{t}{2R_1} - \frac{t}{2R_2}\right) \tag{9}$$

and

$$\sigma_i \cong \bar{\sigma}\left(1 + \frac{t}{2R_1} + \frac{t}{2R_2}\right). \tag{10}$$

This result is general and may be applied to membranes of any arbitrary shape.

The presence of surface charges on the outer and inner surfaces produces surface stresses or pressures on the membrane due to the attraction of ions of opposite sign. The pressures on the outer and inner surfaces are given by

$$P_o = \frac{\sigma_o^2}{2\epsilon} \tag{11}$$

and

$$P_i = \frac{\sigma_i^2}{2\epsilon}, \tag{12}$$

where ϵ is the permittivity of the membrane (Reitz and Milford, 1960). The net pressure due to electrical forces is

$$\Delta P_e = P_i - P_o, \tag{13}$$

and by substitution of equations 9–12 into equation 13 may be written as

$$\Delta P_e \cong \frac{\bar{\sigma}^2}{\epsilon}\left(\frac{t}{R}\right), \tag{14}$$

where

$$\frac{1}{R} = \frac{1}{R_1} + \frac{1}{R_2}.$$

The surface charge density may be expressed as a function of the membrane potential $|\Delta\Phi|$. If the membrane is plane, the relation between $|\Delta\Phi|$ and $\bar{\sigma}$ is

$$|\Delta\Phi| = \frac{\bar{\sigma} t}{\epsilon}, \tag{15}$$

which is the relation for a parallel plate capacitor (Reitz and Milford, 1960) and according to Woodbury (1965) may be applied to cell membranes. Equation 15 may be used also for a curved membrane surface as long as t is much less than R because of the following considerations. The governing equation for the electric field inside the membrane is Gauss's law (Reitz and Milford, 1960) which may be written as

$$\nabla \cdot \bar{E} = 0, \tag{16}$$

if the net charge density is zero inside the membrane. In a curvilinear coordinate system, the divergence of $\bar{E}$ may be substituted into equation 16 to give

$$\frac{dE(y)}{dy} + \left(\frac{1}{R_1 + y} + \frac{1}{R_2 + y}\right) E(y) = 0, \tag{17}$$

where the y-axis extends along the outward normal direction to the membrane surface. The tangential components of $\bar{E}$ must vanish at the inner and outer surfaces and may be neglected elsewhere if the curvature of the membrane is constant or slowly varying as pointed out by Lew (1970). If the origin of the coordinate system is located on the inner surface of the membrane, the boundary condition on $E(y)$ at $y = 0$ is

$$E(0) = \frac{\sigma_i}{\epsilon}, \tag{18}$$

where the electric field outside the membrane is taken as zero. Equation 18 is a result of Gauss's law. Equation 17 may be integrated directly subject to the boundary condition given by equation 18. The result is

$$E(y) = \left(\frac{\sigma_i}{\epsilon}\right)\frac{R_1 R_2}{(R_1 + y)(R_2 + y)}\,. \tag{19}$$

$\bar{E}$ is related to the electrostatic potential Φ by

$$\bar{E} = -\nabla\Phi \tag{20}$$

or

$$E(y) = -\frac{\mathrm{d}\Phi}{\mathrm{d}y}\,. \tag{21}$$

Therefore, the membrane potential $|\Delta\Phi|$ is from equations 19 and 21

$$\begin{aligned} |\Delta\Phi| &= \left(\frac{\sigma_i}{\epsilon}\right) R_1 R_2 \int_0^t \frac{\mathrm{d}y}{(R_1 + y)(R_2 + y)} \\ &= \left(\frac{\sigma_i}{\epsilon}\right)\frac{R_1 R_2}{(R_1 - R_2)} \ln \frac{\left(1 + \frac{t}{R_2}\right)}{\left(1 + \frac{t}{R_1}\right)}\,. \end{aligned} \tag{22}$$

For wall thicknesses much less than R_1 and R_2 equation 22 can be reduced to

$$|\Delta\Phi| \cong \frac{\sigma_i}{\epsilon}\, t\left(1 - \frac{t}{2R}\right). \tag{23}$$

Further approximation of equation 23 may be made by substitution of equation 10. Eliminating all terms of order $(t/R)^2$ as small compared to unity results in

$$|\Delta\Phi| \cong \frac{\bar{\sigma} t}{\epsilon}\,,$$

which is identical to equation 15.

The net pressure on the membrane due to electrical forces may be expressed in terms of the membrane potential by substitution of equation 15 into equation 14

$$\Delta P_e = \frac{|\Delta\Phi|^2 \epsilon}{tR}\,. \tag{24}$$

The equation of mechanical equilibrium of a membrane element in the direction normal to the membrane surface (Fung, 1966) must now include the net pressure due to electrostatic forces as well as the net hydrostatic pressure difference across the membrane surface, ΔP_h. The equilibrium equation is

$$\frac{T_1}{R_1} + \frac{T_2}{R_2} = \Delta P_e + \Delta P_h, \tag{25}$$

where T_1 and T_2 are membrane tensions and ΔP_h is the difference between internal and external hydrostatic pressures. By substitution of equation 24 into equation 25, the equilibrium equation may be written as

$$\frac{T_1}{R_1} + \frac{T_2}{R_2} = \frac{|\Delta\Phi|^2\epsilon}{t}\left(\frac{1}{R_1} + \frac{1}{R_2}\right) + \Delta P_h. \tag{26}$$

DISCUSSION

An analysis of the red blood cell in its normal biconcave disc configuration has been made by Fung (1966) where electrostatic forces on the cell membrane were neglected. Fung showed that for positive values of ΔP_h the membrane tensions T_1 and T_2 vary around the membrane and are negative in several regions including the polar regions of the cell where the membrane is approximately spherical. Such a loading condition on the membrane would likely lead to local buckling for very small values of ΔP_h (Fung, 1966; Fung and Tong, 1968).

The effect of electrostatic forces on the red blood cell membrane may be estimated by applying equation 26 to the polar regions of the cell. The polar regions are approximately spherical so that T_1 and T_2 are constant and equal as are R_1 and R_2. The curvatures are negative so that equation 26 reduces to

$$\frac{T_1}{R_1} = \frac{|\Delta\Phi|^2\epsilon}{R_1 t} - \frac{\Delta P_h}{2}. \tag{27}$$

Positive values for the membrane tension result when

$$\Delta P_h < \frac{2|\Delta\Phi|^2\epsilon}{R_1 t}. \tag{28}$$

The right-hand side of equation 28 can be estimated using the following data obtained from Woodbury (1965) and Ponder (1948):

$$|\Delta\Phi| = 10 \text{ mv},$$

$$\epsilon \cong 6.6 \times 10^{-11} \text{ farads/m},$$

$$t \cong 100 \text{ A},$$

$$R_1 \cong 6\ \mu.$$

The limiting value for ΔP_h is 2.2 dynes/cm^2.

Direct determinations of ΔP_h have been reported on several types of cells. Rand and Burton (1964) have estimated ΔP_h to be 225 dynes/cm^2 for normal and hypotonically swollen red blood cells and 0 dynes/cm^2 for hypertonically crenated cells. In addition, Cole (1932) has determined ΔP_h to be 40 dynes/cm^2 for the *Arbacia* egg in the normal shape which is spherical. A comparison of these values with the limiting value for ΔP_h of 2.2 dynes/cm^2 suggests that with the possible exception of crenated cells, the electrostatic forces are of little importance in red blood cells and as a consequence, the membrane tension T_1 in the biconcave shape is negative. Therefore, the problem of the buckling of the cell membrane still remains.

Several comments should be made, however, about the experimental data of Cole and Rand and Burton. First, the *Arbacia* egg data is on spherical eggs with positive curvature such that an internal pressure of 40 dynes/cm^2 corresponds to a membrane tension of approximately 0.08 dynes/cm for an egg radius of 40 μ where electrostatic forces are neglected. An estimate can be made from the original data of Cole of the membrane tension corresponding to zero internal pressure. The estimated value is less than 10^{-2} dynes/cm for $\Delta P_h = 0$ assuming the membrane is elastic. This low value for the membrane tension might be the result of electrostatic forces.

The experimental data of Rand and Burton on crenated red blood cells (1964) establishes that $\Delta P_h = 0$. The membrane tension may be determined from equation 27 which reduces to

$$T_1 = \frac{|\Delta\Phi|^2\epsilon}{t} \cong 10^{-3}\ \text{dynes/cm.}$$

This value of tension is due solely to electrostatic forces and is independent of curvature. These results are difficult to compare, but they do indicate that an estimate of membrane tension of the order of 10^{-3}–10^{-2} dynes/cm may not be unreasonable for red blood cells and *Arbacia* egg cells with no hydrostatic pressure drop across the cell membrane.

The value of ΔP_h determined for normal and hypotonically swollen red blood cells by Rand and Burton is probably not a reliable estimate because ΔP_h was determined on cells with part of the membrane sucked into a small pipette. This procedure undoubtedly stretches the membrane thereby causing an increased internal pressure as compared to the normal cell pressure. This increase in pressure apparently does not occur with crenated cells because the required membrane deformations can occur without stretching of the membrane.

SUMMARY

The presence of a double layer of electric charge is shown to produce an effective pressure drop across a curved membrane of finite thickness. The pressure drop is estimated to be 2.2 dynes/cm^2 in the polar regions of the normal red blood cell. This value places an upper limit on the hydrostatic pressure drop the membrane can

withstand without producing negative values of membrane tension which would most likely lead to buckling. In cases where the hydrostatic pressure drop is zero, the electrostatic forces on the red blood cell membrane produce a membrane tension of approximately 10^{-8} dynes/cm. In addition, if the assumptions made are correct, the membrane tension would be independent of curvature.

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