MECHANICAL DEFORMABILITY OF BIOLOGICAL MEMBRANES AND THE SPHERING OF THE ERYTHROCYTE

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ABSTRACT Equations of mechanical equilibrium are applied to the erythrocyte membrane in the normal, hypotonically swollen, and sphered configurations. The hydrostatic pressure drop across the normal cell membrane is shown to be zero for all biconcave shapes if the membrane thickness is uniform. This result leads to the conclusion that the membrane tension is uniform and is a function of membrane potential. A two-dimensional fluid film model for the membrane is introduced to describe the unusual deformability of the erythrocyte during sphering in hypotonic solutions. The model predicts a smooth transition from the biconcave shape to a perfect sphere.

INTRODUCTION

The problem of the sphering of the erythrocyte in hypotonic solutions has been examined extensively by Fung (1966) and Fung and Tong (1968). They introduced a model based on an elastic membrane and showed that in order to sphere the initial biconcave shape, a specific distribution of membrane extensional stiffness is required. In addition, certain requirements are placed on the surface tension distribution in order to produce spheres of a diameter smaller than the maximum diameter of the initial cell. More recently, Danielson (1971) has considered the stability of the solutions of Fung and Tong and has shown that certain restrictions must be placed on the elastic constants of the membrane in order to insure that the sphered configuration is stable.

The sphering problem is reconsidered in this report in order to include the effect of electrical forces in the equilibrium equations as given by Adams (1972). The possible importance of such forces in determining equilibrium shapes of cells has been discussed by Lopez et al. (1968) and Lew (1970). In addition, a two-dimensional fluid film model for the membrane is introduced to describe the mechanics of membrane deformations. The results provide a simplified explanation of the sphering of the erythrocyte that is consistent with experimental evidence. The conditions under which a fluid model for the membrane may be more appropriate than an elastic model are discussed.

EQUILIBRIUM CONSIDERATIONS

Under normal isotonic conditions, the erythrocyte is a biconcave disk as shown in meridional section in Fig. 1. The cell membrane, represented as the cell outline in Fig. 1, is axisymmetric about the z axis. This axisymmetric nature of the membrane surface simplifies somewhat the application of equilibrium conditions because the number of equilibrium equations reduces to two (Fung, 1966).

A general expression for the equilibrium condition in the normal direction to the membrane surface has been given by Adams (1972) as

$$\frac{T_\phi}{R_1} + \frac{T_\theta}{R_2} = \frac{|\Delta\Phi|^2\epsilon}{t}\left(\frac{1}{R_1} + \frac{1}{R_2}\right) + \Delta p_h. \tag{1}$$

For axisymmetric membranes T_ϕ and T_θ are the principal membrane tensions acting in the plane of the membrane in the ϕ and θ directions and R_1 and R_2 are the principal radii of curvature. Δp_h is the difference between internal and external hydrostatic pressures. $|\Delta\Phi|$ is the absolute value of the membrane potential, ϵ is the permittivity of the membrane, and t is the membrane thickness. The effect of electrical forces may also be written as an effective pressure drop, Δp_e, where

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

In applying Eq. 1 to the normal shape of the erythrocyte, as shown in Fig. 1, we note that since the principal curvatures $1/R_1$ and $1/R_2$ are taken to be negative in the polar regions, if the shape is specified by a function $z(x)$, R_1 and R_2 may be determined by the relations

$$R_1 = \frac{-\left[1 + \left(\frac{dz}{dx}\right)^2\right]^{3/2}}{\frac{d^2z}{dx^2}} \tag{3}$$

and

$$R_2 = -\frac{x}{\sin\phi} = \frac{-x\left[1 + \left(\frac{dz}{dx}\right)^2\right]^{1/2}}{\frac{dz}{dx}}, \tag{4}$$

and evaluated at any arbitrary point P on the membrane surface. Eqs. 3 and 4 may also be used for hypotonically swollen cell configurations.

The second equation of equilibrium required in order to determine T_ϕ and T_θ is usually given as a statement of equilibrium in the ϕ direction (Fung, 1966). An alternate method, useful in determining T_ϕ directly, is to consider a free-body section of the membrane formed by passing a plane parallel to the x-y plane as shown in Fig. 2. The pressure drop Δp_e acting on the membrane is drawn in the downward

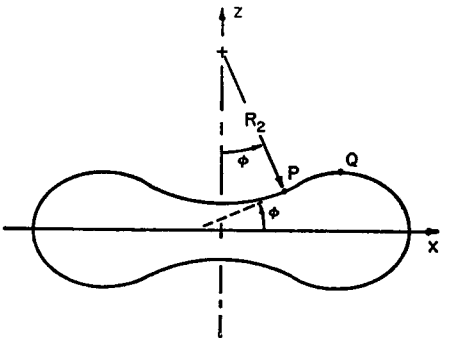

FIGURE 1

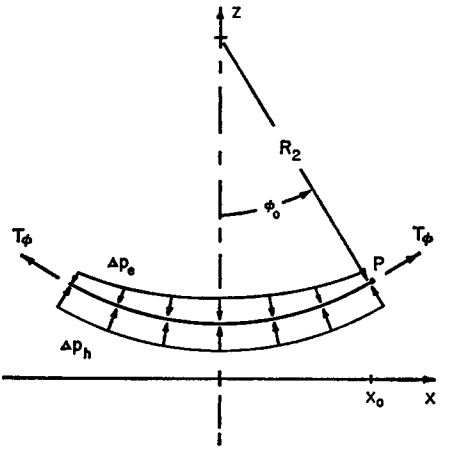

FIGURE 2

FIGURE 1 Normal erythrocyte shown in meridional section.
FIGURE 2 Meridional section of free-body element of membrane.

direction because both curvatures, $1/R_1$ and $1/R_2$, are negative in this region. For the free body to be in equilibrium, the summation of forces in the z direction must be zero which leads to the relation

$$2\pi x_0 \sin \phi_0 T_\phi + \pi x_0^2 \Delta p_h + 2\pi \int_0^{x_0} x \Delta p_e \, dx = 0. \tag{5}$$

Eqs. 2 and 5 may be combined and solved for T_ϕ to give

$$T_\phi = -\frac{x_0}{2 \sin \phi_0} \Delta p_h - \frac{|\Delta\Phi|^2 \epsilon}{x_0 \sin \phi_0} \int_0^{x_0} \frac{x}{t} \left(\frac{1}{R_1} + \frac{1}{R_2} \right) dx, \tag{6}$$

under the assumption that the membrane potential $|\Delta\Phi|$ and permittivity ϵ do not vary with x. In addition, if the membrane thickness t is assumed to be constant along the membrane, Eq. 6 may be reduced to

$$T_\phi = \frac{-x_0}{2 \sin \phi_0} \Delta p_h + \frac{|\Delta\Phi|^2 \epsilon}{t} \tag{7}$$

by noting that

$$x_0 \sin \phi_0 = -\int_0^{x_0} x \left(\frac{1}{R_1} + \frac{1}{R_2} \right) dx$$

or

$$x_0 \sin \phi_0 = \int_0^{x_0} \frac{x \dfrac{d^2z}{dx^2} + \dfrac{dz}{dx} + \left(\dfrac{dz}{dx} \right)^3}{\left[1 + \left(\dfrac{dz}{dx} \right)^2 \right]^{3/2}} dx. \tag{8}$$

Eq. 8 may be shown by differentiation of the function $x \sin \phi$ with respect to x.

For an arbitrary point P on the membrane surface, Eq. 7 may be written as

$$T_\phi = -\frac{x}{2 \sin \phi} \Delta p_h + \frac{|\Delta\Phi|^2 \epsilon}{t}, \tag{9}$$

or in terms of R_2 as

$$T_\phi = \frac{R_2}{2} \Delta p_h + \frac{|\Delta\Phi|^2 \epsilon}{t}. \tag{10}$$

This is a general result for the principal membrane tension T_ϕ of an axisymmetric membrane as a function of hydrostatic pressure drop assuming that the membrane potential, permittivity, and thickness are uniform and not functions of x. The corresponding relation for the second principal membrane tension T_θ is obtained by substitution of Eq. 10 into Eq. 1 which gives

$$T_\theta = R_2 \left(1 - \frac{R_2}{2R_1}\right) \Delta p_h + \frac{|\Delta\Phi|^2 \epsilon}{t}. \tag{11}$$

Eqs. 10 and 11 are applicable to the biconcave and hypotonically swollen and sphered cell configurations.

For biconcave shapes, an additional condition may be imposed on the problem by a requirement that the transverse shear force in the membrane be zero at point Q as shown in Fig. 1 (Fung, 1966). At point Q, the slope is zero so that the equilibrium condition given by Eq. 5 for the free-body element shown in Fig. 2 leads to the result that

$$\Delta p_h = -\frac{2}{x_1^2} \int_0^{x_1} x \Delta p_e \, dx, \tag{12}$$

assuming no transverse shear force in the membrane. x_1 is the value of x at point Q. For a membrane of constant thickness, membrane potential, and permittivity, Eq. 12 by substitution of Eqs. 2 and 8 reduces to

$$\Delta p_h = \frac{2}{x_1} \frac{|\Delta\Phi|^2 \epsilon}{t} \sin \phi_1 = 0, \tag{13}$$

where ϕ_1 is the value of ϕ at point Q and is equal to zero. Eq. 13 indicates that the electrical forces on the membrane balance out in the region between the polar axis and point Q. Subject to the condition given by Eq. 13, the membrane tensions from Eqs. 10 and 11 are

$$T_\phi = T_\theta = \frac{|\Delta\Phi|^2 \epsilon}{t} \tag{14}$$

at every point on the membrane except at Q where both are indeterminate. However, if T_ϕ is assumed to be continuous at Q, the equation of equilibrium in the ϕ

direction as given by Fung (1966) may be used to show that $T_\phi = T_\theta$ at point Q. This result combined with Eq. 1 shows that the membrane tensions at Q are also given by Eq. 14.

MEMBRANE DEFORMABILITY

The uniform, pure, biaxial nature of the membrane tensions, as given by Eq. 14 for biconcave shapes of constant wall thickness, suggests that an analysis of membrane and cell deformability based on a fluid drop model would be appropriate because fluid interfaces are in a similar state of membrane tension under equilibrium conditions. The possibility of such a fluid drop model for the erythrocyte has been noted by Rand and Burton (1964) and Schmid-Schonbein and Wells (1969). In addition, an erythrocyte membrane with fluid properties has been argued for on the basis of rheological considerations by Dintenfass (1964), and on the basis of molecular structure by Singer and Nicholson (1972).

The model to be proposed here is consistent with the fluid drop model except that the total surface area of the cell is assumed to be constant and independent of cell volume changes which accompany swelling and sphering in hypotonic solutions. Area changes before sphering have not been observed experimentally (Rand and Burton, 1964; Canham and Parkinson, 1970). A membrane model based on a two-dimensional, incompressible fluid film of constant thickness conforms to the constant area assumption and is the basis for the following analysis of membrane deformability.

If we now consider small deformations of the membrane model consistent with the incompressible, constant thickness assumption, we find that the principal membrane strains, ϵ_ϕ and ϵ_θ, are related by

$$\frac{\Delta A}{A} = \epsilon_\phi + \epsilon_\theta = 0, \tag{15}$$

because volume dilatation and thickness changes are zero. A is an element of area in the plane of the membrane and $\Delta A/A$ is the area dilatation. The requirement that the area dilatation be zero at each point is sufficient to insure that the total membrane area remains constant.

The state of strain of the membrane at 45° to the principal strain axes and in the plane of the membrane is obtained by transformation of principal strain components, ϵ_ϕ and ϵ_θ, and gives

$$\epsilon_{12} = \epsilon_{21} = \epsilon_\phi,$$
$$\epsilon_1 = \epsilon_2 = 0, \tag{16}$$

where the (1, 2) axes are located in the plane of the membrane at 45° to the (ϕ, θ) axes. ϵ_1 and ϵ_2 are normal strain components and ϵ_{12} and ϵ_{21} are shear strain com-

ponents. The relations given by Eqs. 16 show that the membrane model is restricted to pure shearing deformations as a consequence of the condition imposed on area dilatation given by Eq. 15.

The ability of cell membranes to resist deformations such as those given by Eqs. 16 is not known, but viscoelastic models are suggested by the work of Rand (1964) and Katchalsky et al. (1960) on erythrocytes. If, however, we restrict our attention to slow changes in cell size, the viscous effects in such viscoelastic models would be expected to be controlling because of relaxation effects (Prandtl, 1949).

Restricting our attention to slow changes in cell volume and assuming newtonian behavior for the membrane model, we would expect a relation between membrane shear and shear strain rate of the form

$$T_{12} = 2\mu_s \dot{\epsilon}_{12}, \tag{17}$$

where T_{12} is the membrane shear (shear stress times film thickness) in the plane of the membrane referred to the (1, 2) coordinate system. μ_s is a coefficient of surface shear viscosity (Scriven, 1960). By stress transformation, T_{12} can be written as

$$T_{12} = \frac{T_\phi - T_\theta}{2}, \tag{18}$$

and, therefore, Eq. 17 may be expressed as

$$T_\phi - T_\theta = 4\,\mu_s \dot{\epsilon}_{12}. \tag{19}$$

Eq. 19 indicates that stationary states in the sphering process are reached when $T_\phi = T_\theta$ if the membrane behaves as a viscous fluid film.

A stationary state must also satisfy the equilibrium conditions given by Eqs. 10 and 11. By equating these expressions for T_ϕ and T_θ we find that either

$$R_1 = R_2$$

and

$$T_\phi = T_\theta = \frac{\Delta p_h R_1}{2} + \frac{|\Delta\Phi|^2 \epsilon}{t}, \tag{20}$$

or

$$R_1 \neq R_2,$$

$$\Delta p_h = 0,$$

and

$$T_\phi = T_\theta = \frac{|\Delta\Phi|^2 \epsilon}{t}. \tag{21}$$

The case given by Eq. 20 is the spherical solution, whereas Eq. 21 represents all other configurations. The result for these nonspherical configurations is identical with that found for biconcave shapes of uniform thickness as given by Eq. 14.

DISCUSSION

In the present analysis of the normal erythrocyte, we have established from mechanical equilibrium conditions that for a constant thickness membrane in any biconcave configuration, the hydrostatic pressure drop across the membrane is zero and the principal membrane tensions are equal to

$$T_\phi = T_\theta = \frac{|\Delta\Phi|^2\epsilon}{t}.$$

This factor has previously been estimated to be approximately 10^{-3} dyn/cm for a membrane of 100 Å thickness (Adams, 1972). This is a useful result because of the observed shapes found experimentally in the sphering process by Rand and Burton (1964) and Canham and Parkinson (1970). They report that shape changes occur slowly and that the curvature at the poles reverses gradually as the cell approaches an ellipsoid. The analysis presented here could be applied to any of the biconcave shapes observed with the same conclusions.

The possibility of membrane thickness variations has been suggested by Fung and Tong (1968). They found that for an elastic membrane to sphere from a biconcave shape, the extensional stiffness or product of Young's modulus and thickness must vary in a specified manner, and that for the resulting sphere to be of smaller diameter than the initial major cell diameter, the surface tension (surface energy per unit area) would also have to vary.

If thickness variations are introduced in the present analysis, we find that in general $\Delta p_h \neq 0$ and $T_\phi \neq T_\theta$ for biconcave shapes. However, if thickness variations are specified, Δp_h may be determined from Eq. 12 and the membrane tensions from Eqs. 1 and 6. Since the effect of wall thickness variations does not depend on any assumed mechanical properties for the membrane, the present analysis may be applied to the elastic case.

In comparing the elastic and fluid models for the initial biconcave configuration, we now have an important result: namely, for the elastic membrane of constant Young's modulus, since the membrane thickness must vary, the hydrostatic pressure drop is not zero and membrane tensions are not equal. For the two-dimensional fluid film membrane analyzed here, the deformability of the film maintains a pure, biaxial state of membrane tension and zero hydrostatic pressure drop, independent of the cell configuration in the sphering process. Therefore, in the fluid model, the wall thickness must be uniformly distributed.

The fluid model as applied to the sphering process predicts a smooth change in curvature as cell volume increases with constant cell area. Conditions on hydro-

static pressure drop and membrane tensions do not change until the spherical shape is attained. At this point the cell membrane could be expected to develop an appreciable degree of stiffness since further deformations must increase the total cell area. Under such conditions, an analysis based on the elastic membrane model would be required.

The solutions examined here are presented as stationary, equilibrium solutions and as such they do not assure stability. A fluid film membrane model allows the normal cell to assume an infinite number of shapes of the same area and volume. This degree of freedom for the cell shape is consistent with the observed ease of cell deformability found, for instance, in the passage of cells through small pore diameter sieves (Chien et al., 1971) and filters (Prothero and Burton, 1962). A stability analysis might explain why the erythrocyte has a preferred shape under normal conditions and why the final transition from swollen ellipsoid to sphere in hypotonic solutions is so rapid as to indicate an instability (Rand and Burton, 1964).

SUMMARY

A zero value for the hydrostatic pressure drop and a constant, pure, biaxial state of membrane tension are shown for the normal biconcave shape of the erythrocyte if the membrane thickness, membrane potential, and permittivity are uniform. This result is shown to be valid for any biconcave shape. The mechanical deformability of the erythrocyte membrane is modeled as a two-dimensional, incompressible fluid film of constant thickness. The fluid film model predicts a smooth transition of the erythrocyte from the biconcave to spherical shape in hypotonic solutions with no change in surface area. A pure, biaxial state of membrane tension is maintained during sphering as well as a vanishing hydrostatic pressure drop under stationary, equilibrium conditions. A uniform membrane thickness is shown to be a requirement of the fluid film model. The stability of the equilibrium solutions obtained is not assured.

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