A NEW MATERIAL CONCEPT FOR THE RED CELL MEMBRANE

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ABSTRACT The proposition is made that the red cell membrane is a two-dimensional, incompressible material and a general stress-strain law is developed for finite deformations. In the linear form, the character of such a material is analogous to a two-dimensional Mooney material (e.g., rubber), indicating that the molecular structure in the plane of the membrane would consist of long chains, randomly kinked and cross-linked in the natural state. The loose network could be provided by the protein component and the lipid phase could exist interstitially as a liquid bilayer, giving the membrane its two-dimensional incompressibility. The material provides the capability of large deformations exhibited by the discocyte and yet the rigidity associated with the osmotic spherocyte state. It is demonstrated that a membrane of this type can form a sphere at constant area. An illustrative example of the application to single cell discocyte-to-osmotic spherocyte transformations is presented.

INTRODUCTION

The purpose of this paper is to propose a new concept for the continuum mechanical behavior of membranes. The development is based on red blood cell observations and shape transformations. The mammalian red cell has been extensively studied because of its geometric simplicity and nearly homogeneous interior. It must be remembered that the red cell has a passive membrane unlike the dynamic membranes of motile cells. However, the precursory forms of the red cell (e.g., reticulocyte) were also active, motile organisms. Therefore, generalization of any proposition to all membranes should be undertaken carefully.

The mammalian red blood cell exhibits a variety of morphological states in response to the physical and chemical environment. The spectrum of erythrocyte shape ranges from a biconcave disk, or discocyte, to a spherocyte; intermediate states include cup-shaped cells and spiculated (also called crenated) cells known as echinocytes (from the Greek: sea urchin). Observations of red cell shape changes were first documented by Hamburger (1895), extensively studied by Ponder (1971), and recently categorized by Bessis and co-workers (1970, 1972) using the scanning electron microscope. It is generally accepted that the normal red cell interior is in the liquid state, offering negligible resistance to deformation; however, the puzzling question remains: how can the red cell membrane permit such large local deformations and return to its original shape? Indeed, there is an apparent contradiction associated with the shape transformations. First of all, estimates of the length changes produced by echinocyte spicules (Bessis and Lessin, 1970) are as large as 100% and 10–30% for the equatorial region during osmotic sphering (Evans and Leblond, 1972). On the other hand, the same single cell osmotic-sphering experiments showed no observable increase in surface area (the area measurements were limited to 2% accuracy); indicating that the surface is nearly incompressible. Keep in mind, however, that the two-dimensional incompressibility of the surface does not imply inextensibility.

Knowledge of the geometric properties of the shape transformations has been recognized as prerequisite to understanding the membrane behavior. Because of the irregularity of the echinocyte geometry and inherent limitations in light microscopy, investigations have been restricted to the discocyte-to-spherocyte transformation. Even though scanning electron microscopy can provide direct measurements of the red cell to the desired accuracy, the cells must be fixed and dried before observation, which precludes osmotic and all single cell experiments. Therefore, visible light microscopy is necessary. Ponder's initial measurements of surface area during osmotic swelling indicated little change. Subsequent single cell observations by Rand and Burton (1963) showed surface area increases of 10% or more. However, the optical techniques employed by Rand and Burton created significant uncertainties of this order due to the defocusing technique and optical system imaging limitations (Evans and Fung, 1972). A quantitative optical method has been developed that provides better than 1% accuracy in cell dimensions with 2% accuracy in surface area (Evans and Fung, 1972). Using this method, single cell osmotic-swelling experiments were analyzed and no surface area change was detected (Evans and Leblond, 1972). Single cell transformations are essential because of the large variation in geometric properties of red cells (Evans and Fung, 1972).

There are many published observations of large length changes in one direction for red cells (Hochmuth and Mohandas, 1972; Branemark and Lindstrom, 1963; and Skalak and Branemark, 1969). Hochmuth's results for shear-deformed, attached cells are especially interesting. Not only do they support other observations of large unidirectional strains but the additional characteristic that the product of the two extension ratios equals one is compatible with two-dimensional incompressibility.

In view of the experimental evidence, the proposition is made here that the red cell membrane behaves like a two-dimensional, incompressible material. The proposition is consistent with the biological concept of membranes as thin phospholipid interfaces, possibly bilayers (whose structure may be significantly complicated because of additional constituents and local molecular arrangements [Singer and Nicolson, 1972] or unusual matrices of molecules [Dintenfass, 1969]). A material of this type would be totally anisotropic: a continuum in two dimensions, molecular character in the third dimension. In addition, the thermodynamic conditions that restrict the phospholipid to the thin interfacial region, immiscibility, also resist area changes; if the area increases, effectively more of the membrane must go into "solution" because of the increased water interaction. If the membrane were an ordinary three-dimensional material to which we are accustomed, it would simply change thickness in response to the area change; but it apparently is not.

Numerous mechanical analyses of the red cell membrane have appeared in the literature (some recent publications are Hochmuth and Mohandas, 1972; Rand and Burton, 1964; Lopez et al., 1968; Canham, 1970; Lew, 1972; Fung, 1966; and Fung and Tong, 1968). However, with a few notable exceptions (Hochmuth and Mohandas, 1972; Fung, 1966; and Fung and Tong, 1968), these studies have been primarily concerned with explaining the normal, biconcave beauty of the red cell. Again, with the few notable exceptions, the analyses have ignored the equations of mechanical equilibrium for the membrane, choosing only the normal component equation. This choice is the same as the liquid, free interface assumption although it is not specified as such in any of the investigations where it is employed. A closed, liquid, free interface (with uniform surface tension) is stable in the spherical form (Landau and Lifshitz, 1959). If the interface is a two-dimensional, incompressible liquid, then the uniform surface tension has no effect and possibly a simplified approach to bending resistance (Canham, 1970; Lew, 1972) can be employed (discussed further in the theoretical section). The desire in this paper is to investigate the possible elastic character of a two-dimensional, incompressible material; therefore, the plastic or liquid nature of the interface will not be treated here (there is evidence supporting the plastic or even fluid hypothesis [Singer and Nicolson, 1972], but strong evidence against the importance of bending resistance). Hochmuth and Mohandas (1972) ignore the equation for normal forces and use only the in-plane component equation involving the fluid, interfacial viscous shear stress.

Only the papers of Fung (1966) and Fung and Tong (1968) have rigorously utilized the equations of equilibrium and a stress-strain relation for the membrane. The latter specifically treats the problem of the discocyte-to-osmotic spherocyte transformation. The authors point out the "special property" associated with the ability to form a sphere from the original discocyte shape. Using finite deformation strains and an isotropic, elastic stress-strain relation, they reduce the problem to the equivalence of the two principal strains for the spherical state. However, the result is inconsistent with the observation of a smaller sphere radius than the original major radius of the discocyte, unless the surface tension is greater at the rim than at the dimples of the cell.

Using the proposition that the membrane is two-dimensional, incompressible, elastic material, a stress-strain law for finite deformations will be developed and single cell osmotic-swelling experiments will be used to illustrate the application. In the experiments, the swollen cells were observed to be oblate spheroids and not perfect spheres. Therefore, a linear perturbation approach will be used to consider the departure from spherical symmetry. It will be assumed that the normal discocyte state is stress-free (or nearly so in comparison with the large deformation state produced by swelling). The resulting linear form of the stress-strain law is shown to be the two-dimensional analogue of rubber. Using the molecular model of Treloar (Green and Adkins, 1970), the membrane would consist of a two-dimensional matrix of randomly kinked, cross-linked chains which are elongated and oriented upon stretching, thereby decreasing entropy content (Katchalsky and Curran, 1967). The loosely connected, two-dimensional network could be the role of a protein component of the membrane, the two-dimensional incompressibility could be provided by the phospholipid phase existing as a liquid bilayer in the matrix (this would be compatible with the current "mosaic" model of Singer and Nicolson). It is important to recall, however, that the simple fluid version of the mosaic model, i.e. "proteins floating in a sea of fat," cannot account for any elastic solid property of the red cell membrane.

STRESS-STRAIN LAW FOR FINITE DEFORMATION OF A TWO-DIMENSIONAL MATERIAL

In this section, the stress-strain law for a two-dimensional material will be established following the general three-dimensional development of Prager (1961). The relationship will involve the use of extension ratios; Fung and Tong (1968) produced a similar result using strains.[1] However, the advantage of Prager's approach will become apparent in the next section where incompressibility is assumed. For finite strains, it is necessary to make the distinction between eulerian and lagrangian strains. The former are based on the metric of the final state and the latter are relative to the metric of the initial state. Here, the lagrangian strains will be used.

The principal extension ratios are defined by,

$$\lambda_1 = d\bar{x}_1/dx_1\,,$$
$$\lambda_2 = d\bar{x}_2/dx_2\,, \qquad (1)$$

where the metric for the initial state is,

$$ds = (dx_1^2 + dx_2^2)^{1/2}.$$

Using Eqs. 1, the lagrangian strains are given by,

$$\epsilon_1 = \tfrac{1}{2}(\lambda_1^2 - 1),$$
$$\epsilon_2 = \tfrac{1}{2}(\lambda_2^2 - 1). \qquad (2)$$

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[1] After the present article was submitted for review, a similar development by Skalak et al. appeared in the March 1973 issue of *Biophysical Journal*. The general theoretical development is the same however there are some significant differences in final stress-strain laws (see Appendix). Therefore, the general development has been shortened here. The incompressible case is briefly mentioned by Skalak et al. but not explicitly treated.

Because a two-dimensional material is being considered, it is convenient to introduce a strain tensor that represents area change. This tensor, used by Prager, is called Finger's strain tensor and is defined by,

$$\beta_{ij} = \delta_{ij} + 2\epsilon_{ij}, \tag{3}$$

where δ_{ij} is the unit matrix and ϵ_{ij}, the lagrangian strain tensor. The invariants of the two-dimensional tensor are,

$$B_1 = \lambda_1^2 + \lambda_2^2,$$

$$B_2 = -\lambda_1^2\lambda_2^2. \tag{4}$$

The second invariant is minus the area ratio squared.

Simplifying Prager's analysis, the stress-strain law for the two-dimensional material can be obtained from an elastic potential energy function E.

$$T_{ij} = 2(\bar{\rho}/\rho)[B_2(\partial E/\partial B_2)\delta_{ij} + (\partial E/\partial B_1)\beta_{ij}], \tag{5}$$

where $\bar{\rho}$ and ρ are the material densities in the final and initial states. The only assumption implicit in this relation is that the material is isotropic in two dimensions, but not necessarily homogeneous or linear.

STRESS-STRAIN LAW FOR A TWO-DIMENSIONAL, INCOMPRESSIBLE MATERIAL

Obviously no material is truely incompressible. However, from a practical point of view, if the bulk modulus of the material is much greater than the shear modulus, then the material behaves as if it is incompressible (Landau and Lifshitz, 1970). The condition of incompressibility for a two-dimensional material is given by,

$$\lambda_1\lambda_2 = 1, \tag{6}$$

or

$$B_2 = -1.$$

Eq. 6 is recognized as a constraint relation. The derivation of the stress-strain relation from the elastic potential, Eq. 5, involves taking the variation of the elastic potential with respect to variations in the strain tensor. Therefore, a lagrangian free multiplier and the constraint relation must be included in the derivation. The result is given by (noting that $\rho = \bar{\rho}$),

$$T_{ij} = -p_M\delta_{ij} + 2(\partial E/\partial B_1)\beta_{ij}. \tag{7}$$

The free multiplier p_M is the two-dimensional analogue to hydrostatic pressure and will be referred to as membrane tension. The elastic potential only depends on the single invariant B_1. The incompressibility relation, Eq. 6, gives,

$$\epsilon_1 + \epsilon_2 + 2\epsilon_1\epsilon_2 = 0,$$

and

$$B_1 = 2(1 - 2\epsilon_1\epsilon_2),$$

the elastic potential is an analytic function of B_1; therefore, it can be expanded in a power series in B_1.

$$(E - E_0) = \sum_{n=1}^{\infty} E_n (B_1 - 2)^n,$$

$$\partial E/\partial B_1 = \sum_{n=1}^{\infty} nE_n (B_1 - 2)^{n-1}.$$

Using the relation for B_1 in terms of the second invariant of the strain tensor,

$$\partial E/\partial B_1 = E_1 + \sum_{n=1}^{\infty} (n + 1)E_{n+1}(-2\epsilon_1\epsilon_2)^n.$$

Because the function is to first-order quadratic in the strains, only the E_1 term will be important for small strains.

$$(E - E_0) \cong E_1(B_1 - 2).$$

This is the two-dimensional analogue of a Mooney material (Green and Adkins, 1970) or vulcanized rubber. For small strains and constant temperature, one finds that entropy must decrease with stretching (Katchalsky and Curran, 1967), compatible with randomly cross-linked molecular chains becoming ordered as they elongate and align with the stretch direction.

EQUATIONS OF EQUILIBRIUM FOR THE RED CELL MEMBRANE

As Fung (1966) has pointed out, the stresses in a shell can be represented by membrane stresses and bending stresses. Also, for very thin shells like the red cell, Fung (1966) demonstrates that the bending stresses are several orders of magnitude less than the membrane stresses; therefore the equations of equilibrium for an axisymmetric membrane can be used (Flügge, 1966). For the membrane under a constant pressure load (as in the case of an osmotic spherocyte), the equations are given by,

$$K_1T_s + K_2T_\theta = P,$$

$$(\partial/\partial s)(rT_s) + (\partial T_{\theta s}/\partial\theta) - T_\theta(\partial r/\partial s) = 0,$$

$$(\partial/\partial s)(rT_{s\theta}) + (\partial T_\theta/\partial\theta) + T_{s\theta}(\partial r/\partial s) = 0, \quad (8)$$

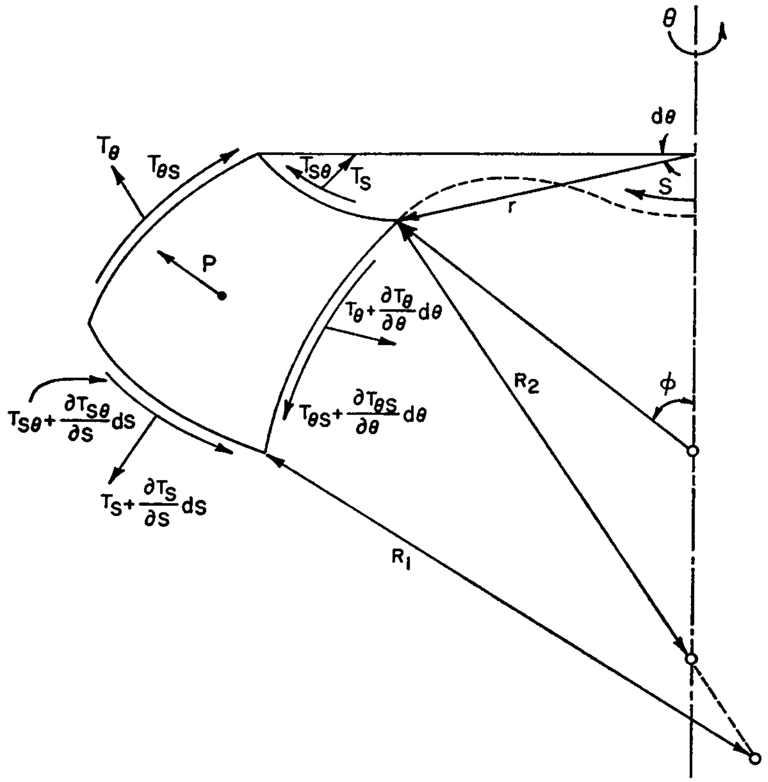

FIGURE 1 Surface element illustrating the curvilinear coordinate system and the stress resultants.

where the curvilinear coordinates (s, θ) are illustrated in Fig. 1; P is the pressure difference across the membrane; K_1 and K_2 are the principal curvatures at the point (s, θ). In the first equation, P can only be nonzero if the gaussian curvature (K_1K_2) is greater than zero over the whole surface (Fung, 1966; Flügge, 1966). Also, if the gaussian curvature is greater than zero everywhere, the shear stress $T_{\theta s}$, $T_{s\theta}$ vanishes. However, for a discocyte, the gaussian curvature changes sign on going from the dimple region to the outer, toroidal region. The pressure difference must be zero and shear stress does not vanish. The intermediate states of swelling are, therefore, more difficult to treat (see Flügge, 1966).

For the osmotic spherocyte with positive gaussian curvature, Eqs. 8 can be integrated to yield,

$$K_2T_s = (P/2) + (C/r^2). \tag{9}$$

The constant C for the homogeneous solution must be zero if the surface is smooth at the origin.

$$T_s = P/2K_2$$

$$T_\theta = \frac{(2K_2 - K_1)}{2K_2^2} P. \qquad (10)$$

Now, the stress-strain law (Eq. 7) is introduced. The total interfacial surface tension γ_T (surface free energy per unit surface area) is a scalar and can be added to the membrane tension.

$$T_s = \left[-p_M + \gamma_T + \frac{F(B_1)}{2}\right] + F(B_1)\epsilon_s,$$

$$T_\theta = \left[-p_M + \gamma_T + \frac{F(B_1)}{2}\right] + F(B_1)\epsilon_\theta, \qquad (11)$$

where,

$$F(B_1) \equiv 4(\partial E/\partial B_1).$$

Using Eqs. 10 and 11, the scalar quantities can be eliminated to give a relation between the principal strains, curvatures, pressure difference, and material elastic properties.

$$(\epsilon_s - \epsilon_\theta) = \frac{P}{F(B_1)} \frac{(K_1 - K_2)}{2K_2^2}. \qquad (12)$$

The condition for attaining the spherical state is observed to be, $P \gg F(B_1)K_2$ that implies, $K_1 \cong K_2$.

If the membrane were a two-dimensional liquid $F(B_1) = 0$, then the membrane tension p_M would be constant and equal to the total interfacial surface tension. The red cell could not sustain a pressure differential and remain axisymmetric without being spherical. In this case, additional consideration would have to be given to "bending resistance," possibly the result of a surface dipole distribution (Lew, 1972). Because the surface would be free to relocate as a two-dimensional liquid, a simple model (such as the "electro-torque" model of Lew) could be employed. However, there is direct observational evidence that supports the concept that the "bending" effects are indeed negligible. First of all, red cells are observed to tumble and follow a trajectory in a flowing medium that is indicative of a solid interface or membrane (Goldsmith, 1971). Second, if one observes attached red cells in a flowing medium, the curvature at the point of attachment is very large (nearly infinite) even for very low strain rates, as if there were no bending resistance at all (Hochmuth and Mohandas, 1972).

PERTURBATION APPROACH TO SPHEROIDAL GEOMETRY

In general, curvature relations for even simple geometries (e.g., ellipsoidal) are complicated nonlinear, algebraic functions. However, if the surface is close to being spherical, a linear perturbation analysis can be used. The radius is defined,

$$R(\phi) = R_0 + \zeta(\phi) \tag{13}$$

where, $R_0 \gg \zeta$ and ϕ is the polar angle. Using Eq. 13 and keeping only first-order terms, the principal curvatures are given by,

$$K_1 \simeq \frac{1}{R_0}\left(1 - \frac{\zeta}{R_0} - \frac{1}{R_0}\frac{\partial^2\zeta}{\partial\phi^2}\right),$$

$$K_2 \simeq \frac{1}{R_0}\left(1 - \frac{\zeta}{R_0} - \frac{\cos\phi}{R_0 \sin\phi}\frac{\partial\zeta}{\partial\phi}\right),$$

$$\frac{K_1 - K_2}{K_2{}^2} \simeq -\frac{\partial^2\zeta}{\partial\phi^2} + \frac{\cos\phi}{\sin\phi}\frac{\partial\zeta}{\partial\phi}. \tag{14}$$

Because of bilateral symmetry and Eq. 13, the perturbation term can be expanded in a series in $\sin^2\phi$.

$$\zeta \equiv R_0 \sum_{m=1} b_m X^m, \tag{15}$$

where $X \equiv \sin^2\phi$. Using the transformation, Eq. 13 becomes,

$$\frac{K_1 - K_2}{2K_2{}^2} = X\frac{d\zeta}{dx} - X(1 - X)\frac{d^2\zeta}{dx^2}. \tag{16}$$

LINEAR APPROXIMATION TO STRESS-STRAIN LAW

Here, the assumption will be made that only the linear term in the elastic potential E_1 is important. In this case, the strain difference $(\epsilon_s - \epsilon_\theta)$ can be expanded in a series similar to Eq. 15. The summation commences at $n = 1$ because the strains $(\epsilon_s, \epsilon_\theta)$ are both zero at $X = 0$ (for a smooth surface).

$$(\epsilon_s - \epsilon_\theta) \equiv \sum_{n=1}^{\infty} a_n X^n. \tag{17}$$

Using Eqs. 12, 15, and 16, the following relation is obtained for the coefficients a_l, b_l, (for $l \geq 1$),

$$a_l = (PR_0/\mu)l[lb_l - (l + 1)b_{l+1}], \tag{18}$$

where $\mu \equiv 4\,E_1$. Normally, the coefficients a_l terminate at some value $l = N$. If $a_l = 0$ for $l > N$, then the coefficients b_l are related by,

$$b_l = (N/l)b_N, \qquad l > N.$$

However, at the equator, $X = 1$; therefore, the coefficients b_l $(l \geq N)$ must also be zero in order that Eq. 15 converge. It is apparent from Eq. 18 that, given a specific value for PR_0/μ and an initial shape, the final shape is determined.

EXAMPLES OF SINGLE CELL OSMOTIC-SWELLING EXPERIMENTS

Using the initial discocyte and final osmotically swollen spherocyte geometries of individual cells (Evans and Leblond, 1972), the principal extension ratios and strains can be calculated for each transformation. As described by Fung and Tong (1968), the extension ratio corresponding to the latitude circumference is given by,

$$1 + 2\epsilon_\theta = (r_s/r_D)^2$$

where r_s and r_D are the cylinder radii for the same material point in the spheroid and discoid geometries, respectively. In order to establish the common material point, the surface areas were integrated, starting at the poles of the cells; the radial positions of equal surface area for the two shapes were determined (two-dimensional incompressibility). It was necessary to perform the computations with a digital computer. The other strain is easily calculated using the incompressibility relation.

$$\lambda_1^2\lambda_2^2 = 1,$$

$$1 + 2\epsilon_s = (r_D/r_s)^2.$$

Fig. 2 shows the scaled cross sections for the discocyte-to-osmotic spherocyte single cell transformations (Evans and Leblond, 1972). Figs. 3–5 contain the plots of $(\epsilon_s - \epsilon_\theta)$ for the three single cell swelling experiments. The strain difference is plotted on a log–log scale against $\sin^2 \phi$. From the plots, the coefficients a_l can be obtained. Table I lists the coefficients for the three cell experiments. The first coefficient is small and negative because the polar region of the spheroid has smaller absolute curvature than the dimple of the discocyte (the polar region membrane must spread out in going to the spheroidal shape). There is essentially only one other coefficient that is significant. As Eq. 18 indicates, the last coefficient is the only one that is proportional only to b_l and not the difference in $[lb_l - (l + 1)b_{l+1}]$.

Eq. 18 can be easily inverted to obtain the shape coefficients b_l. Two coefficients (a_N, a_1) are nonzero.

$$b_N = (\mu/PR_0)(a_N/N^2)$$

$$b_l = (1/Nl)(\mu/PR_0)a_N \qquad (1 < l < N).$$

$$b_1 = (\mu/PR_0)[a_1 + (a_N/N)]$$

Using these equations, the corresponding $(PR_0/\mu)b_l$ coefficients can be calculated

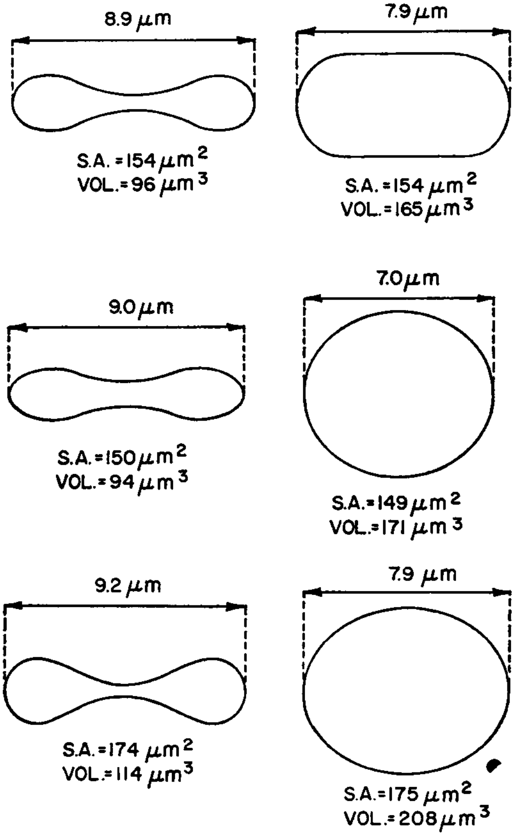

FIGURE 2

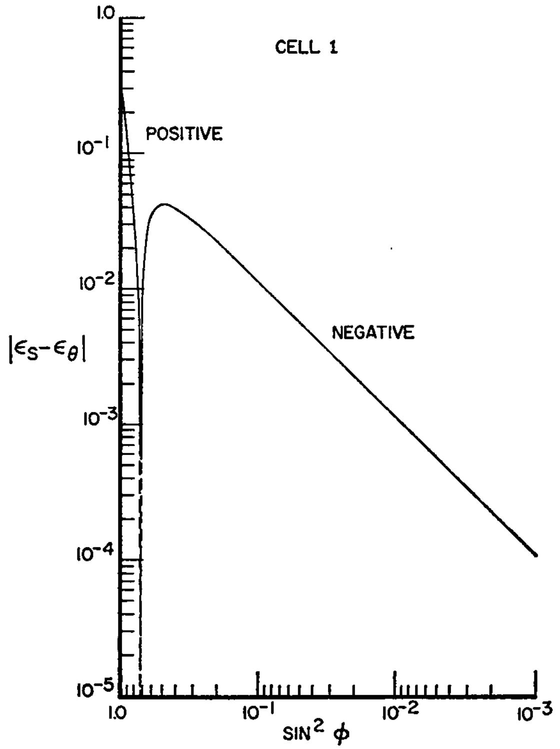

FIGURE 3

FIGURE 2 Scaled cross sections of three single cell discocyte-to-osmotic spherocyte trans-transformations. S. A., surface area.

FIGURE 3 Absolute value of the principal strain difference $|\epsilon_s - \epsilon_\theta|$ for the single cell exp. no. 1 presented in Fig. 2, plotted as a function of the sine squared of the polar angle ϕ.

from the a_l's, Table II. In order to have a pressurized oblate spheroid, the first coefficient b_1 must be positive. The cell number 1 in Fig. 2 has zero curvature at the poles and cannot sustain a pressure differential; in addition, it is observed that the coefficient $[(PR_0/\mu)b_1]$ for cell number 1 is also negative, compatible with its shape. Coefficients $[(PR_0/\mu)b_l]$ for cells 2 and 3 are positive; therefore, a pressure difference can exist. Even though the computerized methods used by Evans and Leblond (1972) provide a 10–20-fold improvement in resolution (~0.04 μm) over ordinary optical microscopy, it is not sufficient to evaluate the separate coefficients b_l from the curvature variations. However, the semimajor and semiminor axes can be used to obtain the ratio PR_0/μ.

$$A_R = [R(1)/R_0] - 1 = \sum_{l=1}^{\infty} b_l,$$

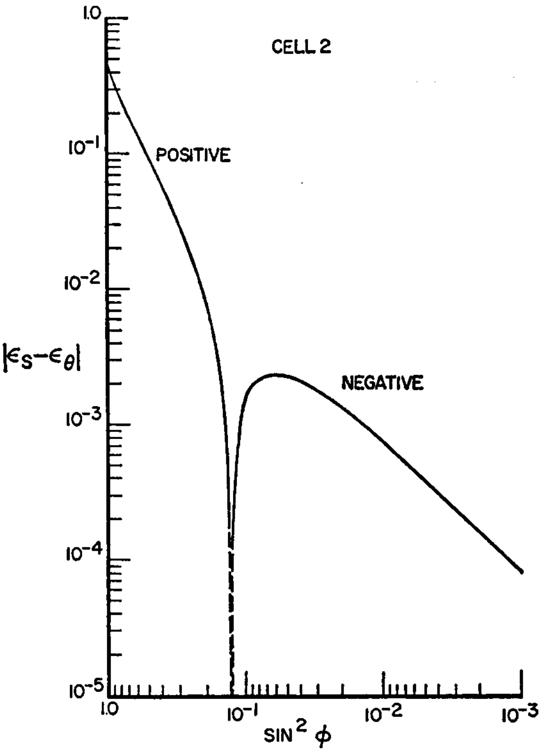

FIGURE 4

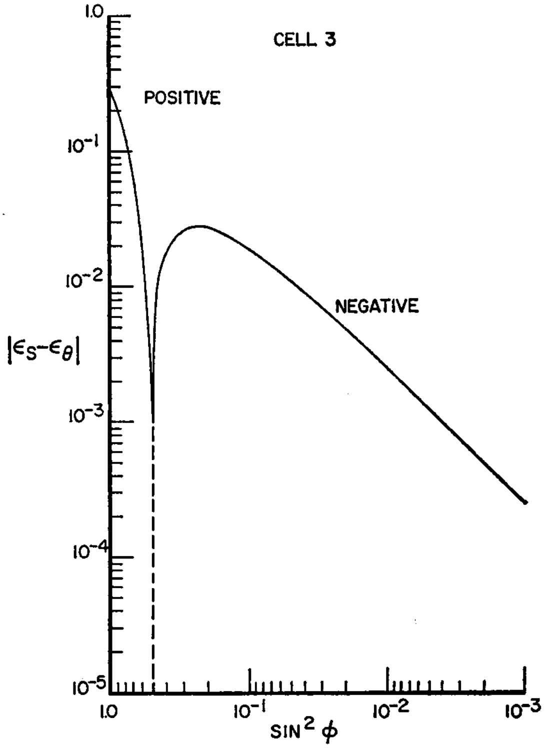

FIGURE 5

FIGURE 4 Absolute value of the principal strain difference $|\epsilon_s - \epsilon_\theta|$ for the single cell exp. no. 2 presented in Fig. 2, plotted as a function of the sine squared of the polar angle ϕ.
FIGURE 5 Absolute value of the principal strain difference $|\epsilon_s - \epsilon_\theta|$ for the single cell exp. no. 3 presented in Fig. 2, plotted as a function of the sine squared of the polar angle ϕ.

where $R(1)$ is the semimajor axis; the values for A_R are given in Table II. For cells number 2 and 3, there are only two coefficients (b_1, b_2),

$$\frac{PR_0}{\mu} = \frac{(a_1 + \frac{3}{4}a_2)}{A_R}.$$

The values of PR_0/μ for cells 2 and 3 are 4.4 and 0.7, respectively.

In order to obtain a constant corresponding to a modulus of elasticity, the parameter μ must be divided by the membrane thickness (assumed to be ~100 Å). Assuming two values for the "modulus of elasticity," 10^4 and 10^6 dyn/cm^2, the values of pressure difference P for cell 2 would be either of the order of 10^2 dyn/cm^2 or 10^4 dyn/cm^2 and for cell 3 either of the order of 10 dyn/cm^2 or 10^3 dyn/cm^2. An estimate of the maximum pressure difference that can exist without lysing the cell was given by Rand (1964), 10^5 dyn/cm^2. In addition, Rand and Burton (1964), estimated the pressure difference for a partially swollen ellipsoid at 2×10^2 dyn/cm^2.

TABLE I

COEFFICIENTS FOR THE THREE CELL EXPERIMENTS

Cell no.	a_1	a_2	a_3	a_4
1	−0.1	0	0	0.34
2	−0.1	0.6	0	0
3	−0.2	0.5	0	0

TABLE II

CALCULATION OF $(PR_0/\mu)b_i$ COEFFICIENTS FROM a_i

Cell no.	$(PR_0/\mu)b_2$	$(PR_0/\mu)b_2$	$(PR_0/\mu)b_3$	$(PR_0/\mu)b_4$	A_R
1	−0.015	0.042	0.028	0.021	0.78
2	0.2	0.15	0	0	0.08
3	0.05	0.13	0	0	0.26

Based on this information, a reasonable upper limit to the "shear modulus" (μ divided by the membrane thickness) would be 10^5–10^6 dyn/cm^2. (This could be lower because Katchalsky et al. use a value of 10^4 dyn/cm^2 for the value of P at hemolysis.)

SUMMARY

The model of the red cell membrane as a two-dimensional, incompressible material has been proposed and a general stress-strain law has been developed for finite deformation. The character of such a material resembles a two-dimensional rubber, indicating that the molecular structure would consist of long chains, randomly kinked and cross-linked in the natural state with an incompressible liquid phase. The material would resist uniform dilatation and store energy in the form of a membrane tension analogous to hydrostatic pressure. The material could easily stretch in any direction with a commensurate shortening in the other dimension. The material could provide the capability of large deformations exhibited by the discocyte and yet appear rigid in the osmotic spherocyte state. Indeed, it has been demonstrated that a membrane of this type can form a sphere at constant area, a task that is especially difficult if not impossible for an ordinary three-dimensional, istropic material. Unfortunately, there is no method for accurately measuring the internal pressure of the cell near the spherical state; therefore, the discocyte-to-osmotic spherocyte transformation cannot provide a good estimate of the material elastic constant (however, the transformation does provide a very useful conceptual study). Experiments such as Hochmuth's shearing of attached red cells and even micropipette studies can be very useful if the proper equations are used. Because the material elastic constant is only a function of one invariant of the strain tensor, it is an easier task to establish a general stress-strain relation experimentally. As Fung and Tong (1968) have pointed out, the uncoiling of some molecular chain arrangement could provide the basis for microfilament formation and other unusual electron microscopic evidence.

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APPENDIX

The recent publication by Skalak et al. (1973) contains an excellent development of a general two-dimensional elastic material. The result is the same as Eq. 5 in the text with the "strain energy" function W used as an alternate label for the elastic potential E. The invariants are directly analogous. The special case of a two-dimensional, incompressible membrane is briefly mentioned but was not developed along with the ensuing simplifications.

The significant difference is apparent in the choice of strain energy function, which was assumed to be quadratic in invariant B_1 by Skalak et al. As will be discussed in a companion article on application of the stress-strain law to micropipette and fluid shear experiments, only the linear or first-order term is discernible from available data even for the very large extension ratios, e.g., "tethering" of attached cells and micropipette aspiration of discocytes. This is very important because of the statistical thermodynamic relation to molecular structure. The character of such a linear material is that of a weak two-dimensional elastomer. In addition, Skalak et al. did not consider the shear modulus in their discussion of osmotic sphering nor in their treatment of the inflation of a membrane stretched over an orifice.