

ON THE SHAPE OF THE ERYTHROCYTE

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ABSTRACT A model is postulated which attributes the distinctive biconcave shape of the human erythrocyte to a balance of forces acting on the membrane. The forces considered are electrostatic forces due to a charge distribution on the membrane, a hydrostatic pressure difference acting across the membrane, and forces arising from a constant tension in the membrane. A numerical study indicates that the postulated model will produce an equilibrium shape which is very similar to the observed shape of the human erythrocyte.

INTRODUCTION

The mechanism maintaining the distinctive biconcave shape of the red blood cell has been the subject of considerable curiosity. The numerous mechanisms which have been proposed can be grouped into two general classifications: (a) those attributing the shape to an internal mechanical structure and (b) those which postulate that the shape is maintained by the membrane. Ponder (1) discusses many of the postulated models. To date the actual mechanism has not been established, but some evidence does exist which supports, or at least makes not unreasonable, the idea that the shape is membrane dependent.

Rand and Burton (2) point out in their study of the mechanical properties of the membrane that studies with the electron microscope have failed to produce evidence of an internal mechanical structure. The work of Weed et al. (3) shows that the erythrocyte maintains its shape even though nearly all of the hemoglobin has been removed. This is supported by other work with red blood cell ghosts.

The work of Rand and Burton (2) establishes the fact that the membrane of the cell is elastic and it follows, therefore, that it can produce shaping forces. There exists in the literature some conjectures that the membrane of the cell has variable thickness and different elastic properties over the cell surface. Evidence to support this, however, is inconclusive. Rand and Burton (2) have shown experimentally that if such variations do exist, they are small and of secondary importance.

The simplest model which can be postulated for the shape of the cell, based on the observations above, is one in which the shape is due to tension in the membrane and a uniform pressure difference across the membrane. To explain the difficulty with this model, we assume that the pressure acts inward, i.e., the pressure outside the

cell is higher than inside. The membrane of the cell is treated as a thin elastic shell which has a uniform tension. This model for the membrane is often referred to as the soap bubble analogy.

The model for the cell which has so far been postulated is insufficient to explain the shape of the cell. The difficulty can be seen by considering Fig. 1. In the biconcavity of the cell the tension force, F_t , and the pressure force act in opposite directions¹ and it is possible to obtain force equilibrium. Towards the outer portion of the cell, however, F_t and F_p are both in the same direction and therefore force equilibrium does not exist. Because of this it is impossible to maintain force equilibrium with only tension and pressure forces. It should be noted that this latter statement is true even if the membrane has a variable thickness and tension, provided that one does not allow the membrane to have compressive stresses. In order to produce force equilibrium on the membrane it is essential to consider another force.

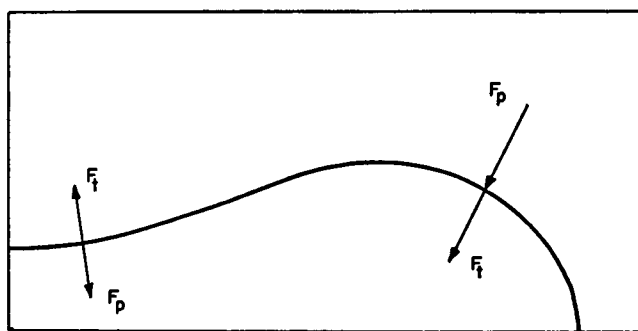


FIGURE 1 Forces acting on a simplified model of the erythrocyte.

It is known from microelectrophoretic studies that the erythrocyte carries an electrical charge (4). Although microelectrophoresis only gives information about the cell surface, it is possible that there is also a volume charge distributed within the cell. In addition, the charged cell will attract ions from the plasma so that a dipole-type electrical distribution will be induced across the membrane. It seems possible that these electrical charge distributions could produce the required additional force.

Because of the complexity of the electrical distributions in and on an erythrocyte, it was decided to consider whether the Coulomb forces, which arise from a monopole distribution on a conducting surface, can provide the additional force required with the soap bubble analogy model. The consideration of the monopole distribution and of a conducting surface was motivated by a desire to obtain a working hypothesis which would simplify the calculations. Both assumptions may be questionable on physiological grounds, but the simplifications which are produced make their use

¹ The tension force acts in a direction towards the center of curvature.

most attractive in a preliminary study on the effect of electrical forces on the shape of a membrane. Although we consider the charge to be located on the membrane, the charge could be considered to be on a conducting surface immediately interior to the membrane. This view would permit the membrane to be an insulator without affecting the analysis. Regardless of the point of view one takes, we neglect forces due to charges external to the cell.

We assume that the difference in hydrostatic pressure between the inside and outside of the cell acts inward. This assumption appears to contradict the work of Rand and Burton (2) in which the pressure was reported to be higher inside the cell. However, they state that "A rigorous analysis of the equilibrium shapes of the cell must of course take into account all the external forces acting on the membrane" but that they will investigate "only one of these possible forces, i.e., pressure within the cell". In a rough sense one can consider their internal pressure to be the sum of the hydrostatic and electrostatic forces considered in this paper. The comparison is not exact because the electrostatic forces are not uniform and will vary as the membrane is perturbed. A more precise explanation of the discrepancy is that the pressure reported by Rand and Burton is a derived quantity and is based on a model which differs from the model considered in this paper.

ANALYSIS

In conducting the analysis, we will consider dimensionless quantities. In practice we use the major diameter of the cell and the hydrostatic pressure difference to obtain the dimensionless quantities. Although this procedure will give a pressure of 1, we will use the symbol P . This should make the analysis easier to follow and has the advantage that some other set of quantities could be used to form the dimensionless quantities without affecting the analysis.

The charge density which appears in the equations is made nondimensional with the pressure and permittivity, ϵ_0 , i.e., $\sigma = (\text{charge density}) / (2 \times \epsilon_0 \times \text{pressure})^{1/2}$.

The cell will be treated as a body of revolution having the z -axis as the axis of revolution. The tension force is given by equation 1

$$F_t = T(1/R_1 + 1/R_2) \quad (1)$$

where F_t is the tension force and is normal to the surface, T is the tension, and R_1 and R_2 are the principal radii of curvature. The radii of curvature are negative if the surface is concave inward and positive if the surface is concave outward. Note that the radii of curvature may have opposite signs at some points. For bodies of revolution, R_2 is the signed distance measured along the normal to the cell surface between the cell surface and the axis of revolution.

The force on a conducting surface due to a charge distribution on the surface is

given by

$$F_q = \sigma^2 \quad (2)$$

where σ is the surface charge density.

A force balance on an element of cell surface gives

$$F_q - P + F_t = 0 \quad (3)$$

where the sign convention is taken that P is positive when directed inward and F_q and F_t are positive when directed outward. Using equations 1 and 2 in 3 one obtains

$$\sigma = [P - T(1/R_1 + 1/R_2)]^{1/2}. \quad (4)$$

Because the cell surface is treated as a charged conductor, it is an equipotential surface. It must now be determined whether the biconcave shape of the cell is an equipotential surface having a charge distribution given by equation 4. Because of difficulties which arise in evaluating equation 4 and in calculating surface potentials for an arbitrarily shaped body, a numerical procedure was used to perform the necessary computations.

NUMERICAL PROCEDURE

The cell surface is approximated by a number of mesh points and the radii of curvature determined by passing a circle through three adjacent points. The radius of the circle is R_1 . The second radius of curvature, R_2 , is determined by finding the intercept with the z -axis of the line which passes through the center of the circle and the middle of the three points used to fit the circle. After specifying values for T and P , equation 4 is used to find σ at each mesh point and this charge distribution is then used to numerically calculate the surface potential. If the surface potential is not constant within some specified accuracy the shape is modified.

The numerical procedure used to calculate the surface potential makes use of the symmetry of the cell. The charge density calculated at any mesh point is constant along a circular ring having its center on the z -axis and lying on the plane, perpendicular to the z -axis, which passes through the mesh point. The potential at any point on the cell surface, V_i , is calculated by summing the contribution to the potential from all of the N rings.

$$V_i = V_{s_i} + V_{t_i}$$

where V_{s_i} is the potential at the i th ring due to the charge on the i th ring, and V_{t_i} is the potential at the i th ring due to all the other charged rings.

Referring to Fig. 2, V_{t_i} is given by equation 5.

$$V_{t_i} = \sum_{j=1}^N \int_0^{2\pi} \frac{\sigma_j r_j d\theta}{d_j} \quad j \neq i. \quad (5)$$

This equation may be written as the elliptic integral

$$V_{t_i} = \sum_{j=1}^N \frac{4r_j \sigma_j}{l_j} \int_0^{2\pi} \frac{d\phi}{(1 - k^2 \sin^2 \phi)^{1/2}} \quad j \neq i \quad (6)$$

where $\phi = \theta/2$, $k^2 = 2A/(1 + A)$, and $A = 2r_i r_j / (d_j^2 + 2r_i r_j \cos \phi)^2$. The potential V_{si} is given by equation 7 (refer to Fig. 3).

$$V_{si} = \int_{2\alpha_i}^{2\pi-2\alpha_i} \frac{r_i \sigma_i d\theta}{d_i} = \sigma_i \ln \left[\frac{1 + \cos \alpha_i}{1 - \cos \alpha_i} \right]. \quad (7)$$

Because the self potential at a point on the ring causes an infinite potential at that point, α_i cannot be made zero.

The procedure just described was tested by calculating the surface potential on a sphere having a uniform charge distribution and on a sphere having a charge distribution given by

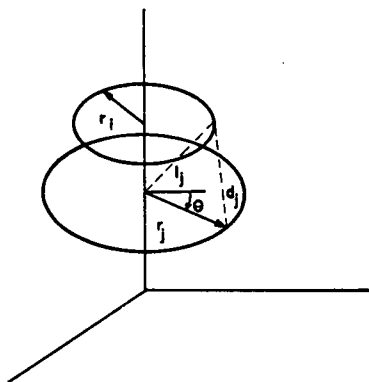


FIGURE 2 Notation used to calculate the potential at the i th ring due to the charges on all the other rings.

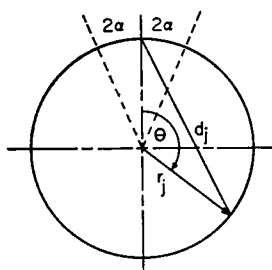


FIGURE 3 Notation used to calculate the potential at the i th ring due to charges on the i th ring.

the zeroth and second Legendre polynomials. The surface potential can be calculated analytically for both of these test cases. In testing the numerical procedure, the sphere was approximated by 72 charged rings. Three methods for choosing α_i were tested. These methods were: (a) Select α_i so that the same charge is excluded from each ring, (b) use the same value of α_i for all rings, and (c) select α_i so that the same area is excluded from each ring. The best results occurred when α_i was selected in such a way that the same area was excluded from each ring. The area neglected was equal to 3% of the area of the smallest ring. The potentials calculated using the numerical procedure were less than the exact potentials by $1\% \pm 0.01\%$.

The procedure used to search for the shape was one developed by Powell (5). This procedure minimizes the sum of the squares of a set of n functions each of which is a function of m variables. For the present problem, the variables are the coordinates of the mesh points. The functions are the deviations from constant potential, the variation in total charge, and the variation in the volume of the cell. A weight factor less than one is used on the constraints

on total charge and volume. This ensures that in the search procedure the deviations from a constant volume and constant charge have much less effect than any of the deviations from a constant potential.

The constraints on volume and charge were introduced to ensure that the volume and charge did not increase without bound, or alternately, that they did not become zero. It should be emphasized that these constraints do not completely fix the charge and volume, but rather that they ensure that large changes in these quantities do not occur. The constraint on volume may be viewed as stating that the volume of the cell is fixed by physiological properties. Since the surface area is not constrained, the constraint on volume does not preclude a sphere. The constraint on charge may be viewed as stating that the erythrocyte carries some fixed quantity of charge which is distributed over the membrane. Since it is possible to determine a charge which would produce a sphere for a given pressure, tension, and volume, one must postulate that the charge on a normal cell is less than the spherical shape value. This reasoning would suggest that the spherizing agents used on erythrocytes produce the swelling by changing the tension of the membrane, the intracellular pressure, the total electrical charge, or by some combination of the three effects. The charge used in the constraint was chosen to be less than the spherical cell charge.

Regardless of the physiological interpretation attached to the constraints, we wish to determine if the biconcave shape of the cell is a shape in which there exists a balance of pressure, tension, and electrostatic forces. If it is shown that the biconcave shape gives rise to force equilibrium, the fact that certain quantities were constrained in the mathematical computations does not alter the final result.

In carrying out the actual computations, a starting shape was selected which was similar to the expected final shape so as to keep to a minimum the required number of iterations. The volume and total charge calculated for this initial shape were used as constraints in the search for the final shape. A nondimensional pressure of 1 was taken and a value for T was selected. With this information, the procedure previously outlined was begun.

RESULTS

Figs. 4 and 5 show the shapes resulting from the procedure described above. The solid circles are the measurements of the erythrocyte obtained by Ponder (6). Fig. 4 is the result obtained for $T = 3$. The surface potential is constant to within $\pm 1.1\%$. Fig. 5 is the result obtained for $T = 4$. The potential is constant to within $\pm 0.9\%$.

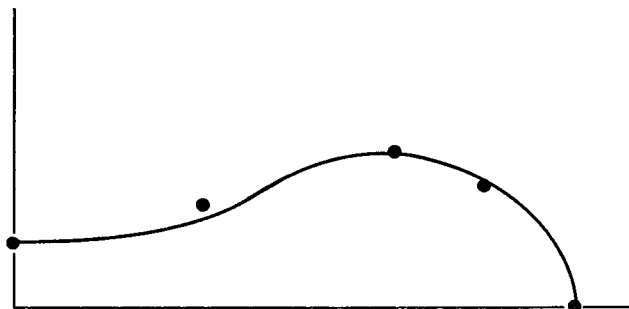


FIGURE 4 Calculated shape for $T = 3$. The potential is constant within $\pm 1.1\%$. Solid circles are data from Ponder (6).

In both of these cases the weight factors on both the total charge on the volume were 10^{-4} . For the cases reported, the starting shapes were close to the final shapes. The variations in potentials for the initial shapes were approximately 3.5%.

Starting shapes which were biconcave but which had a very slight biconcavity were used during the earlier stages of the work. The calculations showed a potential variation of approximately $\pm 30\%$. The computations which were performed showed that the shape was changing towards the shapes presented in this paper, i.e., the points near the axis of revolution were moving inwards and the outer edge of the cell was increasing in curvature. Because of the very slow convergence, the computations were stopped and the starting shapes were modified to be closer to Ponder's (6) measurements. It should be emphasized that equations 2 and 4 are valid only for an equipotential surface. This means that the potentials given for the starting shapes

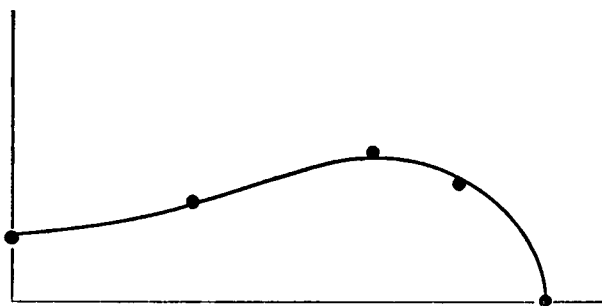


FIGURE 5 Calculated shape for $T = 4$. The potential is constant within $\pm 0.9\%$. Solid circles are data from Ponder (6).

are approximations, the approximation improving as the shape approaches the equipotential shape.

CONCLUSION

It has been shown that the model considered in this paper does give rise to a shape which is very similar to that of an erythrocyte. The actual mechanism maintaining the shape of the erythrocyte is undoubtedly more complex than the one considered in this work. Future work should consider the effects of volume charge distributions and of a dipole distribution on the membrane. The interaction between cells should also be investigated because the presence of electrical forces may have a strong bearing on the formation of rouleaux.

It should be mentioned that membrane shaping forces which arise from electrical considerations may have major implications in the somewhat related problem of mitosis.

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