RED BLOOD CELL SHAPES AS EXPLAINED ON THE BASIS OF CURVATURE ELASTICITY

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ABSTRACT Assuming that the shape of red blood cells is controlled by the curvature elasticity of the surrounding membrane, we fit theoretical shapes to the contours Evans and co-workers determined by interference microscopy. Very good agreement is obtained for disc shapes. The fit is not so good for less common shapes, which may result from Evans' parametric representation and from the interference of shear elasticity.

INTRODUCTION

Human red blood cells in their normal state form very regular biconcave discs, as has been known since the invention of the microscope. In the last few decades many attempts have been made at a theoretical explanation. There is general agreement that the shape is determined by the elastic properties of the red cell membrane whose thickness is very small (of the order of 100 Å) as compared with the cell diameter (~ 8 μm). In the early theories the membrane was postulated to behave like a rubber skin with either the sphere or the disc representing equilibrium (1). In a more recent approach, inspired by the virtual constancy of area, the membrane was assumed to be essentially a fluid lipid bilayer and the red cell shape to be determined by the elasticity of curvature or bending (2–4). Subsequently, it was shown that this model predicts disc shapes only if there is a spontaneous membrane curvature of sufficient strength and negative sign, i.e. opposed to that of the sphere (5). If the condition is not satisfied red cells could be prolate instead of oblate. Although the concept of a spontaneous curvature seems indispensable (6, 7), recent experiments in which red blood cells were partially sucked into micropipettes have demonstrated quite convincingly that curvature elasticity is not the only factor controlling red cell shape (8). Another important influence appears to be the shear elasticity of the membrane. The elastic modulus of shearing is much smaller than that of area dilation. Accordingly, the membrane area can be considered constant, as is also done in the lipid bilayer model. The sucking experiments further provided evidence that the response to shear may be an elastoviscous effect in the sense of a Maxwell body with a very long molecular relaxation time, perhaps of the order of hours (and probably due to a hampered rearrangement of membrane proteins). As a result, one may have to distinguish between slow and fast deformations. If a certain shape has days or hours to develop it is likely to be con-

trolled solely by curvature elasticity including spontaneous curvature. If a deformation takes place within a shorter time, shear elasticity can in general not be neglected. However, mild shape changes such as the transition between the disc and the osmotic spherocyte are, perhaps, only weakly affected by shear stresses.

In the present paper we fit theoretical shapes to red cell contours which E. A. Evans and co-workers (9–11) determined by interference microscopy. Only curvature elasticity is taken into account. We feel justified in doing so as most comparisons are made for discs, i.e. for shapes close to normal shape. For these, the agreement of theory and experiment will be seen to be very satisfactory. Deviations for less common shapes are appreciable; they may be due to the parametrized representation of the experimental data as well as to the neglect of shear elasticity.

THEORY

The curvature energy per unit area of a lipid bilayer or a membrane is given by a kind of Hooke's law

$$g = (\tfrac{1}{2}) \cdot k_c \cdot (c_1 + c_2 - c_o)^2 + (\tfrac{1}{2}) \bar{k}_c \cdot c_1 \cdot c_2, \tag{1}$$

where c_1 and c_2 are the two principal curvatures, k_c and $\bar{k}_c$ are elastic moduli and c_o is the spontaneous curvature (4). The latter may be caused by an asymmetry of the membrane or its environment with respect to the center plane of the membrane. The integral of the second term in Eq. 1 over a closed surface is known to be independent of the shape of the surface. Therefore only the first term in Eq. 1 is relevant for the shape of red cells or lipid bilayer vesicles. In the present paper we shall restrict ourselves to shapes having rotational symmetry. Their principal curvatures are those along the meridians (c_m) and the parallels of latitude (c_p). Let the contour of a cell be given by a function $z(x)$ (see e.g. Fig. 1), the z-axis being the rotational axis. By ψ we denote the angle made by the rotational axis and the surface normal of the cell. With this notation we find

$$c_p = (\sin \psi / x);\ c_m = \cos \psi \cdot (\mathrm{d}\psi / \mathrm{d}x);\ (\mathrm{d}z/\mathrm{d}x) = -\tan \psi. \tag{2}$$

Combination of the two equations eliminates ψ and leads to

$$\mathrm{d}c_p/\mathrm{d}x = (c_m - c_p)/x. \tag{3}$$

We now wish to find the contour for which the total elastic energy $E = (\tfrac{1}{2})\, k_c \cdot \int \mathrm{d}S\, (c_m + c_p - c_o)^2$ is minimal at given volume and surface area. The problem is solved by conventional calculus of variation. We may write

$$\delta\{(\tfrac{1}{2})\, k_c \int \mathrm{d}S\, (c_m + c_p - c_o)^2 + \Delta p \cdot V + \lambda \cdot S\} = 0, \tag{4}$$

where Δp and λ are Lagrange multipliers for the constraints of constant volume V and surface area S. They represent an osmotic pressure difference $\Delta p = p_e - p_i$ between the outside and the inside of the cell and a tensile stress λ.

Expressing dV and dS by

$$dV = \pi \cdot x^3 \cdot c_p (1 - x^2 \cdot c_p^2)^{-1/2}\, dx,$$
$$dS = 2 \cdot \pi \cdot x \cdot (1 - x^2 \cdot c_p^2)^{-1/2}\, dx, \tag{5}$$

and using Eq. 3 to eliminate c_m, we have

$$\delta \int_0^{x_m} x (1 - x^2 \cdot c_p^2)^{-1/2} \cdot \{[x(dc_p/dx) + 2 \cdot c_p - c_o]^2 + (\Delta p / k_c) \cdot x^2 \cdot c_p + 2\lambda/k_c\}\, dx = 0, \tag{6}$$

where $c_p(x)$ is the function to be varied. Doing the variation and expressing the second derivative of $c_p(x)$ by Eq. 2, we obtain

$$dc_m/dx = x \cdot (1 - x^2 \cdot c_p^2)^{-1} \cdot \{(\tfrac{1}{2})\, c_p [(c_p - c_o)^2 - c_m^2] + (\lambda/k_c) \cdot c_p + (\tfrac{1}{2}) \cdot \Delta p / k_c\} - (c_m - c_p)/x. \tag{7}$$

The two independent Eqs. 3 and 7 can be solved numerically (5) and the contour $z(x)$ found by a further integration

$$z(x) - z(o) = -\int \tan \psi\, dx = -\int x \cdot c_p \cdot (1 - x^2 \cdot c_p^2)^{-1/2}\, dx. \tag{8}$$

It is interesting to note that the elastic constant k_c enters the equations only as a scaling factor for the Lagrange multipliers Δp and λ. Consequently, the shape of the cell is independent of the magnitude of k_c and controlled solely by the volume. The maximum volume is $V_o = (4\pi/3)\, R_o^3$ with R_o being defined by $S = 4\pi \cdot R_o^2$. We express x and z in units of R_o, all curvatures in units of R_o^{-1} and the volume in units of V_o. When the cell is nearly spherical, i.e. $V \approx V_o$, it should have the shape of either a prolate or an oblate ellipsoid of revolution. It was reported earlier (5) that the oblate form has lower elastic energy than the prolate one as long as the spontaneous curvature c_o is below a critical value $c_{oc} = -(\frac{39}{23})\, R_o^{-1}$. The details of this calculation will be given elsewhere.[1] Theory (4) also shows that as V approaches V_o the pressure difference Δp approaches the value $\Delta p_c = 2 \cdot k_c \cdot (6 - c_o R_o) \cdot R_o^{-3}$. With the estimate $k_c = 5 \cdot 10^{-13}$ erg one has $\Delta p_c \approx 1$ dyn $\cdot$ cm^{-2} for red cells (4). Accordingly, Δp should be completely negligible as compared to osmotic forces. When the volume is reduced sufficiently below V_o the prolate ellipsoids become dumbbells in our theory. For all values of V/V_o the prolate and the dumbbell-like forms were found to have a higher elastic energy than the oblate and the biconcave discoid forms, provided $c_o < c_{oc}$. For $c_o > c_{oc}$ the prolate ellipsoids have lower energy than the oblate ones. If, however, the volume is sufficiently reduced the dumbbells finally have higher elastic energies than discs of equal volume. For $c_o > c_{oc}$ there would have to be a hysteresis

[1] Deuling, H. J., and W. Helfrich. The curvature elasticity of fluid membranes: a catalogue of vesicle shapes. Submitted for publication in *J. Phys.*

in the transition between discocytes and osmotic spherocytes, which is not found experimentally. It is gratifying that our analysis of experimental data, to be discussed below, gives values for c_o well below c_{oc}. However, the fact that deformations of red cells into elongated bodies are only rarely observed may be evidence for the influence of shear elasticity. It is readily seen that less shear is required for the formation of flattened than elongated shapes if one starts from the sphere or a disk.

NUMERICAL RESULTS AND COMPARISON WITH EXPERIMENTAL CONTOURS

We have compared our model with experimental data by Evans and co-workers (9–11) obtained from human erythrocytes by means of interference microscopy. This method allows to measure the phase change $\varphi(x)$ which a light wave undergoes when passing through a blood cell along the direction parallel to the rotational axis. The phase change $\varphi(x)$ is not simply proportional to the thickness of the cell but is related to the contour $z(x)$ in a much more complicated way due to diffraction effects. To allow for diffraction effects Evans et al. calculated the phase shift $\varphi_m(x)$ for the class of contours given by the following expression

$$z(x) = (1 - (x/R)^2)^{1/2} \cdot (C_o + C_2(x/R)^2 + C_4(x/R)^4). \quad (9)$$

The free model parameters R, C_o, C_2, C_4 were determined by fitting $\varphi_m(x)$ to the measured phase shift $\varphi(x)$. Obviously, this method gives accurate results only if expression 9 represents a good description of the contour of the cell which has been measured.

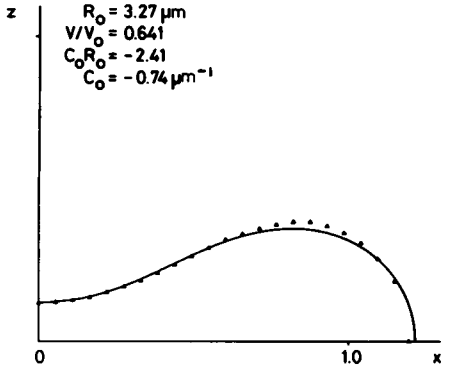

FIGURE 1

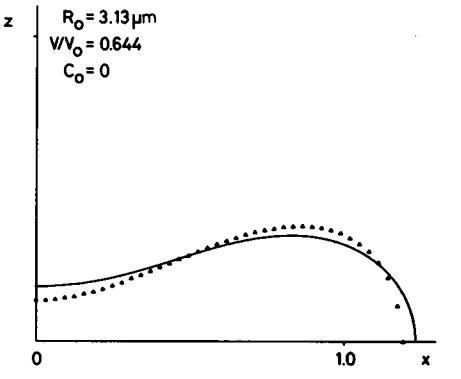

FIGURE 2

FIGURE 1 Contour of a red blood cell calculated with the curvature elastic model (solid curve) in comparison to average contour (Δ) of normal red blood cells at 300 mosmol as obtained by interference microscopy. Only one quadrant of the contour is shown. Experimental data taken from ref. 10.

FIGURE 2 Contour of a red blood cell calculated with Canham's model (curvature elasticity without spontaneous curvature) in comparison with the data of Fig. 1.

Fig. 1 shows the best fit possible with the curvature-elastic model (solid curve) to data obtained by Evans and Fung (10) from 50 normal red blood cells at 300 mosmol. The data (marked by Δ) are plotted in reduced form, i.e. x and z are in units of the equivalent sphere radius $R_o = 3.13\ \mu m$. The volume was found to be $V = 0.644\ V_o$. The only free parameter in the curvature elastic model is the spontaneous curvature c_o (not to be confused with the coefficient C_o in expression 9). Fitting the theoretical contour to the data gives $c_o = -0.73\ \mu m^{-1}$. The deviation of the theoretical curve from the data is within experimental error.[2]

Fig. 2 compares the theory for $c_o = 0$ to the data of Fig. 1. The theoretical curve of Fig. 2 represents the stable form since its elastic energy is slightly smaller than that of the dumbbell of equal volume. The poor agreement indicates that a curvature elastic model without the assumption of a spontaneous curvature does not render the shape of erythrocytes satisfactorily. Fig. 3 shows data of 55 normal red blood cells (10) at 217 mosmol. We get good agreement with the data choosing $c_o = -1.94\ \mu m^{-1}$.

When the cell volume is reduced sufficiently the two membrane halves touch each other at the center of the cell thus forming torocytes. (Our nomenclature is essentially that of Bessis [12]). The two halves have contact not only at one point but over an entire circular area which increases as the volume is reduced further. Such cells were reported by Evans and Leblond (11) as intermediate forms in the discocyte to cup cell transformation. Two such cells are shown in Fig. 4. The parametrization (9) (with the first coefficient C_o put equal to zero) used by the authors permits the two halves of the cell to be in contact at a single point only in the center of the cell. The contour for torocytes being derived from the measured phase shift with an inadequate parametrization can therefore not be as accurate as the data for discocytes. This seems to be the

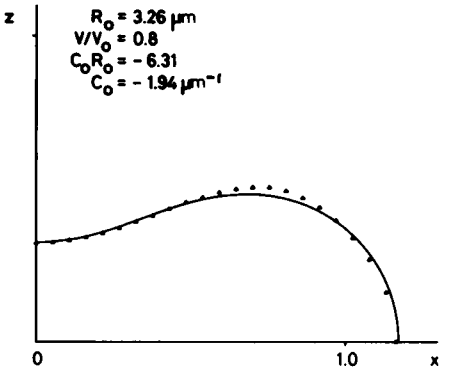

FIGURE 3

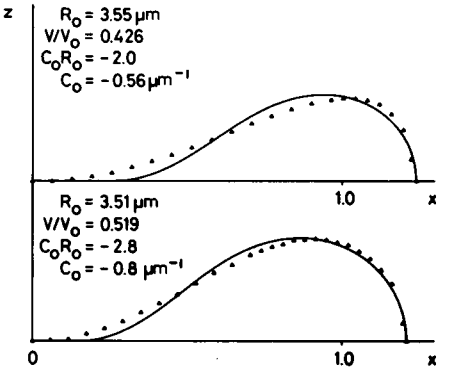

FIGURE 4

FIGURE 3 Comparison of theoretical contour to average red blood cell at 217 mosmol. Data taken from ref. 10.

FIGURE 4 Theoretical contours of torocytes as compared to experimental data taken from ref. 11.

[2]Evans, E. A. 1975. Comment at the Fifth International Biophysics Congress, Copenhagen. August 4–9.

main reason for the discrepancy of theoretical and experimental shapes. The best agreement was obtained for $c_o = -0.56\ \mu m^{-1}$ and $c_o = -0.8\ \mu m^{-1}$, respectively.

So far we have considered only solutions which besides rotational symmetry also have reflection symmetry with respect to the equatorial plane. In addition to these symmetric forms we have calculated "asymmetric" solutions which lack reflection symmetry. Fig. 5 shows a cup-shaped cell for $c_oR_o = -4.0$. The energy of this form is only slightly higher than that of a discocyte having the same volume. The energy is given in units of $E_o = 2\pi k_c(2 - c_oR_o)^2$ which is the curvature elastic energy of the sphere. We found the asymmetric, probably metastable, form to exist only above a critical volume V_c. With decreasing volume the cup cell becomes more and more symmetric and at the critical volume V_c it reaches the symmetric discocyte shape. When the volume is increased the cup cell becomes more and more asymmetric assuming a nearly spherical form with an indentation at one pole. When the volume is increased further the cup cell becomes a stomatocyte, i.e. a body with a concavity, as shown in Fig. 6 for $c_oR_o = -2.0$. The critical volume V_c depends strongly on spontaneous curvature and decreases with increasing c_o. The smallest value we could obtain without contact of the upper and lower membranes was roughly $V_c/V_o = 0.5$.

Very recently Jay (13) has studied the effect of albumin on the geometry of human erythrocytes. Tracing out edge-on photographs of blood cells he obtained contours of discocytes and cup cells from which he calculated surface area and volume. From his numbers we find for a typical cup cell $V/V_o = 0.55$.

Fig. 7 shows the theoretical contour of a cup cell for $V/V_o = 0.55$ and $c_o = 0$. The experimental data shown in the same figure were obtained by Evans and Leblond (11) using interference microscopy. The volume corresponding to the experimental contour is $V/V_o = 0.43$. Since we have not been able to reproduce cup cells with such a small volume we were forced to adopt a new type of solution representing an asymmetric torocyte, also called codocyte. Fig. 8 shows a theoretical codocyte together with

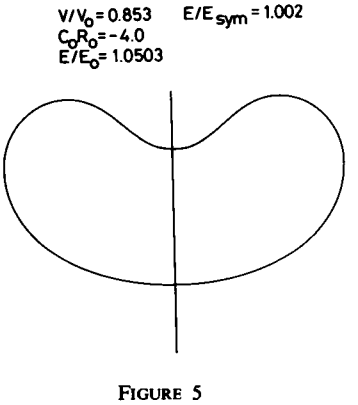

FIGURE 5

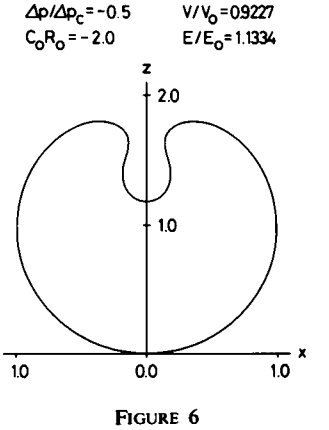

FIGURE 6

FIGURE 5 Theoretical contour of a cup cell.
FIGURE 6 Theoretical contour of a stomatocyte.

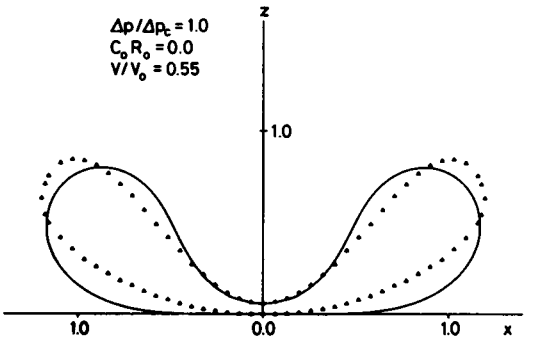

FIGURE 7

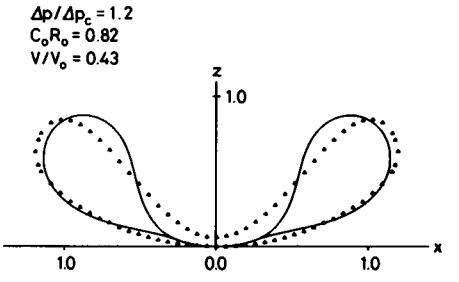

FIGURE 8

FIGURE 7 Theoretical contour of a cup cell compared to experimental data taken from ref. 11. The volume corresponding to the experimental contour is $V/V_0 = 0.43$.
FIGURE 8 Theoretical contour of a codocyte compared to the data of Fig. 7.

the data of Evans and Leblond. Its shape cannot be described by the class of contours used by Evans and Leblond in the analysis of their phase shift data. So there is a good chance that the discrepancy between the experimental and the theoretical contour shown in Fig. 8 is due to an inadequate parametrization in much the same way as we found to be the case for torocytes (Fig. 4). On the other hand, Jay's data for cup cells and even more so those by Evans and Leblond show a rather strong curvature at the brim of the cup in contrast to our theoretical curves (Fig. 7 and Fig. 8). This could be an indication that the shear elasticity of the membrane must not be neglected here. Cup cells may therefore be well suited to study how the shear elasticity of the membrane affects red cell shape.

Skalak and co-workers (14) have taken an approach to the problem of red cell shape which employs curvature elasticity as one element. They do not draw upon the concept of spontaneous curvature, but take shear elasticity into account. First numerical results on the spherocyte-discocyte transition have been published recently (15). Either a biconcave disc or the sphere were considered to be the equilibrium shape and the shear elastic modulus was used as an adjustable parameter. At present, it is not possible to evaluate the relative importance of spontaneous curvature and shear elasticity for the actual spherocyte-discocyte transition of red blood cells.

We are pleased to acknowledge very informative discussions with R. Skalak and E. A. Evans at the Fifth International Biophysics Congress Copenhagen, August 4–9, 1975.

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