LATERAL ORGANIZATION OF MEMBRANES AND CELL SHAPES

VLADISLAV S. MARKIN, *Institute of Electrochemistry, Academy of Sciences of the U. S. S. R., Moscow, U. S. S. R.*

ABSTRACT The relations among membrane structure, mechanical properties, and cell shape have been investigated. The fluid mosaic membrane model used contains several components that move freely in the membrane plane. These components interact with each other and determine properties of the membrane such as curvature and elasticity. A free energy equation is postulated for such a multicomponent membrane and the condition of free energy minimum is used to obtain differential equations relating the distribution of membrane components and the local membrane curvature. The force that moves membrane components along the membrane in a variable curvature field is calculated. A change in the intramembrane interactions can bring about phase separation or particle clustering. This, in turn, may strongly affect the local curvature. The numerical solution of the set of equations for the two-dimensional case allows determination of the cell shape and the component distribution along the membrane. The model has been applied to describe certain erythrocyte shape transformations.

INTRODUCTION

In recent years the relation of membrane composition and lateral organization to mechanical properties and shapes has been the object of keen interest. This problem is important because any one of the above characteristics may strongly affect the functioning of the whole cell or its parts.

Investigations of cell shape are often done with special reference to erythrocytes. The various hypotheses so far advanced have ranged from the elastic inner matrix concept to the liquid-filled flexible membrane shell (1). According to current thinking (2–22), properties of an erythrocyte membrane are uniform throughout and the membrane is not divided into independent regions. Membrane components move freely laterally, so that forces acting in the membrane plane can easily redistribute them. Another point of view treats the erythrocyte membrane as a superelastic shell (10–16). The curvature elasticity may be due either to charge interaction on the membrane (23–26) or to simple compression and tension on its opposite sides (22, 27). Canham (28) was one of the first authors to give close attention to the hypothesis of the effect of membrane curvature elasticity on the erythrocyte shape. Since the bending energy of an elastic shell depends on its shape, the equilibrium shape should be attained at minimum energy.

A more detailed study, however, showed that two cell forms exist that correspond to the bending energy minimum: an oblate shape (biconcave disk) and a prolate shape (dumb-bells) (29). To overcome this contradiction, Helfrich alone (30, 31) and with Deuling (32, 33) suggested a spontaneous membrane curvature. In this case, in the absence of stresses, the surface is curved and not planar. As a result, Helfrich and Deuling (33, 34) succeeded in

obtaining good agreement between the calculated and observed cell outlines, especially for discocytes. The experimental studies of Evans et al. (20, 21, 35) have usually been used as a standard reference against which to check the calculated contours. The work of Jenkins (36, 37) follows the same line.

The reason for the spontaneous membrane curvature may be an asymmetry of membrane molecules (38), asymmetric molecular distribution between the inner and outer monolayers (39), or both. Another interesting approach to erythrocyte stability and the transformation problem has been suggested by Glaser and Leitmannova (40). It is based on a "fluid mosaic" membrane model (41), where the cell shape is controlled by the state of contractile proteins and their aggregation on the interior surface of the membrane.

In the present paper we deal with the relation between membrane composition and cell shape. We investigate this concept in terms of the fluid mosaic model. The fluid mosaic model reflects many important features of erythrocytes. For this reason erythrocytes as well as lymphocytes and lipid vesicles will be used to illustrate the general conclusions.

PROBLEM FORMULATION

We will assume that the cell membrane is a liquid crystal shell; that is, it consists of individual elements which move comparatively easily (relative to each other) in the lateral direction. Generally speaking, the membrane components may have an asymmetric (for instance, conical) shape which is responsible for the spontaneous membrane curvature. The geometric membrane curvature does not always coincide with the spontaneous curvature. It is when they are different that stresses arise in the membrane. The curvature is not only characterized by its absolute value but also by a sign. We shall assume the curvature of a sphere to be positive; protrusions on the cell surface will also have a positive curvature and invaginations a negative one. Accordingly, the membrane component asymmetry (i.e., intrinsic spontaneous curvature) will be either positive or negative. The distribution of components in the membrane is assumed to be initially uniform. The membrane maintains a constant surface area but can be bent by external forces; the bending curvature depends on the bending moment applied. Correspondingly, the bending energy is a quadratic function of curvature:

$$(D/2)\,(K-K_s)^2$$

where D is curvature elasticity; K is total geometric curvature of the membrane equal to the sum of the reciprocals of major curvature radii; and K_s is the spontaneous, i.e., unstressed, curvature (30). Not to overburden the analysis, we confine the treatment to the case of a two-component membrane. Its composition can then be characterized by only one variable: the concentration of one of the components, c.

The free energy formula will contain the usual terms:

$$F_1 = bc + kTc \ln c$$

where k is the Boltzmann constant, T the absolute temperature, and b is a constant. Assuming that the membrane component concentration has an upper bound c_m, the free energy formula must further include the term

$$F_2 = kT(c_m - c) \ln (c_m - c).$$

The components may also interact with each other producing an additional contribution to the energy $F_3 = wc^2$. The equation for free energy per unit membrane surface area then takes the form:

$$F = \frac{D}{2}(K - K_s)^2 + bc + kTc \ln c + kT(c_m - c) \ln (c_m - c) + wc^2. \tag{1}$$

The local membrane properties, i.e., the parameters D and K_s, will depend on the membrane component concentration which, in turn, will vary over the membrane as a function of the local curvature and will thus depend on the membrane shape. The problem of determining the membrane shape, therefore, involves determining the particle distribution pattern over the membrane. The total membrane free energy is obtained by integrating Eq. 1 over the entire surface:

$$F_{tot} = \int F \mathrm{d}s. \tag{2}$$

In the equilibrium state F_{tot} is a minimum, subject to the following three limitations: a fixed cell volume, a fixed cell surface, and a fixed total number of membrane elements. Using the Lagrange method of undetermined multipliers we obtain

$$G = \int F \mathrm{d}s - p \int \mathrm{d}v - \lambda_1 \int \mathrm{d}s - \lambda_2 \int c \mathrm{d}s \tag{3}$$

whose first variation in the equilibrium state must be zero:

$$\delta G = 0. \tag{4}$$

From this condition, the equations determining the cell membrane shape can be obtained.

For the sake of clarity we first consider a two-dimensional case, in which the problem reduces to a consideration of the shape of an elastic ring (i.e. a uniform cylinder) with variable properties around the contour. It is convenient to use the curvilinear coordinate running along the ring. The contour shape will be defined by an angle between the tangent to the contour and some arbitrary axis; curvature K is defined by

$$K = \frac{\mathrm{d}\varphi}{\mathrm{d}l}. \tag{5}$$

Applying Eq. 4 we obtain a set of two equations containing the unknown functions $\varphi(l)$ and $c(l)$:

$$D\left(\frac{\mathrm{d}\varphi}{\mathrm{d}l} - K_s\right) + \frac{p}{2}(x^2 + y^2) + A = 0 \tag{6}$$

$$\gamma c + \ln \frac{c}{1 - c} - \rho\left(\frac{\mathrm{d}\varphi}{\mathrm{d}l} - K_s\right) + \nu\left(\frac{\mathrm{d}\varphi}{\mathrm{d}l} - K_s\right)^2 + B = 0. \tag{7}$$

Here c is a normalized value c/c_m; A and B are integration constants; and x and y are Cartesian contour coordinates:

$$x = x_0 + \int_0^l \cos \varphi\,(t)\mathrm{d}t;$$

$$y = y_0 + \int_0^l \sin \varphi\,(t)\mathrm{d}t. \tag{8}$$

The coefficients of the second equations are

$$\gamma = \frac{2wc_m}{kT};\ \rho = \frac{D}{kT}\frac{dK_s}{dc};\ \nu = \frac{1}{2kT}\frac{dD}{dc}. \tag{9}$$

The dimensionless coefficient γ describes the direct interaction intensity between membrane particles in kT units. The coefficients ρ and ν are, generally speaking, functions of the concentration, the first of these having the dimension of length and the second of area.

Eqs. 6 and 7 have a rather simple physical meaning. Eq. 6 reflects mechanical equilibrium of the membrane. The left-hand-side of Eq. 7 corresponds to the chemical potential of the membrane particles; this equation implies that the chemical potential of particles must be constant over the whole membrane.

It is now assumed that the spontaneous curvature may be expressed in the form

$$K_s = \xi_1 c + \xi_2 (1 - c) = \xi c + K_0. \tag{10}$$

ξ_1 and ξ_2 are the "intrinsic" spontaneous curvatures of the individual components, $K_0 = \xi_2$ is the membrane curvature in the absence of a second component, and ξ is the variation due to membrane particles.

An analysis of the equilibrium relationships in the membrane shows that the curvature elasticity can be written in the form

$$\frac{1}{D} = \frac{c}{D_1} + \frac{(1 - c)}{D_2} \tag{11}$$

where D_1 and D_2 are elasticities of individual components.

Using Eqs. 10 and 11 it is possible to express the parameters ρ and ν

$$\rho = \frac{D}{kT}\xi,\ \nu = \frac{D_1 D_2 (D_1 - D_2)}{2\,kT\,[c(D_2 - D_1) + D_1]}. \tag{12}$$

We are now in position to carry out a more detailed treatment of Eq. 7, which determines the membrane component distribution in a field of given curvature. The presence of a logarithmic term is known to produce more than one solution with respect to c. When the coefficient of the linear term is < -4, the system can experience a phase transition resulting in coexisting phases: a "condensed" phase and an "expanded" phase.

Suppose that the Eqs. 6 and 7 can be solved separately, i.e., K_s is constant. This is the situation when the coefficients ρ and ν in Eq. 7 are zero, which occurs at an elevated temperature, or when the membrane component curvatures are identical, i.e., $\xi = 0$. We can then solve Eq. 6 separately.

$$\frac{d\varphi}{dl} + qr^2 + \left(\frac{A}{D} - K_s\right) = 0 \tag{13}$$

where $q = p/2D$ is reduced pressure with a dimension of centimeter^{-3}; r is the distance from a given contour point to the origin; and A is an unknown constant.

In this limiting case the problem reduces to that of an elastic ring equilibrium under a pressure differential. For the case of circular ring this problem was considered back in 1913 (42, 43) in connection with the problem of underwater pipe collapse, and has often been

referred to since (44–46). Interest in this problem has recently been renewed in connection with blood vessel statics and dynamics (47–49).

From the solution of Eq. 13, it follows that the deformation of elastic ring can begin only after the pressure differential has exceeded a certain threshold value. The absolute value of the threshold depends on the membrane elasticity D and ring radius r_0; and can be expressed as $q_{th} = -3/2r_0^3$. The negative sign means that inside pressure must be less than outside. This threshold value is however quite independent of the constant spontaneous membrane curvature.

A Membrane Consisting of Particles with the Same Elasticity but Different Spontaneous Curvatures

Because the set of Eqs. 6 and 7 is rather complex, we shall try to deal with the effects of different elasticities and different spontaneous curvatures of membrane components separately. We begin with the case where the membrane components have equal elasticities $D_1 = D_2 = D$ but differing spontaneous curvatures, and put $\xi_2 = 0$. Then

$$K_3 = \xi c, \quad \rho = \frac{D\xi}{kT}, \quad \nu = 0. \tag{14}$$

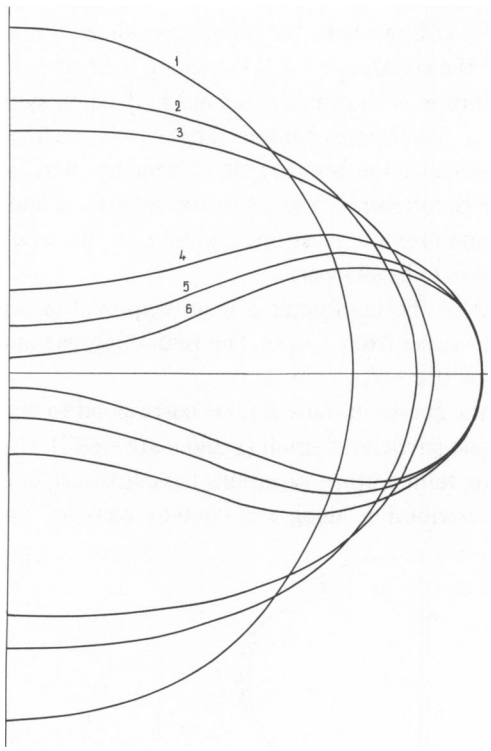

FIGURE 1 The cross-sectional area variation of a zero spontaneous curvature elastic ring as function of pressure difference: (1) $\geqslant -1.5$; (2) -1.55; (3) -1.6; (4) -2.1; (5) -2.2; (6) -2.3. Only the right half of the symmetric contour is shown. The minus sign means that the pressure in the interior of the ring is smaller than on the exterior. A further pressure reduction inside the ring rapidly leads to contour self-intersection, i.e. a contact between the opposite sides of the membranes.

The set of the fundamental equations can be rewritten as

$$\frac{d\varphi}{dl} - \xi c + q(x^2 + y^2) + A = 0 \tag{15}$$

$$\gamma c + \ln \frac{c}{1-c} - \rho\left(\frac{d\varphi}{dl} - \xi c\right) + B = 0. \tag{16}$$

Here A and B are the unknown constants; q is reduced pressure; all the other coefficients are also constant. The contour coordinates $x(l)$ and $y(l)$ are found via the Eq. 8. Eqs. 15 and 16 were solved numerically by computer. In what follows, the initial radius of the circle was $r_0 = 1$.

We shall first consider the contour properties in the absence of asymmetric membrane particles, that is, we put $c = 0$. In Fig. 1 we show how the cell shape varies with the pressure differential. The results agree closely with those published previously, in particular the threshold pressure happened to be $q_{th} = -1.5$. We now introduce the membrane particles and consider how the shape is affected by the particle symmetry, i.e. by ξ. Let us now assign fixed values to all the other parameters, for instance $q = -2.1$, $\gamma = 0$, $\rho = 1.0$, and the average concentration $\bar{c} = 0.1$.

We shall be concerned not only with the cell form but also with the distribution of the membrane component. Fig. 2 shows both the membrane shape and particle concentration in the membrane. In Fig. 2 *b* the coordinate l is taken along the contour shown in Fig. 2 *a*. Since only one half of the membrane is shown (the second half being symmetric), Fig. 2 *b* shows only half of the distribution. The figures contain three curves each, corresponding to ξ values from 0.1 to 3. It is readily seen that as the particle asymmetry increases in the membrane, the cell undergoes a stronger deformation: the particles tend to accumulate in the equatorial portion of the membrane and promote its strong bending. In this case the distribution of more asymmetric particles appears to be sharper.

Consider now the effect of the parameter ρ (the reciprocal of temperature $1/T$) on the membrane shape. Let ρ decrease from 1 to 0. The remaining parameters have the following values: $q = -2.1$, $\xi = 1$, $\gamma = 0$, $\bar{c} = 0.1$ (Fig. 3).

The larger numbers of the curves in these figures correspond to higher temperatures. Since the asymmetry of membrane particles is small (ξ and c are small), their effect on cell shape is insignificant, and therefore, temperature variations have little effect on the cell shape. At the same time, the particle distribution along the contour changes radically. The higher the

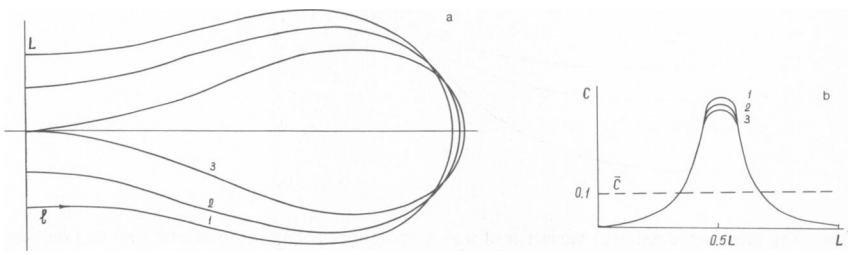

FIGURE 2 Variations of contour shape (*a*) and membrane component distribution (*b*) as function of ξ (for spontaneously curved components). The system parameters are: $q = -2.1$, $\gamma = 0$, $\rho = 1$, $\bar{c} = 0.1$. ξ can take values of (1) 0.1; (2) 1.0; (3) 3.0.

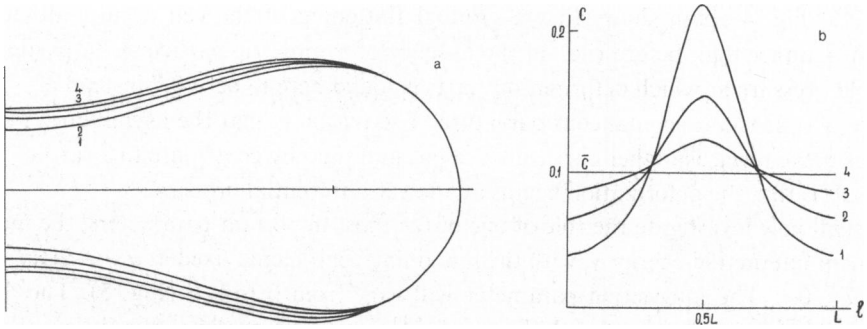

FIGURE 3 The effect of ρ running through the values: (1) 1.0, (2) 0.5, (3) 0.25, (4) 0.0 on the contour shape (*a*) and the membranes component distribution (*b*). The constant system parameters are $q = -2.1$, $\xi = 1.0$, $\gamma = 0$, and $\bar{c} = 0.1$.

temperature, the more uniform is the distribution. At very high temperatures Curve 4 tends to a constant. This indicates that the spontaneous curvature remains constant along the contour and, for this reason, has no effect whatsoever on the shape. The cell outlines shown in Fig. 3 *a*, Curve 4, therefore coincide with the contour (Fig. 1, Curve 4) of a cell with no asymmetric membrane particles.

Consider now the cell shape variation with pressure in the presence of membrane particles. With parameters $\xi = 10$, $\gamma = 0$, $\rho = 1$, $\bar{c} = 0.1$, and the pressure q varying between 0

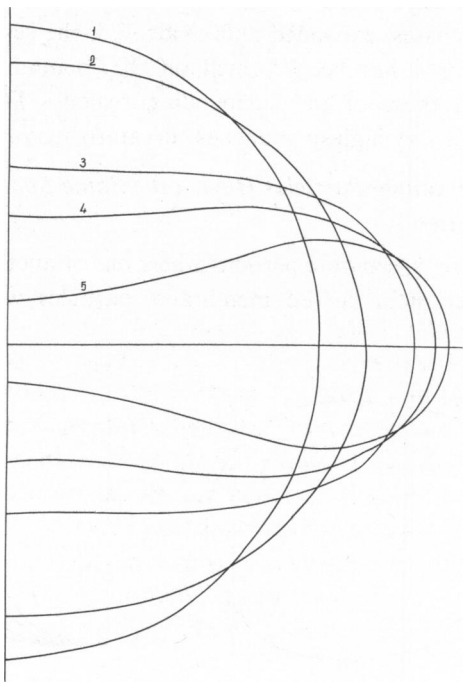

FIGURE 4 Effect of pressure differential on the shape in the presence of membrane components with spontaneous curvature. The constant system parameters are $\xi = 10$, $\gamma = 0$, $\rho = 1$, and $\bar{c} = 0.1$. The pressure q takes the following values: (1) -0.70, (2) -0.75, (3) -0.8, (4) -0.9, (5) -1.0, and (6) -1.2.

and -2.1, Fig. 4 again shows that a gradual flattening of the cell occurs, although in a different manner than before (i.e., in the absence of symmetric particles). In particular, the threshold pressure at which deformation starts is found here to be -0.7 instead of -1.5 as in the case of a constant spontaneous curvature. The reason is that the asymmetric membrane particles move towards higher curvature regions and thereby contribute to a further bending. The result is that the deformation begins at a lower differential pressure.

We shall now investigate the role of one of the most important parameters, the membrane component interaction energy γ, with the remaining coefficients fixed at $q = -2.1$, $\xi = 0.001$, $\rho = 1$, $\bar{c} = 0.1$. The interaction parameter will vary from 0 to -6 (Fig. 5). The degree of asymmetry, ξ, is small here and the presence of membrane particles has little effect on the membrane shape. The curves in Fig. 5 finally merge into a single curve; but the membrane particle distribution changes in a radical way as the interaction changes. The interaction energy changes between Curve 1 and Curve 2 of Fig. 5 *b* from 0 to -3, i.e., attraction between the particles begins to manifest itself. As a result, the distribution curve becomes narrower and higher.

The critical value of the interaction parameter γ is -4. Beyond this point a phase transition will be possible in the system. Indeed, Curve 3, corresponding to $\gamma = -5$, is discontinuous. The particle concentration given by this curve is small along the major portion of cell contour, and only on a short segment near the cell "equator" does it attain very high, almost limiting, values. A lateral phase separation has occurred in the system: over a major portion of the membrane the membrane component in question is in the expanded state, while near the equator a cluster or a condensed-state domain is formed. Curve 4 corresponds to $\gamma = -6$. Here, too, there are two phases: expanded and condensed, the separation being even more pronounced. Thus, a domain has been formed on the membrane whose properties are drastically different from those of the surrounding regions. This sharp point, a small membrane portion having a very high spontaneous curvature, may give rise to a spicule.

A Membrane Containing Particles Having the Same Spontaneous Curvature but Different Elasticities

In a flat membane there are no separate portions where one or another particle species would be concentrated. However, in a curved membrane, particles with different mechanical

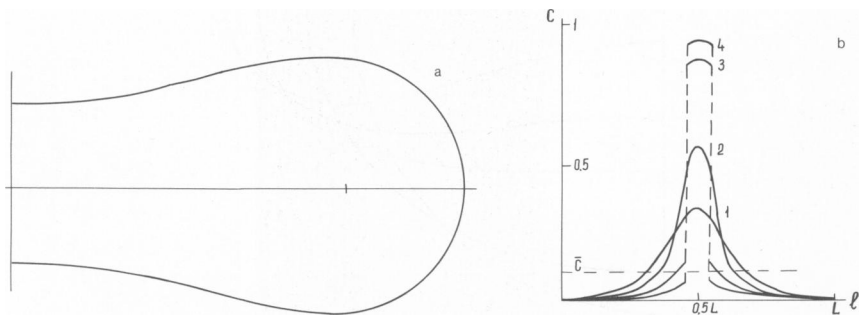

FIGURE 5 The effect of the interaction energy between the components, γ, which takes the following values (1) 0, (2) -3, (3) -5, (4) -6, on the contour shape (*a*) and membrane component distribution (*b*). The system parameters are $q = -2.1$, $\xi = 0.001$, $\rho = 1$, and $\bar{c} = 0.1$. Because the spontaneous curvature of the membrane components was small in this case, the contour shape varied very little.

properties will be displaced towards a greater or smaller curvature. We now calculate the force causing this movement. The calculations will cover a simple case and the results will be generalized. In a two-dimensional membrane model only one curvature radius has to be considered and the problem is to describe an elastic ring incorporating one or more inserts of another material. Let the elasticity of the whole ring be D_0, its initial radius r_0 and let there be one portion having a different elasticity D_i. For the sake of simplicity we shall neglect the difference between the internal and external pressure. Then the problem of determining the shape of such membrane reduces to finding the minimum of the bending energy,

$$W = \tfrac{1}{2} \int DK^2 \mathrm{d}l, \tag{17}$$

in which K is the local curvature and integration is done along the entire contour. The bending energy of the deformed ring is obtained as

$$W = \frac{\pi D_0}{r_0} + E_i. \tag{18}$$

The first term gives the energy of ring having no inserts, while the second term accounts for the effect of an insert on the total ring energy:

$$E_i = \frac{aD_0}{2}\left(1 - \frac{D_0}{D_i}\right) K^2. \tag{19}$$

When the insert is "soft," i.e., $D_i < D_0$, the additional energy E_i is negative and the total energy therefore decreases. In case of a "stiff" insert the total energy increases.

Extending the result to a three-dimensional case we use the notation K to denote the total curvature, and a the particle area. To have some idea of the orders of magnitude involved, we shall have recourse to a simple estimate. Putting $D \sim 10^{-11}$ erg, $a \sim 100 \times 100$ Å, $K \sim 10\,\mu^{-1}$, we find $E \sim 10^{-13}$ erg $\sim 1\ kT$. This means that when a cell undergoes deformation, i.e., invaginations or spicules are formed, the mechanical energy of a particle is comparable to the thermal energy. This may cause substantial restructuring.

We see that when the membrane curvature is different from point to point the system energy depends on the location of the insert. Therefore, a force arises which tends to shift the particle towards a region of greater or smaller curvature. This force is

$$F = -\nabla E_1 = aD_0\left(\frac{D_0}{D_i} - 1\right) K \nabla K. \tag{20}$$

Thus a soft particle tends to move towards a greater (in absolute value) curvature.

Another reason for movement may be a difference in the spontaneous curvature. If one considers a particle with the asymmetry (spontaneous curvature) ξ, taking the spontaneous curvature of the surrounding membrane as zero, then the energy of bending will be

$$E_i = -D\xi aK\,. \tag{21}$$

It is of interest to compare this with Eq. 19. The most striking difference is the different dependence on curvature. While in Eq. 19 the additional energy was quadratic with respect to K, in Eq. 21 it is linear. Thus, while the sign of the curvature was of no importance in Eq. 19, it makes a difference in Eq. 21. The reason is quite obvious. A particle whose elasticity differs from that of the remaining part of the membrane is "sensitive" only to the absolute value of

the curvature, whereas an asymmetric particle should of course be expected to be additionally sensitive to the sign of the curvature. As can be seen from Eq. 21, when the sign of the asymmetry ξ is the same as that of the curvature, the additional energy is negative. The reason is evident. Introduction of a particle having asymmetry of the same sign as the membrane curvature will reduce the elastic stresses. When, on the other hand, the signs are different, the elastic stress in the membrane increases, and the additional energy is positive.

Such particles should tend to move towards regions with the greatest positive or the greatest negative curvature, tending to bring down the energy of the whole system. The force causing such movement is

$$F = -\nabla E_i = D\xi a \nabla K. \tag{22}$$

Finally, when the two factors—the differing elasticities and spontaneous curvatures—are present concurrently, a more complex situation may arise, in which the particles do not move towards extremal curvature regions but remain in some optimum intermediate position. As an example, we shall consider a rigid particle with a positive spontaneous curvature. As noted above, the difference in elasticities causes such a particle to move towards the membrane region having zero curvature, while its own asymmetry induces it to shift towards the maximum positive curvature. Since the two energies are different functions of curvature, the sum of the energies will attain its minimum at some intermediate point. This is actually the point where the particles in question should tend to collect.

In the preceding section we have shown that when the interaction between some particles is large enough, domains of different particle concentration may be expected to arise. Obviously, a similar phenomenon should be expected in the case of particles having different curvature elasticities. We shall not dwell on the special problem of how the particle separation in the membrane occurs but proceed to investigate the effect of the resulting domains on cell shape.

Suppose a domain of length l_2 and elasticity D_2 has been formed on a membrane, while the rest of the membrane has an elasticity D_1 (Fig. 6). The other membrane half, not shown in this figure, has the same structure. Let us now analyze how the cell shape varies as pressure, relative elasticities, and domain lengths are varied. The problem reduces to solving Eq. 6 in which the spontaneous curvatures K_s will be assumed to be zero. We consider the role of the elasticity ratio D_1/D_2 by fixing $q = -1.2$, $l_1/L = 0.5$, $l_3 = 0$ and varying D_1/D_2 from 2 to 4. The resultant contours are shown in Fig. 7. The increase of D_1/D_2 corresponds to a situation in which the top portion of the cell becomes softer. This is why it bends more, as is particularly apparent at its extremeties. Similarly, the cell outline changes when the location of the domain of different elasticity changes. Putting $q = -1.2$, $D_1/D_2 = 4$, $l_2/L = 0.5$, we shall vary the relative lengths l_1 and l_3 maintaining their sum constant $(l_1 + l_3)/L = 0.5$. The calculated results are given in Fig. 8. It is clear that by varying the parameters in question it is possible to come up with a broad variety of shapes, all however resembling stomatocytes.

DISCUSSION

We have shown how, in a uniform membrane containing a number of different components, a nonuniform organization may arise and how this affects the membrane shape. The underlying assumptions of this model have been that the membrane is a multicomponent system; the particles constituting the membrane are laterally mobile; the membrane components interact

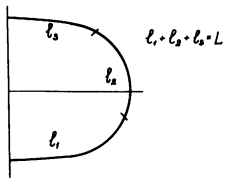

FIGURE 6

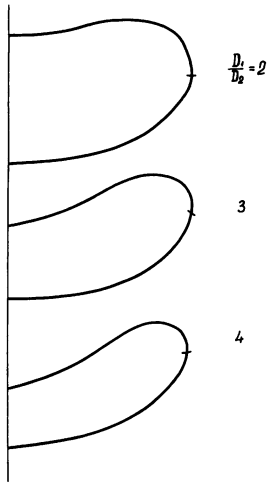

FIGURE 7

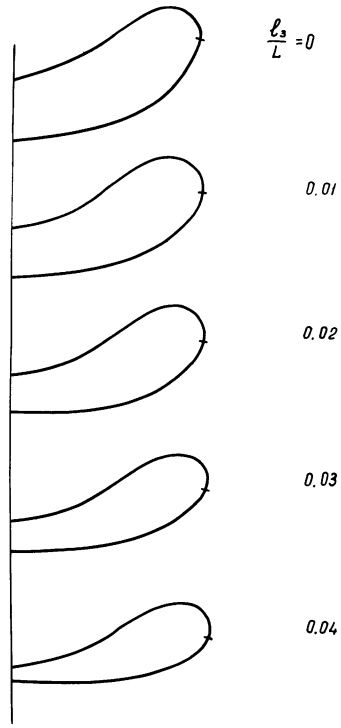

FIGURE 8

FIGURE 6 A composite membrane. The segment l_2 represents a domain with curvature elasticity D_2, the rest of the membrane having elasticity D_1.
FIGURE 7 Effect of the different elasticities of two segments constituting a membrane on its shape. The elasticity ratio is indicated in the figure. The system parameters are $q = -1.2$, and $l_1/L = 0.5$.
FIGURE 8 Effect of the location of the intermediate domain having a constant length $l_2/L = 0.5$ on the cell form. The system parameters are $q = -1.2$, and $D_1/D_2 = 4$.

with each other; the components may be asymmetric; and the different components have different mechanical properties. Such assumptions appear to be quite plausible. The real biological membranes are indeed composed of a large number of different molecules that are more or less mobile.

We have assumed that the membrane components can interact with each other. Eq. 7 is a formula for the chemical potential of particles in which the direct interaction is accounted for by the term γc, where γ represents the strength of the intereaction. Eq. 7 also contains the terms $\rho\,(\mathrm{d}\varphi/\mathrm{d}l - K_s)$ and $\nu\,(\mathrm{d}\varphi/\mathrm{d}l - K_s)^2$ which incorporate the concentration of the particles in question. These terms account for the interaction between the particles via elastic interactions, i.e., the long-range forces. The interaction is stronger for greater curvature elasticity of the membrane. This effect can be demonstrated by Eq. 16 incorporating the term $\rho\xi c = \mathrm{D}\xi^2 c$; the term $D\xi^2 c$ is always positive. Thus similar particles in an elastic membrane should repel each other with a force proportional to the membrane curvature elasticity and the square of the asymmetry of particles.

The results can be easily generalized to the case of interactions of unlike particles. The interaction energy formula would then contain a product of their asymmetries $\xi_1\xi_2$. When the

signs of ξ_1 and ξ_2 are different, the interaction energy will be negative, i.e. particles of unlike asymmetry will be attracted to each other.

The possibility of such interaction has already been suggested. Gruler (50) hypothesized that asymmetric (conical) particles floating in the "lipid sea" of the membrane should either attract or repel each other depending on what distortions they introduce into this elastic medium. This effect has been experimentally demonstrated on liquid crystals (51).

A direct interaction between the structural components of a membrane may be due to various mechanisms; very often it is of purely steric nature. It may be due to the electric charges of the polar heads of lipids or protein charges. It is essential to life processes that such interaction can be easily controlled by the aqueous environment, especially by the presence and concentration of specific and nonspecific electrolytes (52–54). The effect caused by the adsorption of ligands is an example, i.e., sufficiently large molecules selectively reacting with only the specific membrane components is equivalent to the indirect attraction between the respective membrane components.

Another important characteristic of the membrane used as a model in the present paper is its geometric curvature. It can affect the membrane structure, provided that the latter has some effect on curvature elasticity. The membrane can have a spontaneous curvature, for example, when it is composed of asymmetric (conical—in the simplest case) blocks. The presence of asymmetric components in a cell membrane is more the rule than exception. This is the case for lipid molecules, and probably for membrane proteins (38). The spontaneous curvature could be due to the different surface areas of the exterior and interior monolayers, i.e., the different amounts of matter in each of these monolayers. Such a "bilayer couple" hypothesis has been suggested by Sheetz and Singer (55) and by Evans (27).

A variation of a property of an individual component, sometimes even a small variation, can give rise to a radical restructuring of the organization of a membrane. We have shown above that a cluster or domain of particles of one type has dimensions that depend upon the conditions. The formation of such domains has been observed many times both in biological and artificial membranes. An investigation in artificial membrane has been carried out (52–54, 56–59) on lipid vesicles and monolayers composed of mixed lipids of several different types. Phase separation occurred as a result of environmental factors, such as temperature, presence of divalent cations, adsorption of the external proteins such as polylysine, etc. Each of the above conditions ultimately results in a change of interaction between the membrane components, and in this sense such phase separation is described by the model adopted here.

In cell membranes phase separation and formation of domains occur very frequently and radically affect the salient biological processes (60). Lateral phase separation, for example, affects the mobility of the membrane antigens (61), is necessary for the functioning of certain membrane proteins (62), changes the susceptibility of erythrocytes to the action of phospholipase-C (63), etc. The clustering of the membrane components apparently affects the fusion of natural membranes making possible the fusion of regions of a pure lipid bilayer from which the intramembrane proteins have been removed (64, 65). The fact that phase separation is necessary for membrane fusion has been indirectly demonstrated in a study of the fusion of artificial vesicles from a mixture of phosphatidylserine and phosphatidylcholine under the action of calcium ions (66); the conditions required for the separation of these lipids coincide with the optimum vesicle fusion conditions.

Protein clustering can, for example, occur in the course of an exocytosis process (67). One of the mechanisms may be that the formation of lipid domains of different composition are accompanied by displacements of the membrane proteins, provided that the latter have different solubilities in one or another domain type. This has been demonstrated in experiments involving vesicle formation from human erythrocytes (68). Their membrane composition differs radically from that of the "mother" erythrocytes. The concentration of phosphoric acid in the vesicles is 10 times as high as that in the erythrocyte membrane, while the concentration of phosphatidyl ethanolamine is somewhat below the norm, acetylcholine-esterase concentration increases twofold.

When we analyzed Fig. 5 we noted that the resulting domain may have a spontaneous curvature quite different from that of the adjacent membrane regions. This has been experimentally verified (57, 58, 69). The pictures of artificial vesicles shown there demonstrate clearly that marked protrusions and invaginations have appeared in certain membrane regions after phase transition. These protrusions and invaginations may eventually give rise to vesicles.

A phase separation in a membrane may result in a spontaneous curvature even in cases where the membrane components, and consequently the resulting domains are symmetrical. A separation of the layer into individual phases involves an increase in its area (39). If the phase transition occurs in only one of the monolayers surrounding the cell, the resultant extension of this monolayer will produce a positive or negative spontaneous curvature.

A similar effect may be produced by the intrusion of foreign molecules into the exterior or interior membrane monolayers. Indeed, an addition of amphifilic chemical substances to an erythrocyte solution converts the discocytes either into stomatocytes or echinocytes, depending on the substance type (70). For example, "anion crenators" build themselves into the exterior monolayer thereby expanding it. The membrane is caused to have an additional spontaneous curvature resulting in cell crenation. In contrast, the "cation cup-formers" prefer to invade the interior monolayer, expanding it and creating a negative spontaneous curvature. This promotes the formation of invaginations and results in stomatocytes. The same effect was obtained by treating erythrocytes with detergents that extract a part of the lipids from the exterior monolayer. This also produces a negative spontaneous curvature resulting in the formation of stomatocytes. The combined action on a cell of cup-formers and crenators does not affect the spontaneous curvature of membrane, and does not lead to transformations (71).

The distribution of a number of membrane components, such as enzymes, over the cell surface should be expected to depend on the local curvature. The data available today support this suggestion. In many cells whose forms are subject to variation during their lifetime it has been often observed that the normally uniformly distributed mobile molecules suddenly begin to concentrate at certain specific places. Such concentration usually occurs in strongly curved regions. In reference 72 the concentration of antigens on echinocytic spicules obtained from type A human erythrocytes was observed. de Petris (73) studied the transformation of lymphocytes. The observed formation of microvilli on the spherical cell surface was accompanied by the movement of the surface immunoglobulins and by their concentration on the microvillum peaks. It is important that the surface immunoglobulins concentrated on the microvilli are as motile as before because they are not permanently bonded to any of the

structural components of a microvillum, for example, a strand of microfilaments passing along the microvillus axis. In the same paper, de Petris (73) lists other cases of such nonuniform redistribution of surface molecules due to membrane shape variation. The mechanism discussed above may be regarded as the simplest explanation of this phenomenon.

It is natural to expect that in cell membranes there are regions of unfavorable curvature that completely lack a certain molecular species. The analysis of spectrin distribution over membranes of the echinocytic red blood cells carried out by Ziparo et al. (74) shows that this protein occurs more or less uniformly over the entire surface, with the exception of areas near the spicule bases (i.e., areas of negative curvature where there was practically none of it).

Thus the curvature conception allows explanation of a range of phenomena. It can be used to account for the distribution of intramembrane particles on the cell surface (69, 75), the origination of the myelin shapes produced by erythrocytes (76), and the origin and size of bilayer lipid vesicles generated under ultrasonic irradiation of the lecithin suspension in water (77). Sheetz and Chen (78) have suggested that the curvature should affect the dynamic behavior. The phase transitions in a flat and curved lipid bilayer were found to be considerably different (79–81), and the lateral phase separation was found to occur in a different way (82).

It is interesting to note that there are differences not only between flat and curved bilayers, but also between two monolayers of a curved bilayer. Two reasons may be responsible for this. The first is that topologically they are curved in the opposite directions, the inner monolayer being curved towards the polar heads and the outer monolayer towards the hydrocarbon tails. Second, the curvature of the interior monolayer is obviously higher than that of the exterior one. The difference should be more tangible in small vesicles. Indeed, the experiments involving dipalmitoyl phosphatidylcholine vesicles (83) showed that the structure was substantially asymmetric. The total thickness of the membrane of such vesicles with an outer radius of about 109 Å is practically the same as that of a flat bilayer but the thicknesses of the outer and inner monolayers are widely different, being 15 and 20 Å, respectively. This means that the lipid molecule tails in the outer layer are much longer than in the inner layer where they are partially folded. No wonder that the phase transitions in such layers occur differently and at different temperatures. This supports the hypothesis of the bilayer couple hypothesis as one of the mechanisms governing cell shape variations.

The development of a local curvature on a cell membrane may serve as a triggering mechanism actuating certain physiological processes. The external protein adsorption on a membrane can cause a radical restructuring in the outer and then in the inner monolayer of the cell membrane (54). Thus, every projection on the outer surface of membrane will have its counterpart cavity on the inner surface. This form may happen to be favorable for the adsorption on the interior membrane surface of some other molecule of the cytoplasm. This will be a simplest example of the transmembrane interaction of two adsorbed molecules whereby they come closer in space and may then take part in some reaction. The spontaneous curvature thus produced may affect the curvature elasticity of membrane (84). Given certain relations among the membrane constituents, a very high entailing monolayer collapse into micelles is predicted (84).

A local phase transition in a membrane, followed by the development of spontaneous curvature, may trigger the development of vesicles, as in the case of pinocytosis (38). Such is

apparently the endovesiculation mechanism in the erythrocytes of newborn babies under the effect of certain ligands (85). The process may take place in the following manner (85). The ligand binds to the receptors on the erythrocyte surface and causes their clustering. The resulting cluster exhibits a negative spontaneous curvature which entails invagination followed by endocytosis. The fact that very small vesicles are produced is apparently due to limitation of receptor mobility by certain domains. These limitations are possibly even stricter in the erythrocytes of adult humans, since no endocytosis has been observed in them under similar conditions.

The model developed here is most clearly applicable to the simplest lipid vesicles. However, lipid vesicles sometimes represent a good simulation of the real cells, including erythrocytes. Thus lecithin in water spontaneously separates into membranes consisting of one or more bilayers which merge together to form vesicles. The contour of these vesicles resembles the various forms of erythrocytes, including stomatocyte and dumb-bell shapes (86). This probably indicates that the mechanism of erythrocyte-shape maintenance is similar in this regard to that of lipid vesicles, even though the structure of the erythrocyte is much more complex.

There is another difficult point in the present theory. Strictly speaking the model describes a monomolecular layer of a liquid crystal with another molecular species floating in it. The biological membranes are not exactly monomolecular layers of liquid crystals. Nor are they simple bilayers, because the intrinsic proteins spanning the membranes give them some properties of a monolayer. For this reason the rather simplified model may have something in common with the biological membranes.

Insofar as we have confined our attention only to an equilibrium membrane structure, we have completely disregarded its viscoelastic and other time-dependent properties, although there are indications that these properties may have an important role in maintaining or varying the erythrocyte shapes (14, 87, 88). There are a number of papers which treat the erythrocyte membrane as a superelastic shell and explain successfully some erythrocyte deformations. It seems that in the analysis of large deformations of real erythrocytes, one also has to take into consideration the shear elasticity of its membrane, especially when the transformation proceeds rather fast, i.e., when the membrane is not in an equilibrium state at any moment. The reason for that is the very complicated structure of a real erythrocyte membrane.

Indeed the membrane structure of the real erythrocyte differs in many details from the model discussed in this paper, primarily by the spectrin-actin network underlying the membrane. It should be noted that such a cytoskeleton is a two-dimensional, rather than a spatial structure, spanning the cytoplasmic side of the membrane (89). The structure and function of the spectrin-actin networks are currently being studied in many laboratories. The explanations so far advanced are as follows (90): the lipid layer per se, containing the integral protein inclusions, is apparently insufficiently strong to serve as a shell for such a large cell as an erythrocyte; the lipid layer would not be stable enough and would disintegrate into small vesicles.

The spectrin network has, most probably, a twofold function. First, it connects the separate portions of the membrane, thereby preventing fragmentation; second, it imparts a high bending strength to the system. It is interesting that this cytoskeleton stratum can exist

practically without any membrane. If the erythrocyte ghosts are treated with the nonionic detergent Triton X-100, which extracts a major portion of lipids and integral proteins, only the cytoskeletons with the same size and shape as the initial ghosts will remain (91, 89). These cytoskeletons consist primarily of spectrin and actin with traces of other proteins and a minor amount of phospholipids.

This spectrin net is not a rigid permanent structure, however. In the course of erythrocyte transformations it can be destroyed (and rebuilt), as indicated by the movements of the integral proteins whose mobility under normal conditions is quite limited (92). It seems that the translocation of membrane particles occurs just in this transitive state. It may be caused by different reasons such as shape changes under the external forces, for example. The rate of such translocation is determined by membrane viscosity and was calculated in reference 93. The question of dynamic nature of spectrin-actin network and its ability to disrupt and to rebuild in the new form is of special interest. But it must be a subject of separate investigation. The presence of the spectrin network, therefore, does not undermine the model of the present paper, but rather imparts to it new features which must be considered when describing the nonequilibrium effects and the rheological properties of cells.

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