

ON THEORETICAL SHAPES OF BILIPID VESICLES UNDER CONDITIONS OF INCREASING MEMBRANE AREA

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ABSTRACT Certain standard properties, including spontaneous curvature, are assumed for the membrane of a bilipid vesicle. Then, if there is a mechanism that causes the membrane area to increase, vesicle cleavage is found to occur.

INTRODUCTION

A simple mathematical model of the red blood cell membrane was proposed by Canham (1970) and, in a generalized form that includes spontaneous curvature, by Helfrich (1973). That model, based on minimization of bending energy, has been remarkably successful in the description of the biconcave shape of the red blood cell (Deuling and Helfrich, 1976a). In that model shear stresses within the membrane are assumed negligible. The membrane is in essence a two-dimensional fluid, with energy per unit area equal to

$$\frac{1}{2}k_c(c_1 + c_2 - c_0)^2, \quad (1)$$

where k_c is an elastic constant, c_1 and c_2 are the principal curvatures, and c_0 is the constant of spontaneous curvature.

In model 1 the membrane is assumed homogeneous but asymmetric, as seems appropriate to the fluid mosaic model proposed by Singer and Nicolson (1972). Several authors (Evans, 1974; Helfrich, 1974; Sheetz and Singer, 1974) have concluded that membrane asymmetry is essential to an explanation of observed red blood cell shapes, and have proposed various mechanisms to account for it. Such an asymmetry of the lipid bilayer, and the resulting nonzero value of c_0 , could arise from differences of composition or effective area of the two monolayers, or from the effects of membrane proteins. Indeed, asymmetry might be anticipated simply because of the different fluid environments on the inside and outside of the lipid bilayer, but there is also substantial evidence that the inner and outer monolayers differ in composition and structure (Singer and Nicolson, 1972). Thus c_0 cannot in general be assumed to be zero; in fact, some progress has also been made toward an understanding of mechanisms that could cause the spontaneous curvature to change with time (see Sheetz and Singer, 1977). It is of course possible that model 1 is too idealized and that shear stresses should indeed be taken into account, at least over short time intervals (see Evans and La Celle, 1975; Deuling and Helfrich, 1976b). In any case, the model is useful be-

cause of its simplicity and elegance. Because it has only one unspecified parameter, c_0 , model 1 has correspondingly great predictive value. By contrast, in membrane models based on the more traditional thin shell theory (e.g., Fung and Tong, 1968), it is usually necessary to specify a priori an intrinsic membrane shape.

We now apply membrane model 1 to a bilipid vesicle where c_0 remains constant, but where there is some kind of mechanism that increases the membrane area. We are not concerned here with the precise nature of the growth or synthesis mechanism; however, one mechanism that has been observed in connection with biological membranes and widely discussed is the fusion of small vesicles with the membrane. In the process their area is incorporated into the membrane and their contents expelled across the membrane (see reviews: Berlind, 1977, p. 216; Morré and Ovtracht, 1977, p. 135).

FORMULATION OF THE MODEL

Let us then consider a vesicle whose area increases, and let p be the pressure difference (external pressure minus internal pressure). Although one might assume a general functional dependence

$$\begin{aligned} dA/dt &= f(A, V, p) \\ dV/dt &= g(A, V, p) \end{aligned} \quad (2)$$

for the time derivatives of the vesicle area and volume, we prefer for concreteness to mention the following two special cases: in case 1, dA/dt and dV/dt depend linearly on A (or, more generally, on a linear combination of A and V), so that A and V increase exponentially with time. Case 1 might apply to a nearly impermeable membrane where the rate of fluid transport across the membrane is essentially related to or limited by the membrane area. A more interesting situation, case 2, is where dV/dt is proportional to $p - p_0$, and the volume increases in such a way that p tends toward the constant pressure p_0 ; in fact, if the time constant is sufficiently short, the time dependence of A is unimportant since p is approximately equal to p_0 , and A enters the problem only as a parameter. Case 2 applies if the membrane is relatively permeable, so that fluid is transported quickly across the membrane whenever p differs from the osmotic pressure p_0 . We ignore for the present any change in p_0 that might be caused by dilution of the fluid within the vesicle. In either of the above cases we assume Eq. 1 and use energy considerations to determine the vesicle shape at each instant; thus we are assuming that other dynamic effects in the system (e.g., membrane acceleration, motion of fluid with the vesicle) can be neglected at the time scale of interest.

We are primarily concerned with the changes in vesicle shape and volume that occur as the area increases, but to solve the problem we need information about the family of static vesicle shapes. The numerical calculation of static vesicle shapes is of itself an interesting but rather lengthy undertaking; however, the underlying mathematical problem can be stated quite simply: find the closed surface of given area and volume for which the integral over area of the energy (1) is a minimum. Deuling and Helfrich (1976*a, b*) and Jenkins (1977) have each described methods of doing this. Assuming axial symmetry, they each set up a variational problem and use the calculus of variations to derive a system of Euler equa-

tions, which are then solved numerically. Deuling and Helfrich use a Newton-Raphson method of solution, whereas Jenkins uses the technique of quasi-linearization. Because our method of solution for static vesicle shapes is sufficiently similar to those of Deuling and Helfrich (1976a, b) and Jenkins (1977), we will omit the details here, but it has some computational advantages that will be described elsewhere.

RESULTS FROM NUMERICAL COMPUTATION

Results for case 2 when $c_0 = 5$ are shown in Fig. 1, Fig. 2, and Fig. 3. Case 2 is for constant pressure, so for convenience we present results in nondimensional form with $k_c = 1$, $p_0 = 1$; however, the problem can easily be rescaled for other values of k_c and p_0 if desired. In that case distances are to be multiplied by $(k_c/p_0)^{1/3}$, areas by $(k_c/p_0)^{2/3}$, volumes by k_c/p_0 , and curvatures c_0, c_1, c_2 by $(p_0/k_c)^{1/3}$. In Fig. 1 the broken line ABC shows the free energy (bending energy, plus pressure times volume) of the family of spherical solutions. A family of elongated solutions, $CDBE$, bifurcates from the family of spherical solutions at point C . Near C the elongated solutions approximate prolate ellipsoids, but from B to E , where they have lower energy than the spherical solutions, they are dumbbell-shaped. The sequence of vesicle shapes under conditions of increasing vesicle area is shown in Fig. 2, and the corresponding volumes are shown in Fig. 3; letters correspond to those in Fig. 1. The spherical solution A grows until it reaches size B . Let us suppose for the moment that the spherical solutions become unstable at B , so that a transition occurs to the dumbbell shape

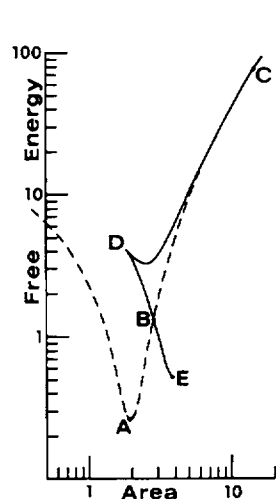


FIGURE 1

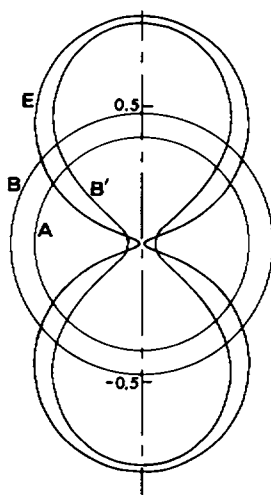


FIGURE 2

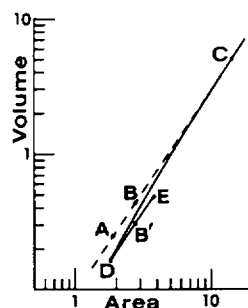


FIGURE 3

FIGURE 1 Free energy of spherical solutions (broken line) and elongated solutions (solid line) as functions of vesicle area. All quantities have been nondimensionalized so that $p = 1$.

FIGURE 2 Sequence of vesicle shapes showing vesicle cleavage. Solutions are axially symmetric about the vertical axis. The scale in nondimensional units is indicated on the vertical axis, and letters correspond to Fig. 1.

FIGURE 3 Volume of spherical solutions (broken line) and elongated solutions (solid line) as functions of vesicle area. Letters correspond to Fig. 1.

B' of the same area and free energy. Upon further increase in vesicle area the shape approaches E and the necklike region of the dumbbell becomes very narrow. We have not been able to obtain complete closure of the neck, perhaps because of computational inaccuracies near the singularity, or perhaps because a somewhat larger value of c_0 is needed. In any case, the neck is so narrow at E that it is natural to assume that the membrane fuses and that two separate vesicles are formed. The process then repeats itself from A .

DISCUSSION

For suitable positive values of c_0 , growth of the membrane area thus leads naturally to a vesicle cleavage phenomenon. That result agrees with intuition in that the large positive value of the spontaneous curvature drives the configuration to two spheres when the area has become large enough to accommodate them. By contrast, negative values of c_0 typically give the biconcave vesicle shapes associated with erythrocytes (Deuling and Helfrich, 1976a, b). When $c_0 = 0$, we infer from the results of Jenkins (1977) that the vesicle would elongate but cleavage would not occur. The shapes calculated by Deuling and Helfrich (1977) for myelin forms for various c_0 also appear consistent with the above interpretations.

In the mode of vesicle cleavage that we have outlined, the main unresolved problems involve the transition from the sphere B to the dumbbell shape B' ; we believe that these questions deserve further study. Whether the vesicle will indeed assume the shape of lowest free energy as we have supposed depends of course on stability considerations, but because of the rapid increase in free energy of the spherical solutions with area, as shown in Fig. 1, it seems implausible that the spherical solutions could be metastable much beyond B . A transition to the dumbbell shape at B or shortly thereafter is thus highly plausible, but a thorough investigation of this question will be difficult because it necessarily involves vesicle shapes that are not axially symmetric.

The dynamic behavior of the membrane during the transition is also of interest. It is clear that a transition from B to B' cannot occur instantly, since fluid must be transported out of the vesicle to account for the decrease in volume, as can be seen from Fig. 3. To examine the details of the transition one would then need to refer back to Eqs. 2, which govern the transport of fluid through the membrane when p is temporarily less than p_0 . Thus for case 2, the transition from B to B' is the only part of the problem where Eqs. 2 are really needed, since otherwise one can simply use the quasi-static idealization that the vesicle shape depends on the area.

We have not yet obtained results for an impermeable membrane (case 1) because the calculation of the static vesicle shapes turns out to be somewhat more difficult than for case 2. In case 1 it is clear, however, that the vesicle cannot in general be spherical, for even if the vesicle is assumed initially spherical, the volume will be insufficient to allow for a spherical shape once an increase in area has occurred.

SUMMARY

The above model of vesicle cleavage is extremely idealized, and we do not yet know of a physical situation where it is clearly applicable; nevertheless, we feel that it is so simple and

natural that its consequences should be understood. We believe that a clear understanding of the behavior of model membranes under diverse circumstances is especially useful in that, because of the difficulties of direct measurement, membrane properties must often be deduced from the observed vesicle shapes. We also note that Greenspan (1977a, b) has formulated a mathematical model of cell cleavage that is related to the classical experiments in which cleavage of an oil drop is caused by application of a surfactant at the opposite poles. Similarly, we consider our results on vesicle cleavage to be an instructive analogue of cell division, even though cell division is known to be a diverse and highly complex phenomenon. More relevant applications are likely to be found in the study of liposomes (Bangham, Hill, and Miller, 1974) or in the study of various cell organelles.

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