Bending energy of vesicle membranes: General expressions for the first, second, and third variation of the shape energy and applications to spheres and cylinders

Ou-Yang Zhong-can* and Wolfgang Helfrich
Institut für Theoretische Physik, Freie Universität Berlin, Arnimallee 14, D-1000 Berlin 33, West Germany
(Received 28 November 1988)

A general equation of mechanical equilibrium of fluid membranes subject to bending elasticity [reported in Phys. Rev. Lett. **59**, 2486 (1987)] is derived in detail. The second variation of the shape energy, also obtained for arbitrary shapes, is used to analyze stability with respect to deformational modes for spherical and cylindrical vesicles. The former analysis is well known, while the latter is presented here for the first time. The theoretical results are shown to agree very well with previous numerical calculations. In addition, they provide the energies controlling the shape fluctuations and show that spontaneous curvature may transform cylinders into tapes or strings of beads. The study of the energy of infinitesimal deformations is finally extended to include the third variation. Applying the general result to the sphere, we obtain the critical value of spontaneous curvature below which oblate ellipsoids of a deformed sphere are more stable than prolate ones. It is shown to be the same regardless of whether volume or pressure is kept constant.

I. INTRODUCTION

In recent years both experimental and theoretical studies of amphiphilic bilayers and monolayers have been beginning to engage the attention of physicists.[1] Part of the progress made in these fields consists in understanding the role of the bending elasticity for both equilibrium shapes and shape fluctuations of fluid layers. Certain amphiphilic molecules, such as phospholipids, assemble in water to build bilayers which, at low concentration, close to form single shells called vesicles.[2] These structures are simple models for biological membranes and cells.[3,4] Other amphiphiles form surfactant films separating oil and water, thus giving rise to microemulsions.[5]

The equilibrium shape of a vesicle is determined by the minimization of the shape energy which may be written as[6,7]

$$F=\tfrac{1}{2}k_c \oint (c_1+c_2-c_0)^2 dA + \Delta p \int dV + \lambda \oint dA \ . \quad (1)$$

Here dA and dV are surface area and volume elements, respectively, k_c the bending rigidity, c_1 and c_2 the two principal curvatures, and c_0 the spontaneous curvature. The last serves to describe the effect of an asymmetry of the membrane or its environment.[8,9] The first term of Eq. (1) is the curvature-elastic energy of the vesicle membrane.[6] The second and third terms either take account of the constraints of constant volume and area or represent actual work. Depending on the situation, the pressure difference $\Delta_p = p_{\text{out}} - p_{\text{in}}$ and the tensile stress λ serve as Lagrange multipliers or they are prescribed experimentally by volume or area reservoirs. Instead of the last term of Eq. (1) Jenkins[10,11] introduced a local area constraint by $\oint \gamma\, dA$, where γ is a Lagrange function varying with position. He derived a general equilibrium equation, but did not consider spontaneous curvature except recently for the special case of the fluctuating sphere.[12] A generalized equilibrium-shape equation including spontaneous curvature was put forward in our recent paper.[13] The first purpose of the present paper is to give a derivation of this equation.

The second aim of the paper is to provide a general formula for the stability analysis of equilibrium shapes. The deformational energies of a nearly spherical vesicle of given area and volume were studied by Peterson.[14] Another case, instability of a sphere as a function of Δp at variable V, has been considered by us.[13] In the following we calculate the second variation of the shape energy for arbitrary shapes. The formalism is then applied to analyze the stability of cylindrical vesicles by calculating the energies of a complete set of deformational modes. In addition, the results to be obtained provide the energies controlling the shape fluctuations and show that spontaneous curvature may transform cylinders into tapes as well as strings of beads. The results compare very well with numerical examples of strings of beads calculated previously by Deuling and Helfrich.[15]

In a third step, the general theory of infinitesimal deformation is extended to include the third variation of the shape energy. In this way we find a limiting value of spontaneous curvature below which oblate forms of a deformed sphere are more stable than prolate ones. The value is shown to be the same, at the threshold of deformation, whether the volume or the pressure is given. The result, $c_0 r_0 \le -1.2$, where r_0 is the radius of the sphere, agrees with that of Peterson[14] as well as Milner and Safran[16] calculated for the case of fixed volume and also corrects the limit $c_0 r_0 \le -39/23$ given by Deuling and Helfrich[7] for fixed pressure.

The paper is organized as follows. In Sec. II some definitions and basic formulas are given. Section III presents the derivation of the equilibrium equation. The second variation of shape energy and its application to the analysis of the stability of spheres are given in Sec. IV. The stability analysis for cylindrical vesicles is treated in Sec. V. In Sec. VI the third variation of the energy is obtained and applied to calculating critical spontaneous curvature. Section VII concludes the paper.

II. DEFINITIONS AND BASIC FORMULAS

Theoretically, the membrane of a vesicle may be represented as a closed surface in Euclidean three space given by the vector $\mathbf{Y}(u,v)$ depending on the two real parameters u,v. Using the formalism of differential geometry,[17] we introduce the following quantities:

$$\mathbf{Y}_i=\partial_i\mathbf{Y},\quad \mathbf{Y}_{ij}=\partial_i\partial_j\mathbf{Y},\quad g_{ij}=\mathbf{Y}_i\cdot\mathbf{Y}_j\ ,$$
$$g^{ij}=(g_{ij})^{-1},\quad g=\det(g_{ij}),\quad L_{ij}=\mathbf{Y}_{ij}\cdot\mathbf{n}\ , \tag{2}$$
$$L^{ij}=(L_{ij})^{-1},\quad L=\det(L_{ij})\quad (i,j=1,2)\ ,$$

where $\partial_1=\partial_u$, $\partial_2=\partial_v$, g_{ij}, and L_{ij} are associated with first and second fundamental forms of the surface, respectively. The outward unit normal vector $\mathbf{n}$ and the Christoffel symbols Γ^k_{ij} are defined by

$$\mathbf{n}=(\mathbf{Y}_1\times\mathbf{Y}_2)/\sqrt{g}\ ,\quad \mathbf{Y}_{ij}=\Gamma^k_{ij}\mathbf{Y}_k+L_{ij}\mathbf{n}\ . \tag{3}$$

Here and in the following repeated indices imply summation over them. The mean curvature and Gaussian curvature, respectively, may be written as

$$H=-\tfrac{1}{2}(c_1+c_2)=\tfrac{1}{2}g^{ij}L_{ij},\quad K=c_1c_2=L/g\ . \tag{4}$$

We assume $\mathbf{Y}$ to be an equilibrium shape and consider a slightly distorted surface defined by

$$\mathbf{Y}'=\mathbf{Y}+\Psi(u,v)\mathbf{n}\ , \tag{5}$$

where $\Psi(u,v)$ is a sufficiently small and smooth function. First, from Eqs. (2)–(5) we may calculate $\mathbf{Y}'_i,\mathbf{Y}'_{ij},g'_{ij},L'_{ij}$, and so on. For example, we have

$$\mathbf{Y}'_i=\mathbf{Y}_i+\Psi_i\mathbf{n}+\Psi\partial_i\mathbf{n}\ , \tag{6}$$

where $\Psi_i=\partial_i\Psi$. Use of the Weingarten equations[17]

$$\partial_i\mathbf{n}=-L_{ij}g^{jk}\mathbf{Y}_k \tag{7}$$

transforms (6) into

$$\mathbf{Y}'_i=\mathbf{Y}_i+\Psi_i\mathbf{n}-\Psi L_{ij}g^{jk}\mathbf{Y}_k\ . \tag{8}$$

The relationship[17]

$$L_{ij}g^{ik}L_{kl}=2HL_{il}-Kg_{il} \tag{9}$$

and $\mathbf{Y}_i\cdot\mathbf{n}=0$ then lead from (8) to

$$\delta g_{ij}=\mathbf{Y}'_i\cdot\mathbf{Y}'_j-\mathbf{Y}_i\cdot\mathbf{Y}_j=-2\Psi L_{ij}+\Psi_i\Psi_j+\Psi^2(2HL_{ij}-Kg_{ij})\ . \tag{10}$$

The identity

$$g=\det(g_{ij})=\tfrac{1}{2}e_{3ij}e_{3kl}g_{ik}g_{jl} \tag{11}$$

in combination with (10) results in

$$\delta g=g[-4\Psi H+g^{ij}\Psi_i\Psi_j+\Psi^2(4H^2+2K)]+O(\Psi^3)\ . \tag{12}$$

Here and in the following $O(\Psi^3)$ refers to terms of higher than quadratic order in Ψ, and the symbols e_{ijk} are defined as

$$e_{ijk}=\begin{cases}+1, & \text{when}(ijk)\text{ is an even permutation of }(123)\\ -1, & \text{when}(ijk)\text{ is an odd permutation of }(123)\\ 0, & \text{otherwise}\ .\end{cases} \tag{13}$$

In addition, we have

$$\delta g^{ij}=2\Psi(2Hg^{ij}-KL^{ij})+\left[\frac{1}{g}e_{3ik}e_{3jl}-g^{ij}g^{kl}\right]\Psi_k\Psi_l-3\Psi^2[(K-4H^2)g^{ij}+2HKL^{ij}]+O(\Psi^3)\ , \tag{14}$$

$$\delta L_{ij}=\Psi_{ij}+\Psi(Kg_{ij}-2HL_{ij})-\Gamma^k_{ij}\Psi_k+\Psi\Psi_m[K\Gamma^k_{ij}e_{3lk}e_{3mp}g_{pq}L^{ql}+(L_{jl}g^{lm})_i+L_{jk}g^{kl}\Gamma^m_{li}]$$
$$+\Psi_k\Psi_m[g^{lk}(\delta_{im}L_{jl}+\delta_{jm}L_{il})-\tfrac{1}{2}L_{ij}g^{mk}]+O(\Psi^3)\ , \tag{15}$$

with $(L_{jl}g^{lm})_i=\partial_i(L_{jl}g^{lm})$ and

$$\delta_{ij}=\begin{cases}1, & \text{when } i=j\\ 0, & \text{when } i\neq j\ .\end{cases}$$

From Eqs. (4), (14), and (15), one can now obtain the variation of mean curvature

$$\delta H=\Psi(2H^2-K)+\tfrac{1}{2}g^{ij}\nabla_i\Psi_j+\tfrac{1}{2}\Psi\Psi_m[g^{ij}(L_{jl}g^{lm})_i-L_{lk}g^{km}g_{ij}\Gamma^l_{ij}+(KL^{ij}-2Hg^{ij})\Gamma^m_{ij}]$$
$$+\Psi^2(4H^3-3HK)+\tfrac{1}{2}\Psi_i\Psi_j(Hg^{ij}-KL^{ij})+\Psi\Psi_{ij}(2Hg^{ij}-KL^{ij})+O(\Psi^3)\ , \tag{16}$$

where $\nabla_i \Psi_j$ is the covariant derivative of Ψ_j defined by[17]

$$\nabla_i \Psi_j = \Psi_{ij} - \Gamma_{ij}^k \Psi_k \ . \tag{17}$$

The variation of area is given locally by

$$\delta\sqrt{g} = (-2\Psi H + \tfrac{1}{2} g^{ij} \Psi_i \Psi_j + \Psi^2 K)\sqrt{g} + O(\Psi^3) \ , \tag{18}$$

and globally by

$$\begin{aligned} \delta A &= \delta \oint dA \\ &= \oint (-2\Psi H + \tfrac{1}{2} g^{ij} \Psi_i \Psi_j + \Psi^2 K) dA + O(\Psi^3) \ . \end{aligned} \tag{19}$$

The variation of volume is found to be

$$\delta V = \oint (\Psi - H\Psi^2) dA + O(\Psi^3) \ . \tag{20}$$

Evidently, all variations can be expressed by H, K, g_{ij}, L_{ij}, and Γ_{ij}^k which have all been defined at the beginning of this section.

III. SHAPE EQUATION

In order to obtain the equation of mechanical equilibrium of the vesicle membrane we have to calculate the first variation of the shape energy given by Eq. (1). Because of (4) we may write

$$\begin{aligned} \delta^{(1)} F = \Delta p \delta^{(1)} \int dV + \lambda \delta^{(1)} \oint dA \\ + \tfrac{1}{2} k_c \delta^{(1)} \oint (2H + c_0)^2 dA \ . \end{aligned} \tag{21}$$

The first variations of V and A are immediately seen from (19) and (20) to be

$$\delta^{(1)} \int dV = \oint \Psi \, dA \tag{22}$$

and

$$\delta^{(1)} \oint dA = -\oint 2\Psi H \, dA \ . \tag{23}$$

The first variation of the curvature-elastic energy may be written as

$$\begin{aligned} \delta^{(1)} F_c &= \tfrac{1}{2} k_c \delta^{(1)} \oint (2H + c_0)^2 dA \\ &= \tfrac{1}{2} k_c \oint [(2H + c_0)^2 \delta^{(1)} dA \\ &\qquad + 4(2H + c_0)(\delta^{(1)} H) dA] \ . \end{aligned} \tag{24}$$

Because of (16), we have

$$\delta^{(1)} H = \Psi(2H^2 - K) + \tfrac{1}{2} g^{ij} \nabla_i \Psi_j \ , \tag{25}$$

which with (17) becomes

$$\delta^{(1)} H = \Psi(2H^2 - K) + \tfrac{1}{2} g^{ij} (\Psi_{ij} - \Gamma_{ij}^k \Psi_k) \ . \tag{26}$$

We insert (23) and (26) into (24) and integrate Ψ_{ij} and Ψ_k by parts, then use the result and Eqs. (22) and (23) to obtain

$$\begin{aligned} \delta^{(1)} F = \oint \Psi[\Delta p - 2\lambda H + k_c(2H + c_0)(2H^2 - 2K - c_0 H) \\ + (k_c/\sqrt{g})(\partial_i \partial_j + \partial_k \Gamma_{ij}^k) g^{ij} \sqrt{g} \\ \times (2H + c_0)] dA \ . \end{aligned} \tag{27}$$

Considering Eqs. (2) and (3), one may prove

$$\partial_i[(\partial_j g^{ij} \sqrt{g}) f] = -\partial_k (\Gamma_{ij}^k g^{ij} \sqrt{g} f) \tag{28}$$

for arbitrary functions $f(u,v)$. Transforming the last term of Eq (27) accordingly, we arrive at

$$\begin{aligned} \delta^{(1)} F = \oint \Psi[\Delta p - 2\lambda H + k_c(2H + c_0)(2H^2 - 2K - c_0 H) \\ + 2k_c \Delta H] dA \ , \end{aligned} \tag{29}$$

where, except for Δp, Δ is the Laplace-Beltrami operator on the surface $\mathbf{Y}$, i.e.,

$$\Delta = (1/\sqrt{g}) \partial_i (g^{ij} \sqrt{g} \, \partial_j) \ . \tag{30}$$

If $\mathbf{Y}(u,v)$ describes an equilibrium shape, it satisfies $\delta^{(1)} F = 0$ for any infinitesimal function $\Psi(u,v)$. Hence Eq. (29) leads to the equilibrium condition

$$\Delta p - 2\lambda H + k_c(2H + c_0)(2H^2 - 2K - c_0 H) + 2k_c \Delta H = 0 \ . \tag{31}$$

This shape equation has first been shown in Ref. 13. It represents the balance of normal forces per unit area. Apart from the pressure difference Δp and the tensile stress λ it contains the complicated stresses of curvature elasticity. Equation (31) can also be obtained by generalizing and forming the derivative of the shape equation for axisymmetric vesicles which was calculated some time ago.[6]

IV. SECOND VARIATION AND INSTABILITY OF THE SPHERE

In dealing with the stability and deformational energies of vesicles, the membrane area is usually taken to be constant. Unlike other equilibrium shapes, such as cylinders, spheres can then be deformed only if the enclosed volume is variable. They can be destabilized by a pressure difference or by spontaneous curvature. The former may be produced osmotically or by means of a micropipette that opens into the inside of the vesicle.

The shape equation (31) enables one to seek equilibrium configurations. However, only the solutions of Eq. (31) which are stable can be observed in an experiment. Therefore, one has to check whether the second variation of the shape energy (1) is positive definite. We start from

$$\begin{aligned} \delta^{(2)} F = \Delta p \delta^{(2)} \int dV + \lambda \delta^{(2)} \oint dA \\ + \tfrac{1}{2} k_c \delta^{(2)} \oint (2H + c_0)^2 dA \ . \end{aligned} \tag{32}$$

The first two terms are immediately obtained from Eqs. (19) and (20) which give

$$\delta^{(2)} \int dV = -\oint \Psi^2 H \, dA \tag{33}$$

and

$$\delta^{(2)} \oint dA = \oint (\tfrac{1}{2} g^{ij} \Psi_i \Psi_j + \Psi^2 K) dA \ . \tag{34}$$

The last term may be written as

$$\delta^{(2)}F_c=\tfrac{1}{2}k_c \oint [4(2H+c_0)\delta^{(2)}H+4(\delta^{(1)}H)^2]dA + \tfrac{1}{2}k_c \oint [(2H+c_0)^2\delta^{(2)}dA + 4(2H+c_0)(\delta^{(1)}H)\delta^{(1)}dA] \,, \tag{35}$$

where, because of (19),

$$\delta^{(2)}dA=(\tfrac{1}{2}g^{ij}\Psi_i\Psi_j+\Psi^2K)dA \tag{36}$$

and $\delta^{(2)}H$ is the lengthy second-order part of δH given in (16). With Eqs. (16), (23), (25), and (36) substituting for the respective terms, one obtains among the integrands of Eq. (35) some which involve $\Psi\Psi_{ij}$ and $\Psi\Psi_i$. Using the identities

$$\Psi\Psi_i=\tfrac{1}{2}\partial_i\Psi^2 \,, \tag{37}$$

$$\Psi\Psi_{ij}=\tfrac{1}{2}\partial_i\partial_j\Psi^2-\Psi_i\Psi_j \,, \tag{38}$$

and integrating by parts, one transforms them into terms involving Ψ^2 and $\Psi_i\Psi_j$. The final result is

$$\begin{aligned}\delta^{(2)}F = \oint [&\Psi^2(\lambda K-\Delta pH+2k_c(H+c_0/2)(8H^3-5KH+c_0K/2)+2k_c(K-2H^2)(K+2c_0H+2H^2)\\ &+2(k_c/\sqrt{g})\partial_i\partial_j[\sqrt{g}(H+c_0/2)(2Hg^{ij}-KL^{ij})]\\ &-(k_c/\sqrt{g})\partial_m\{\sqrt{g}(H+c_0/2)[g^{ij}\partial_j(L_{jl}g^{lm})-L_{lk}g^{km}g^{ij}\Gamma^l_{ij}-(2Hg^{ij}-KL^{ij})\Gamma^m_{ij}]\})\\ &+\Psi_i\Psi_j\{[\lambda/2+k_c(H+c_0/2)^2]g^{ij}+2k_c(H+c_0/2)(KL^{ij}-3Hg^{ij})\}\\ &-2k_c(K+c_0H)\Psi g^{ij}\nabla_i\Psi_j+(k_c/2)(g^{ij}\nabla_i\Psi_j)^2]dA \,.\end{aligned} \tag{39}$$

An equilibrium shape is stable if $\delta^{(2)}F$ is positive for any $\Psi\neq 0$. In general, this requires that the eigenvalues of an operator acting on Ψ and associated with Eq. (39) satisfy certain conditions. The aim is to write (39) as a diagonal quadratic form in the amplitudes of a set of deformational modes related to the given equilibrium surface, but this is difficult to achieve in the general case. However, we believe Eq. (39) to provide a powerful tool for the numerical stability analysis of any equilibrium shape. In some simple cases, it may be evaluated analytically. A typical example is the spherical vesicle which is now considered in detail.

It is obvious that the sphere is always a solution of Eq. (31) if its parameters satisfy the following equation:

$$\Delta p r_0^3+2\lambda r_0^2-k_c c_0 r_0(2-c_0r_0)=0 \,. \tag{40}$$

We use polar coordinates to describe the sphere, putting $u=\theta, v=\phi$, so that

$$\mathbf{Y}=r_0(\cos\phi\sin\theta, \sin\phi\sin\theta, \cos\theta) \,. \tag{41}$$

Inserting into Eqs. (2), (3), and (4) yields

$$\begin{aligned} &g_{11}=r_0^2,\quad g_{12}=g_{21}=0,\quad g_{22}=r_0^2\sin^2\theta \,,\\ &L_{11}=-r_0,\quad L_{12}=L_{21}=0,\quad L_{22}=-r_0\sin^2\theta \,,\\ &\Gamma^1_{11}=\Gamma^2_{11}=\Gamma^1_{12}=\Gamma^2_{22}=0 \,,\\ &\sin^2\theta\,\Gamma^2_{12}=-\Gamma^1_{22}=\sin\theta\cos\theta\\ &H=-\frac{1}{r_0},\quad K=\frac{1}{r_0^2}\end{aligned} \tag{42}$$

and so on. The Laplace-Beltrami operator is now the usual Laplace operator on the sphere

$$\Delta_s=\left[\frac{1}{r_0^2\sin\theta}\right]\partial_\theta(\sin\theta\partial_\theta)+\left[\frac{1}{r_0\sin\theta}\right]^2\partial_\phi^2 \,. \tag{43}$$

From Eqs. (17), (30), and (42) one obtains for the sphere

$$\Delta_s\Psi=g^{ij}\nabla_i\Psi_j=(-1/r_0)L^{ij}\nabla_i\Psi_j \,. \tag{44}$$

When Eqs. (42)–(44) are inserted in Eq. (39), the lengthy formula for $\delta^{(2)}F$ reduces to the simple form

$$\delta^{(2)}F=\oint \Psi[D+B\Delta_s+(k_c/2)\Delta_s^2]\Psi\, dA \,, \tag{45}$$

where

$$\begin{aligned} D&=\tfrac{1}{2}\Delta p r_0^{-1}+k_c c_0 r_0^{-3} \,,\\ B&=\tfrac{1}{4}\Delta p r_0+\tfrac{1}{2}k_c(2+c_0r_0)r_0^{-2} \,,\end{aligned} \tag{46}$$

and λ has been eliminated with the help of Eq. (40).

Obviously, the spherical harmonics $Y_{lm}(\theta,\phi)$ provide a convenient basis for the computation of Eq. (45). Use of

$$\Delta_s Y_{lm}=-l(l+1)Y_{lm}/r_0^2 \tag{47}$$

results in

$$\delta^{(2)}F=\tfrac{1}{2}k_c\sum_{l,m}|a_{lm}|^2 r_0^{-2}[l(l+1)-2] \times[l(l+1)-c_0r_0-\Delta p r_0^3/2k_c] \,, \tag{48}$$

if Ψ is written as

$$\Psi=\sum_{l,m} a_{lm}Y_{lm}(\theta,\phi) \,. \tag{49}$$

Here $l=0,1,2,\ldots, |m|\leq 1$, and $a^*_{lm}=a_{l,-m}$. The latter requirement serves to ensure that Ψ is a real function. Equation (48) is the result reported in our paper[13] We can show that it holds generally regardless of whether area, volume, or radius is kept constant. Equations (27) and (39) give for the curvature-elastic deformational energy

$$\delta F_c=\delta^{(1)}F_c+\delta^{(2)}F_c$$
$$=k_c c_0 r_0(c_0 r_0-2)\sqrt{4\pi}a_{00}r_0^{-1}+\tfrac{1}{2}k_c\sum_{l,m}|a_{lm}/r_0|^2\{[l(l+1)]^2-(2+2c_0r_0-\tfrac{1}{2}c_0^2r_0^2)l(l+1)+c_0^2r_0^2\} \quad (50)$$

which is identical with the expression of Helfrich[18] and Milner and Safran[16]. In the same second-order approximation, Eqs. (22), (23), (33), and (34) give the following variations of area and volume:

$$\delta A=2\sqrt{4\pi}a_{00}r_0+\sum_{l,m}|a_{lm}|^2[1+\tfrac{1}{2}l(l+1)]\ , \quad (51)$$

$$\delta V=\sqrt{4\pi}a_{00}r_0^2+\sum_{l,m}|a_{lm}|^2 r_0\ . \quad (52)$$

For fixed area, i.e., $\delta A=0$, one obtains

$$\sqrt{4\pi}a_{00}=-\tfrac{1}{2}\sum_{l,m}|a_{lm}|^2 r_0^{-1}[1+\tfrac{1}{2}l(l+1)]\ . \quad (53)$$

Inserting this into (50) and (52) and forming

$$\delta F=\delta F_c+\Delta p\,\delta V\ , \quad (54)$$

one finds δF to be identical to $\delta^{(2)}F$ as given by (48). Similarly, for $\delta V=0$, i.e.,

$$\sqrt{4\pi}a_{00}=-\sum_{l,m}|a_{lm}|^2 r_0^{-1}\ , \quad (55)$$

and from Eqs. (40), (50), and (51), one finds

$$\delta F=\delta F_c+\lambda\delta A$$
$$=\delta F_c+\tfrac{1}{2}[k_c c_0 r_0^{-1}(2-c_0r_0)-\Delta p r_0]\delta A \quad (56)$$

which is again identical to $\delta^{(2)}F$. Note that volume or area reservoirs are presupposed if $\delta A=0$ or $\delta V=0$, respectively, and that the Y_{00} mode cannot be separately excited in these cases. Both reservoirs are needed in the case of constant radius, i.e., $a_{00}=0$, which is contained in (48).

In (48) the coefficients of $|a_{lm}|^2$ will be negative above some threshold pressure difference

$$\Delta_{pl}=(2k_c/r_0^3)[l(l+1)-c_0r_0] \quad (57)$$

depending on l but not on m. In other words, by increasing the pressure difference the sphere can be destabilized with respect to ever higher l's. The trivial case $l=1$, characterized by $\delta^{(2)}F=0$, means a translation of the sphere. The least stable case, $l=2$, was first discussed by Helfrich.[6] Rotational symmetric equilibrium shapes of variables have been numerically calculated by Deuling and Helfrich.[7] The agreement of the calculated pressures with the theoretical threshold is excellent, as was shown in our paper.[13]

V. CYLINDER INSTABILITY

Swelling of lecithin in a large excess of water produces not only spherical vesicles but also tubular shapes, among them cylinders and modulated cylinders including strings of beads.[19] The bending fluctuations of long cylindrical vesicles have been used repeatedly to measure the bending rigidity k_c of lecithin membranes.[20,21] The same shapes are found on the outside of aged red blood cells (see Fig. 31 of Ref. 22). A theoretical study of the stability and the deformational energies of cylindrical vesicles is therefore of some interest. In this section we apply the general formula for the second variation of the shape energy Eq. (39) to this problem.

A circular cylinder is a solution of the equilibrium-shape equation (31) if it satisfies

$$\Delta p\rho_0^3+\lambda\rho_0^2+\tfrac{1}{2}k_c(c_0^2\rho_0^2-1)=0\ , \quad (58)$$

ρ_0 being its radius. The cylindrical vesicle may also be regarded as a cylinder closed by roughly hemispherical caps at both ends. Neglecting the influence of the ends, i.e., assuming $L\gg\rho_0$, where L is the length of the cylinder, one can write the shape energy (1) in the form

$$F=\Delta p\,\pi\rho_0^2L+\lambda 2\pi\rho_0L+\tfrac{1}{2}k_c(\rho_0^{-1}-c_0)^2 2\pi\rho_0L=0\ . \quad (59)$$

Demanding $\delta F/\delta L=0$, one finds as a second equilibrium condition for the cylinder

$$\Delta p\rho_0^3+2\lambda\rho_0^2+k_c(c_0\rho_0-1)^2=0\ . \quad (60)$$

In other words, both the pressure difference Δp and the tensile stress λ are fixed, obeying

$$\Delta p=2k_c\rho_0^{-3}(1-c_0\rho_0) \quad (61)$$

and

$$\lambda=\tfrac{1}{2}k_c\rho_0^{-2}(3-c_0\rho_0)(c_0\rho_0-1)\ , \quad (62)$$

while one of them can be freely chosen in the case of spheres.

Let us now calculate the deformational energies in a quadratic approximation. With cylindrical coordinates $u=\phi, v=z$, a cylinder of length L is defined by

$$\mathbf{Y}=\rho_0(\cos\phi,\sin\phi,z),\quad 0\le\phi\le2\pi,\quad 0\le z\le L\ . \quad (63)$$

Inserting $\mathbf{Y}$ into Eqs. (2) to (4), we obtain

$$g=\rho_0^2,\quad g_{12}=g_{21}=0,\quad g_{22}=1$$
$$\mathbf{n}=(\cos\phi,\sin\phi,0),\quad \Gamma_{ij}^k=0$$
$$H=-\frac{1}{2\rho_0},\quad K=0 \quad (64)$$
$$KL^{11}=KL^{12}=KL^{21}=0,\quad KL^{22}=-\rho_0^{-1}$$

and so on. The Laplace-Beltrami operator is now the usual Laplace operator on the cylinder $\mathbf{Y}$

$$\Delta_c=\rho_0^{-2}\partial_\phi^2+\partial_z^2\ . \quad (65)$$

A slightly distorted cylinder may be described by

$$\mathbf{Y}'=\mathbf{Y}+\psi\mathbf{n}\ , \tag{66}$$

and the real function Ψ expanded into

$$\Psi=\sum_{m,n} b_{mn}\exp\{i[m\phi+n(2\pi z/L)]\}\quad \text{with } b_{mn}^{*}=b_{-m,-n}\ . \tag{67}$$

Using (17), (64) to (67), and the abbreviation $q=2\pi\rho_0/L$, we have

$$\oint \Psi\Delta_c\Psi\, dA=\oint \Psi g^{ij}\nabla_i\Psi_j dA = -2\pi\rho_0 L\sum_{m,n}(m^2+n^2q^2)\left[\frac{|b_{mn}|}{\rho_0}\right]^2 . \tag{68}$$

Let us now insert (64) to (68) into the first and second variations of F_c, A, and V, i.e., into (19), (20), (29), and (39). We obtain

$$\delta A=\pi\rho_0 L\sum_{m,n}(m^2+n^2q^2)|b_{mn}|^2\rho_0^{-2} + 2\pi\rho_0 L(b_{00}/\rho_0)+O(b_{mn}^3)\ , \tag{69}$$

$$\delta V=\pi\rho_0^2 L\sum_{m,n}|b_{mn}|^2\rho_0^{-2}+2\pi\rho_0^2 L(b_{00}/\rho_0)+O(b_{mn}^3)\ , \tag{70}$$

and

$$\delta F_c=\tfrac{1}{2}k_c\delta\oint(2H+c_0)^2 dA = k_c\pi L\rho_0^{-1}\Big[(c_0^2\rho_0^2-1)(b_{00}/\rho_0)+\sum_{m,n}\{[\tfrac{1}{2}(c_0^2\rho_0^2-1)-2c_0\rho_0](m^2+n^2q^2)+2c_0\rho_0 m^2 + (m^2+n^2q^2)^2+1-2m^2\}|b_{mn}|^2\rho_0^{-2}\Big]+O(b_{mn}^3)\ . \tag{71}$$

Accordingly, the variation of the total energy

$$\delta F=\delta F_c+\Delta p\,\delta V+\lambda\delta A \tag{72}$$

becomes, if (61) and (62) are substituted for Δp and λ,

$$\delta F=k_c\pi\rho_0^{-1}L\sum_{m,n}[2c_0\rho_0(m^2-1)+(m^2+n^2q^2)^2-4m^2-2n^2q^2+3]|b_{mn}|^2\rho_0^{-2}\ . \tag{73}$$

In order to check the last formula, we have done a completely independent calculation, maintaining volume and surface area of the cylinder but allowing changes of ρ_0 and L. We have also modified the above calculations at constant L, fixing either V or A. In all the cases, the result is identical to (73) except for the absence of the b_{00} term. In order to understand why reservoirs make no difference it is helpful to imagine the following experiment: We excite a single deformational mode of an equilibrium structure connected to reservoirs. Afterwards, we remove δV and δA due to the deformation, exchanging area, and volume with the reservoirs while keeping the deformation amplitude a constant. Since δV and δA vary as a^2 and Δp and λ are regular functions of a, the energy needed for the adjustment goes with a^3 or a higher power of the amplitude. This argument should equally hold for a single reservoir, but it does not include deformation modes whose δA and δV have linear terms in their dependence on amplitude, such as the a_0 and b_{00} modes of sphere and cylinder, respectively.

The general expression of (73) provides the basis for analyzing the stability and fluctuations of cylinders. For example, in the case of rotational symmetry, i.e., $m=0$, it becomes

$$\delta F=k_c\pi\rho_0^{-1}L\sum_n[-2c_0\rho_0+2 +(n^2q^2-1)^2]|b_{0n}|^2\rho_0^{-2}\ . \tag{74}$$

Obviously, the necessary condition for $\delta F\le 0$ is

$$c_0\rho_0\ge 1\ . \tag{75}$$

The nth mode ($n\ge 1$) is unstable if

$$n^2q^2=(2n\pi\rho_0/L)^2\le 1+\sqrt{2(c_0\rho_0-1)}\ . \tag{76}$$

For a cylinder of practically infinite length, the least stable mode is characterized by $c_0\rho_0=1$ and

$$n^2q^2=1,\ \text{i.e.,}\ T=L/n=2\pi\rho_0\ , \tag{77}$$

where T is the period of the distortion along the z axis. To interpret myelin shapes of red blood cells, more than ten years ago Deuling and Helfrich[15] calculated four rotationally symmetric myelin forms and displayed them in Fig. 2 of their paper. Using their data, we make a check as shown in Table I and find very good agreement with the predictions of (75) and (77).

In the case $m=1$, one Eq. (73) reduces to

$$\delta F=k_c\pi\rho_0^{-1}L\sum_n n^4q^4|b_{1n}|^2\rho_0^{-2}\ . \tag{78}$$

If, in addition $n=0$, we have a simple sideways translation of the cylinder requiring no energy. For $n>0$, the modes represent tube bending. The deformation associated with a single mode resembles a corkscrew because of the particular definition (67) of the modes. It is easy to redefine the modes such that they describe sinusoidal

TABLE I. Comparison of theory with numerical examples calculated by Deuling and Helfrich (Ref. 15). Here $c_0\rho_{\max}$, T, $\rho_{\max}$, and $\rho_{\min}$ are taken from Fig. 2 in Ref. 15 with length unit of cm; ρ_0 is approximated by $\frac{1}{2}(\rho_{\max}+\rho_{\min})$, and $c_0\rho_0$ then calculated by means of $(c_0\rho_{\max})\rho_0/\rho_{\max}$. The last column shows ρ_0 as obtained from T and Eq. (77).

Shape No.	$\frac{1}{2}T$	$\rho_{\max}$	$\rho_{\min}$	$c_0\rho_{\max}$	$\rho_0=\frac{1}{2}(\rho_{\max}+\rho_{\min})$	$c_0\rho_0$	$\rho_0=T/2\pi$
1	1.8	0.6	0.5	1.2	0.55	1.10	0.573
2	1.4	0.6	0.3	1.4	0.45	1.05	0.456
3	1.2	0.6	0.2	1.6	0.40	1.07	0.382
4	1.0	0.6	0.1	1.8	0.35	1.05	0.318

tube bending in orthogonal planes. The energy of tube bending does not depend on spontaneous curvature and should therefore always be positive. The elastic modulus of bending the tube as a whole is $k_c\pi\rho_0$ which agrees with the early calculations of Servuss *et al.*[20]

Whenever $m>1$, the circular cylinder can be destabilized by negative spontaneous curvature. In discussing these remaining modes it is advantageous to rewrite (73) as

$$\delta F=k_c\pi\rho_0^{-1}L\sum_{m,n}[2(c_0\rho_0-1)(m^2-1)+(m^2+n^2q^2-1)^2]|b_{mn}|^2\rho_0^{-2}\ . \qquad (79)$$

We consider only the simplest case $n=0$, i.e., deformations uniform along the tube. The circular cylinder is easily seen to become unstable at $c_0\rho_0\le-\frac{1}{2}$, its cross section turning into an ellipsoid ($m=2$). In the absence of reservoirs this is accompanied by a decrease of its length. As the spontaneous curvature becomes more and more negative, the cylinder may be expected to transform into a tape.

VI. THIRD VARIATION AND CRITICAL SPONTANEOUS CURVATURE

As pointed out in Sec. IV, at $\Delta p=\Delta p_2$ a spherical vesicle becomes unstable and begins to be deformed into a shape which can be described by a linear combination of the functions Y_{2m}. In the rotationally symmetric case the deformations of positive and negative amplitude are physically not equivalent, being prolate and oblate ellipsoids, respectively. Both of them have the same energy to second order in the amplitudes [Eq. (48)]. In order to break this symmetry one has to expand the right-hand side of Eq. (1) to third order, as first pointed out by Deuling and Helfrich.[7] This may be done directly in spherical coordinates. However, with a view to further uses of the theory, we begin with general coordinates as we have done in the preceding sections.

If third-order terms are included, we have for the variations of A and V

$$\delta A=\oint[-2\Psi H+\Psi^2K+\tfrac{1}{2}g^{ij}\Psi_i\Psi_j+(Hg^{ij}-KL^{ij})\Psi\Psi_i\Psi_j]dA\ , \qquad (80)$$

$$\delta V=\oint(\Psi-\Psi^2H+\tfrac{1}{3}\Psi^3K)dA\ . \qquad (81)$$

The variation of F_c is divided according to orders

$$\delta F_c=\tfrac{1}{2}k_c\delta\oint(2H+c_0)^2dA=\delta^{(1)}F_c+\delta^{(2)}F_c+\delta^{(3)}F_c\ , \qquad (82)$$

where $\delta^{(1)}F_c$ and $\delta^{(2)}F_c$ may be taken from Eqs. (29) and (39) by putting $\Delta p=0$ and $\lambda=0$. The third-order part is given by

$$\delta^{(3)}F_c=2k_c\left[\delta^{(3)}\oint H^2dA+c_0\delta^{(3)}\oint H\,dA\right]+\tfrac{1}{2}k_cc_0^2\oint(Hg^{ij}-KL^{ij})\Psi\Psi_i\Psi_j dA\ , \qquad (83)$$

where

$$\delta^{(3)}\oint H\,dA=\oint(\Psi^2\nabla_i\Psi_j[(2H^2-K)g^{ij}-HKL^{ij}]+\Psi\Psi_i\Psi_j[(4H^2-\tfrac{3}{2}K)g^{ij}-2HKL^{ij}]+\tfrac{1}{2}\Psi_i\Psi_j\nabla_k\Psi_l[(1/g)e_{3ik}e_{3jl}-g^{ij}g^{kl}]+\tfrac{1}{2}\Psi^2\Psi_k\{-Kg^{ij}\Gamma_{ij}^k+(2Hg^{ij}-KL^{ij})[2(L_{jl}g^{lk})_{,i}+K(g_{jl}L^{lk})_{,i}+KL^{lk}g_{lm}\Gamma_{ij}^m]\})dA \qquad (84)$$

and

$$\delta^{(3)} \oint H^2 dA = \oint \big(\Psi^3(8H^5-12H^3K+4HK^2)+\Psi\Psi_i\Psi_j[(9H^3-4KH)g^{ij}+(K^2-5H^2K)L^{ij}]$$
$$+\Psi^2\Psi_k\{[(6H^2-K)g^{ij}-2HKL^{ij}](L_{jl}g^{lk})_i+(2KH^2g^{ij}-HK^2L^{ij})(g_{jl}L^{lk})_i$$
$$+[(K^2-2H^2K)L^{ij}-KHg^{ij}]\Gamma^k_{ij}+[(4H^2K-K^2)g^{ij}-HK^2L^{ij}]L^{km}g_{ml}\Gamma^l_{ij}\}$$
$$+\Psi^2\Delta'\Psi(12H^3-7KH)+\Psi^2\Delta''\Psi(2K^2H^{-1}-6HK)+\tfrac{3}{2}H\Psi(\Delta'\Psi)^2-\Psi(\Delta'\Psi)(\Delta''\Psi)KH^{-1}$$
$$+\tfrac{1}{2}\Psi_i\Psi_j\Delta'\Psi(Hg^{ij}+KL^{ij})+\tfrac{1}{2}\Psi\Psi_k\Delta'\Psi\{[(L_{jl}g^{lk})_i+KL^{km}g_{ml}\Gamma^l_{ij}]g^{ij}-KL^{ij}\Gamma^k_{ij}\}$$
$$+(H/g)e_{3ik}e_{3jl}\Psi_k\Psi_l\nabla_i\Psi_j\big)dA \ . \qquad (85)$$

Here $\Delta'\Psi=g^{ij}\nabla_i\Psi_j$, $\Delta''=HL^{ij}\nabla_i\Psi_j$. For the sphere the operators Δ, Δ', and Δ'' are identical to the usual Laplacian operator Δ_s for the sphere [Eq. (43)].

Although these formulas seem very lengthy and heavy, they are convenient in the actual computation. Being interested only in ellipsoids of revolution, we put

$$\Psi=r_0[a_0+a_2P_2(\theta)] \ , \qquad (86)$$

where P_2 is the second Legendre polynomial and a_0 serves to renormalize the radius so that the total area is conserved. With Eqs. (42)–(44) and (86), the above formulas reduce to

$$\delta A=4\pi r_0^2(2a_0+a_0^2+\tfrac{4}{5}a_2^2) \ , \qquad (87)$$

$$\delta V=4\pi r_0^3(a_0+a_0^2+\tfrac{1}{5}a_2^2+\tfrac{1}{3}a_0^3+\tfrac{1}{5}a_0a_2^2+\tfrac{2}{105}a_2^3) \ , \qquad (88)$$

$$\delta F_c=4\pi k_c[(c_0^2r_0^2-2c_0r_0)a_0+\tfrac{1}{2}c_0^2r_0^2a_0^2$$
$$+(\tfrac{12}{5}-\tfrac{6}{5}c_0r_0+\tfrac{2}{5}c_0^2r_0^2)a_2^2$$
$$+(\tfrac{6}{5}c_0r_0-\tfrac{24}{5})a_0a_2^2-(\tfrac{24}{35}+\tfrac{12}{35}c_0r_0)a_2^3] \ . \qquad (89)$$

The conservation of area, i.e., $\delta A=0$, gives $a_0=-\frac{2}{5}a_2^2$ which permits rewriting Eq. (88) as

$$a_2^3-\tfrac{21}{2}a_2^2-\tfrac{35}{2}\eta=0 \ , \qquad (90)$$

where $\eta=\delta V/(4\pi r_0^3/3)$, the relative volume variation, is negative and near zero. From this one may find two solutions for a_2 (which are also near zero)

$$a_2^+=7[\cos(\theta_0+\tfrac{4}{3}\pi)+\tfrac{1}{2}]>0 \ ,$$
$$a_2^-=7[\cos(\theta_0+\tfrac{2}{3}\pi)+\tfrac{1}{2}]<0 \ , \qquad (91)$$

where $\theta_0=\frac{1}{3}\arccos(1+\frac{10}{49}\eta)$. From (91) one may prove

$$(a_2^+)^2-(a_2^-)^2=49\sqrt{3}\sin\theta_0(1-\cos\theta_0)>0 \ . \qquad (92)$$

Furthermore, $a_0=-\frac{2}{5}a_2^2$ reduces δF_c to

$$\delta F_c=4\pi k_c[\tfrac{2}{5}(6-c_0r_0)a_2^2-\tfrac{12}{35}(c_0r_0+2)a_2^3] \ . \qquad (93)$$

If Eq. (90) is substituted for a_2^3, it becomes

$$\delta F_c=-4\pi k_c[\tfrac{1}{5}(24+20c_0r_0)a_2^2+6(2+c_0r_0)\eta] \ . \qquad (94)$$

For fixed δV, i.e., fixed η, Eqs. (92) and (94) imply that the oblate shape (a_2^-) has the lower curvature-elastic energy and is more stable than the prolate one (a_2^+) whenever

$$c_0r_0<-1.2 \ . \qquad (95)$$

The result is in agreement with previous stability analyses of Peterson[14] and of Milner and Safran,[16] although their approaches seem different from ours. Peterson seemed to use numerical computation, finding $c_0r_0<\approx-1.2$, while Milner and Safran started from a deformation of the type $\Psi=a_2'(Y_{22}+Y_{2,-2})+a_0'Y_{20}$ and showed that $a_2'=0$ and $a_0'>0$ for $c_0r_0>-1.2$.

Let us briefly consider stability at fixed pressure rather than volume. Inserting $a_0=-\frac{2}{5}a_2^2$, because of $\delta A=0$, in Eqs. (88) and (89), we obtain

$$\delta F=\delta F_c+\Delta p\,\delta V$$
$$=-\delta p\tfrac{4}{5}\pi r_0^3a_2^2-\tfrac{32}{21}\pi k_c(c_0r_0+1.2)a_2^3+\delta p\tfrac{8}{105}\pi r_0^3a_2^3 \ , \qquad (96)$$

where

$$\delta p=\Delta p-\Delta p_2=\Delta p-2k_cr_0^{-3}(6-c_0r_0) \ . \qquad (97)$$

The first term in (96) indicates that $\delta F\le 0$ for $\delta p\ge 0$, i.e., the sphere begins to be unstable for $\Delta p\ge\Delta p_2$, in agreement with the general result of Eq. (57). The second term shows that for infinitesimal δp the oblate ellipsoids are again stable for $c_0r_0<-1.2$. This corrects $c_0r_0<-\frac{39}{23}$ as obtained previously by Deuling and Helfrich[7] under the same constraint. The third term of Eq. (96) indicates that the spontaneous curvature below which oblate ellipsoids are stable depends on δp.

It should be noted that the above expressions for δF are complete only to $O(a_2^3)$ [see Eqs. (93) and (96)] and thus cannot be used to calculate a_2. From the requirement of stability one may infer that the a_2^4 term is positive for $c_0r_0\approx-1.2$ and δp near zero, which has to be proved in the future. But it is certain that the oblate ellipsoids ($a_2<0$) have a lower energy than prolate ones ($a_2>0$) under the same constraints.

VII. CONCLUSION

In this paper we have extended the calculation of the first and second variation of vesicle-shape energy to arbitrary shapes, deriving a general equilibrium condition, i.e., shape equation, and a general stability criterion. The third variation of the energy with shape has also been obtained, again for arbitrary shapes. We have discussed the fluctuation modes of spheres (up to third order) and cylinders (for the first time), in particular stability problems. The fourth variation of the energy remains to be calculated. It would permit a complete treatment of the special critical point which separates oblate from prolate deformations of spherical vesicles, a problem encountered in studies of red blood cells. The present work is restricted to the small deformations occurring near threshold. Svetina and Zeks[23] have recently studies strong deformations of vesicles, including certain limiting shapes.

ACKNOWLEDGMENTS

The authors would like to thank M. Winterhalter for his help in preparing the manuscript. One of us (O.-Y.Z) thanks the Alexander von Humboldt-Stiftung for financial support.

---

*On leave from Institute of Theoretical Physics, Academia Sinica, Beijing, People's Republic of China.

[1]For a brief review, see for example, P. A. Fleuer and S. Leibler, Phys. Today S-**181**, 25 (1988). For a more detailed review, *Physics of Amphiphilic Layers,* edited by J. Meunier, D. Langevin, and N. Boccara (Springer, Berlin, 1987).

[2]For a review, see F. Szoka, Jr. and D. Papahadjopoluos, Ann. Rev. Biophys. Bioeng. **9**, 467 (1980).

[3]W. Helfrich and H. J. Deuling, J. Phys. (Paris) **36**, C1-327 (1975).

[4]F. Brochard and J. F. Lennon, J. Phys. (Paris) **36**, 1035 (1975); W. W. Webb, Q. Rev. Biophys. **9**, 49 (1976).

[5]P. G. de Gennes and C. Taupin, J. Phys. Chem. **86**, 2294 (1982); S. A. Safran and L. A. Turkevich, Phys. Rev. Lett. **50**, 1930 (1983).

[6]W. Helfrich, Z. Naturforsch., Teil C **28**, 693 (1973).

[7]H. J. Deuling and W. Helfrich, J. Phys. (Paris) **37**, 1335 (1976).

[8]W. Helfrich, Z. Naturforsch., Teil C **29**, 510 (1974).

[9]H. J. Deuling and W. Helfrich, Biophys. J. **16**, 861 (1976).

[10]J. T. Jenkins, J. Math. Biol. **4**, 149 (1977).

[11]J. T. Jenkins, SIAM (Soc. Ind. Appl. Math.) J. Appl. Math. **32**, 755 (1977).

[12]M. B. Schneider, J. T. Jenkins, and W. W. Webb, J. Phys. (Paris) **45**, 1457 (1984).

[13]Ou-Yang Zhong-can and W. Helfrich, Phys. Rev. Lett. **59**, 2486 (1987); **60**, 1209 (1987).

[14]M. A. Peterson, J. Math. Phys. **26**, 711 (1985); J. Appl. Phys. **57**, 1739 (1985); Mol. Cryst. Liq. Cryst. **127**, 159 (1985); **127** 257 (1985).

[15]H. J. Deuling and W. Helfrich, Blood Cells **3**, 713 (1977).

[16]S. T. Milner and S. A. Safran, Phys. Rev. A **36**, 4371 (1987).

[17]J. J. Stoker, *Differential Geometry,* Vol. XX in *Pure and Applied Mathematics,* edited by R. Courant, L. Bers, and J. J. Stoker (Wiley, New York, 1969).

[18]W. Helfrich, J. Phys. (Paris) **47**, 321 (1986).

[19]W. Harbich, R. M. Servuss, and W. Helfrich (unpublished).

[20]R. M. Servuss, W. Harbich, and W. Helfrich, Biochim. Biophys. Acta **436**, 900 (1976); G. Beblik, R. M. Servuss, and W. Helfrich, J. Phys. **46**, 1773 (1985)

[21]M. B. Schneider, J. T. Jenkins, and W. W. Webb, Biophys. J. **45**, 891 (1984)

[22]M. Bessis, *Living Blood Cells and their Ultrastructure* (Springer, Berlin 1973).

[23]S. Svetina and B. Zeks, Eur. Biophys. J. (to be published).