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Thermal fluctuations of large quasi-spherical bimolecular phospholipid vesicles

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Résumé. — On mesure la corrélation des fluctuations de forme en fonction du temps des grandes ($\geq 10 \mu\text{m}$) vésicules semi-sphériques de phospholipides hydratés, vésicules dont les membranes consistent en une ou plusieurs couches bimoléculaires. Ces membranes sont flasques, de sorte que la superficie et le volume de la vésicule sont constants. Ainsi la seule contribution à l'énergie associée à une fluctuation de forme vient de l'excès de courbure d'un élément de la membrane. De l'amplitude moyenne quadratique des modes normaux des fluctuations, on obtient une valeur pour le module d'élasticité de courbure, K_C , en employant le théorème d'équipartition. On trouve une expression pour le temps de corrélation en résolvant la dynamique de la relaxation de la membrane en fonction de la résistance visqueuse de l'eau à petit nombre de Reynolds. On calcule la force de rappel de la membrane en suivant la théorie de Jenkins [1], selon laquelle la membrane agit comme un fluide incompressible à deux dimensions. Le temps de corrélation est fonction de K_C et de d_0 , la pression à deux dimensions sur le plan de la membrane. Les mesures donnent pour K_C une valeur de 1.2×10^{-12} erg, ce qui est en accord avec d'autres expériences sur des vésicules artificielles [2, 3], et pour d_0 des valeurs qui s'accordent avec les valeurs théoriques calculées [1]. On suggère une raison pour laquelle les expériences [4] sur des globules rouges ont conduit pour K_C à une valeur moins élevée.

Abstract. — The time correlation function of the fluctuations in shape of large ($\geq 10 \mu\text{m}$) quasi-spherical hydrated phospholipid membrane vesicles consisting of one to several bimolecular layers is measured. These membranes are flaccid, so the vesicle area and volume remain constant and the only contribution to the energy of the fluctuating shape is from the excess curvature of a membrane element. A value for the curvature elastic modulus, K_C , is obtained from the mean-square amplitude of normal modes of the fluctuations using the equipartition theorem. An expression for the correlation time is found by solving the dynamics of membrane relaxation against the low Reynolds number viscous drag of the water. The restoring force of the membrane is calculated following the theory of Jenkins [1] which treats the membrane as a two dimensional incompressible fluid. The correlation time is a function of K_C and d_0 , the two dimensional pressure in the membrane plane. The measurements yield $K_C \sim 1.2 \times 10^{-12}$ ergs, in agreement with other experiments on artificial vesicles [2, 3], and values for d_0 in agreement with the theoretical range of predicted values [1]. A reason for the lower published value of K_C deduced from experiments [4] on the red blood cell is suggested.

1. Introduction.

Thin-walled phospholipid vesicles are of current interest as models of cell membranes [5-8] and as systems of two-dimensional intermolecular ordering [9-12]. For a given area and volume, the equilibrium shape of a flaccid, non-fluctuating vesicle (and also of a red blood cell) is determined by the minimization of the elastic energy due to curvature of a membrane element [1, 13]. The curvature elastic

energy of a vesicle is [14, 15]

$$E_{\text{tot}} = \frac{1}{2} K_C \iint H^2 dA = \frac{1}{2} K_C \iint \left[\frac{1}{R_1} + \frac{1}{R_2} \right]^2 dA, \quad (1)$$

where dA is an area element of the membrane, $H = 1/R_1 + 1/R_2$, is twice the mean curvature of dA , R_1 and R_2 are the two principal radii of curvature of dA [16], and K_C is the curvature elastic modulus.

Equation (1) assumes that the two sides of the membrane bilayer are identical. If they are not identical, the membrane may assume a spontaneous curvature, which can be taken into account by inserting a constant, H_s , in equation (1) so that H is replaced by $H - H_s$ [13, 15]. K_C is expected to be related to the splay elastic constant K_1 [17] of smectic A liquid crystals. Because the fluid-like layers of a smectic A can slide over each other, $K_C = K_1 w$, where w is the lamellar repeat distance.

Because the curvature elastic modulus is very small [2-4], a flaccid vesicle can attain many thermally accessible shapes at constant area and volume. In fact, the shapes of many thin-walled vesicles are observed to fluctuate [2-3, 9], the same phenomenon as the so-called « flicker » of red blood cells [4, 18-19]. Measurements of these thermal excitations have been used to deduce a value for the curvature elastic modulus, K_C , of red blood cells and artificial vesicles. Brochard and Lennon [4] measured the frequency spectrum of the fluctuations of the central thickness (peristaltic mode) of the red blood cell and found $K_C \sim 3 \times 10^{-13}$ ergs, while Servuss *et al.* measured the mean-square amplitude of the bends of long unilamellar cylindrical phosphatidylcholine vesicles and found $K_C \sim 2 \times 10^{-12}$ ergs [2]. Surprisingly, the artificial vesicles appeared to have a larger curvature elastic modulus, despite the fact that the membrane of the red blood cell consists of lipids similar to those in the artificial membrane with the addition of cholesterol, integral proteins, and a polymeric protein cortex. Some of the integral proteins are attached to the spectrin-actin polymeric network [20, 21] beneath the cell membrane. It is anticipated that this additional structure affects the behavior of the red cell membrane, but it should stiffen it, rather than decrease K_C .

We previously reported measurements of the time correlation function of the fundamental bending mode of long cylindrical vesicles [3]. We found $K_C \sim 1-2 \times 10^{-12}$ ergs from both the amplitude and the spectra of the fluctuations, in agreement with Servuss *et al.* [2]. The red blood cell experiments measured the dynamics of a peristaltic mode and were analysed using a planar membrane approximation for the real biconcave discoid. Our theory for the long tubes is not sufficiently refined to use to measure K_C from the peristaltic mode. Because of its simplicity, it predicts the instability of these modes, while we note that we have observed stable small amplitude peristaltic fluctuations in cylindrical vesicles. We believe that a more detailed theory, which better approximates the actual shape of the vesicles and which treats the membrane as an incompressible two-dimensional fluid, is necessary to describe the peristaltic fluctuations and regimes of instability. Here we carry out the corresponding calculation to describe the thermal fluctuations of a quasi-spherical vesicle by extending the curvature

elasticity theory introduced by Jenkins [1] to determine the equilibrium shapes of non-fluctuating vesicles as a function of their area and volume.

The time correlation function of the difference, $r1(t)$, between two perpendicular diameters of a vesicle whose time averaged shape is spherical (Fig. 1) is measured. The mode measured is simply interpreted as the fluctuations of a quasi-sphere into prolate and oblate ellipsoids whose major and minor axes are (roughly) the two perpendicular diameters used to calculate $r1(t)$.

According to our theory, K_C can be determined from the correlation amplitude of these fluctuations. We again find experimentally that $K_C \sim 1-2 \times 10^{-12}$ ergs. The correlation time involves K_C and the two dimensional pressure in the plane of the membrane, d_0 . For the sphere, d_0 can have a range of values [1]. The value of K_C deduced from the amplitude of the correlation function is used to determine d_0 from the correlation time. The results are consistent with the theory.

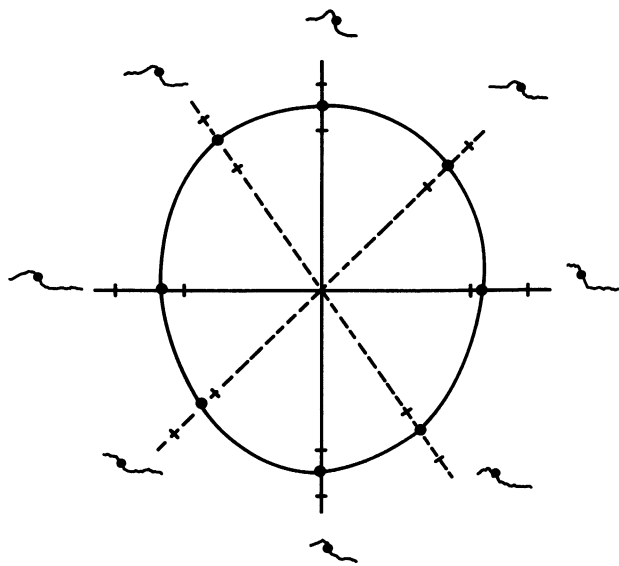


Fig. 1. — Sketch of the measurement on a quasi-spherical vesicle. Two independent measurements of $r1(t)$ are made as indicated by the solid lines and the dashed lines. Also shown are the eight plots of fluorescence intensity *versus* the radial distance for the eight radii used to calculate the two $r1(t)$'s. The computer reads the intensity (starting at the points marked inside the vesicle and proceeding radially outward to the points marked outside the vesicle) and determines the edge of the vesicle by an algorithm which looks for the steepest slope in the intensity graph. The edge is indicated by the filled circle on the scan lines and intensity graphs. The computer is correct about 50 % of the time. At other times, a manual correction is necessary because the algorithm cannot handle optical interference from out of focus vesicles or dirt which happens to lie over part of the vesicle being measured.

2. Theory for the fluctuations of a quasi-spherical vesicle.

A perfect sphere cannot perceptibly fluctuate at constant volume but, if a small amount of volume is removed, large thermal fluctuations of the now flaccid quasi-sphere are possible. Our theory describes the thermal fluctuations of a vesicle whose area-to-volume ratio is greater than that of a sphere but whose time-averaged shape is spherical. The excess area of the vesicle is distributed among the different modes of the thermal fluctuations, and, since each mode uses only a small fraction of the excess area, the modes can be treated as independent. The time-averaged spherical shape of the vesicle is treated as the equilibrium vesicle shape in our analysis.

An expression is needed for the time correlation function of the shape of the fluctuating quasi-sphere. The fluctuations are analysed by examining the linear response of a membrane element to small perturbations. The membrane is considered to be a two dimensional, incompressible, inviscid [1, 5, 14] fluid. Thus, the area of each membrane element remains constant during deformations and the membrane offers no resistance to shear. Because the membrane has a large elastic area compressibility modulus [22], any thermal fluctuations which change the membrane area would have too small an amplitude to be optically observable. The relevant deformations also occur at constant vesicle volume because, on the timescale of the fluctuations, the vesicle walls are impermeable to water flow [23]. The only important contribution to the excess energy of a deformed vesicle is from the curvature elastic energy.

We make the reasonable assumption that the area-to-volume ratio of the time-averaged vesicle determines its time-averaged shape in the same way as the area-to-volume ratio of a non-fluctuating vesicle (i.e. one at $T = 0$ K) determines its shape, as calculated by Jenkins [1]. He shows that, for vesicles of non-uniform curvature, the balance of forces across the membrane requires (a) a uniform hydrostatic pressure difference across the membrane and (b) a uniform surface pressure, d_0 , in the membrane, whose contribution to the normal force per unit area, at a point on the membrane, is the product of d_0 and the curvature at that point. The spherical vesicle is stable for a range of surface pressures, including 0. Note that these surface pressures are extremely small, on the order of $6 K_c/a^2$, or 10^{-5} dynes/cm for a 20 μ m diameter vesicle.

The excess curvature, H_1 , of the fluctuating vesicle can be expanded in a set of normal modes which are linearly independent of the curvature, H_0 , of the time-averaged sphere. The equipartition theorem gives the average excess energy in each mode as $k_b T/2$. The energy of the fluctuating vesicle above that of the time-averaged sphere is the sum over all the modes of the excess curvature energy per mode,

plus the sum over all the modes of $-d_0$ times the excess area per mode. Since the area of the *real* vesicle is fixed, the latter sum is constant in time. Therefore, the only contribution to the excess thermal energy from each mode is its curvature elastic energy.

The fluctuating shape associated with the linear expansion in curvature, which is also consistent with the constraint of local area incompressibility, can be described in terms of the displacement vector of a membrane element from its position on the original sphere to its position on the deformed sphere. The displacement, $\epsilon(\theta, \psi)$, of a point (θ, ψ) from the equilibrium sphere with radius a , consists of a radial component u , and tangential components αa and $\beta a \sin \theta$, as shown in figure 2. Thus,

$$\epsilon = u(\theta, \psi) \hat{r} + \alpha(\theta, \psi) a \theta + \beta(\theta, \psi) a \sin \theta \psi . \quad (2)$$

Defining the outward normal as positive, it is shown in section A.1 of the appendix that the curvature of the membrane element described by equation (2) is, to first order, in the displacements,

$$H(\theta, \psi) = H_0 + H_1(\theta, \psi) = -\frac{2}{a} + 2\frac{u(\theta, \psi)}{a^2} + \nabla^2 u(\theta, \psi), \quad (3)$$

where ∇ is the three dimensional nabla operator.

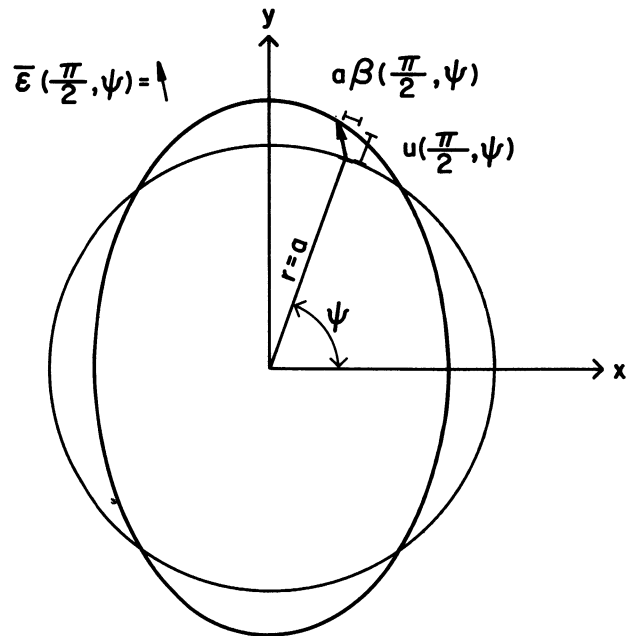


Fig. 2. — Notation used in the calculations for the fluctuations of a quasi-spherical vesicle. The diagram shows the intersection of the vesicles with the xy , ($\theta = \pi/2$) plane. The light line is the equilibrium quasi-sphere with radius a , the dark line is the fluctuating vesicle whose shape is described with the displacement vector $\epsilon(\pi/2, \psi)$. The amplitude of the fluctuation is exaggerated for clarity. In this plane, the radial displacement of a membrane element at $(\pi/2, \psi)$ is $u(\pi/2, \psi)$ and the tangential displacement is $a\beta(\pi/2, \psi)$.

Since $H(\theta, \psi)$ only involves the normal displacement $u(\theta, \psi)$, we choose u to express the local curvature and expand it in surface spherical harmonics [24], $Y_{nm}(\theta, \psi)$, with amplitude $U_{nm}(t)$,

$$u(\theta, \psi) = \sum_{\substack{n=2, \dots \\ m=0, \dots, \pm n}} U_{nm}(t) Y_{nm}(\theta, \psi). \quad (4)$$

(Since $n = 0$ does not conserve volume, and $n = 1$ is a uniform displacement of the entire sphere, the sum starts from $n = 2$.)

The area element of the deformed vesicle, $dA + \delta dA$, may be written, to first order in the displacements (see section A.1 in the appendix) as $dA(1 + \nabla \cdot \boldsymbol{\varepsilon})$, where dA is the area element of the undeformed sphere. Because the membrane is a two dimensional incompressible fluid, the displacement vector must satisfy the continuity equation $\nabla \cdot \boldsymbol{\varepsilon} = 0$.

The curvature energy is derived from equations (1), (3), (4) and the normalization properties of the surface spherical harmonics [24]. The excess curvature energy, E , of the fluctuating sphere, to second order, is found to be

$$E = \sum_{n,m} \frac{1}{2} K_C (n-1)^2 (n+2)^2 \frac{U_{nm}^2}{a^2}. \quad (5)$$

Upon setting the average energy in each mode equal to $(1/2) k_b T$ we find

$$\frac{\langle U_{nm}^2 \rangle}{a^2} = \frac{k_b T}{K_C (n-1)^2 (n+2)^2}. \quad (6)$$

Thus, the curvature energy (Eq. (5)) of each mode is roughly $K_C Q^4 \langle U^2 \rangle A$ [25], where U and Q are, respectively, the amplitude and wavenumber of a mode and A is the area of the vesicle. The brackets denote an ensemble (or time) average. A measurement of the mean-square amplitude of a mode, $\langle U^2 \rangle$ (Eq. (6)), yields a value for K_C . Each of the independent modes contributing to the description of the shape of the vesicle possesses a characteristic correlation time, τ_C . The time correlation function of a mode is [26]

$$\langle U(\tau) U(0) \rangle = \langle U^2 \rangle e^{-\tau/\tau_C}. \quad (7)$$

An expression for the correlation time of a mode is obtained by solving the dynamical problem of the vesicle excited into a normal mode and relaxing to its equilibrium shape. In this process, the restoring force of the membrane's curvature elasticity is balanced by the viscous resistance of the surrounding fluid. The membrane fluid is, in fact, viscous but for long wavelength fluctuations its resistance to shearing is negligible compared to that of the surrounding fluid [27]. The necessary boundary conditions are that the three components of the fluid velocity are continuous across the membrane, that they are equal to the three components of the velocity of the

membrane, and that the normal and tangential components of the membrane forces balance the net viscous tractions exerted by the fluid inside and outside of the membrane.

The velocity of a membrane element is simply the time derivative of its displacement (Eq. (2))

$$\mathbf{V} = \frac{\partial \boldsymbol{\varepsilon}}{\partial t} = \frac{\partial u}{\partial t} \hat{\mathbf{r}} + \frac{\partial \alpha}{\partial t} a \boldsymbol{\theta} + \frac{\partial \beta}{\partial t} a \sin \theta \boldsymbol{\psi}. \quad (8)$$

The calculation of the membrane forces follows Jenkins [1]. The restoring force per unit area is given by the negative of the variational gradient of the curvature energy (Eq. (1)) with respect to the displacement (Eq. (2))

$$\mathbf{F} = - \frac{\delta E}{\delta \boldsymbol{\varepsilon}}. \quad (9)$$

Because the membrane fluid is incompressible, a two dimensional pressure, $\gamma(\theta, \psi)$, appears in the variation of the free energy as the Lagrange multiplier associated with $\delta dA = 0$. With this, the variation of the energy of the vesicle may be written as

$$\delta E = \delta \left[\frac{1}{2} K_C \iint H^2 dA \right] - \iint \gamma \delta dA, \quad (10)$$

$$= \iint \delta \left[\frac{1}{2} K_C H^2 \right] dA - \iint \left[\gamma - \frac{1}{2} K_C H^2 \right] \delta dA;$$

or, upon redefining the Lagrange multiplier to $d = \gamma - \frac{1}{2} K_C H^2$, as

$$\delta E = \iint (K_C H \delta H - d \delta) dA. \quad (11)$$

Retaining only the first order terms (see appendix section A.2), we find the normal and tangential forces acting on a membrane element are

$$\mathbf{F}_{\text{norm}} = (-K_C \nabla^2 H - dH) \hat{\mathbf{n}}, \quad (12a)$$

and

$$\mathbf{F}_{\text{tan}} = -\nabla d. \quad (12b)$$

The normal force has two parts : the first is the restoring force resisting the excess curvature; the second is Laplace's expression for the equilibrium of a spherical element [28] (but with a surface pressure, d , instead of a surface tension). The tangential force is that usually associated with a hydrostatic pressure gradient.

The incompressibility conditions, $\nabla \cdot \boldsymbol{\varepsilon} = 0$ may be written, using equation (2), as

$$2u + \frac{\alpha}{\tan \theta} + \frac{\partial \alpha}{\partial \theta} + \frac{\partial \beta}{\partial \psi} = 0. \quad (13)$$

Equation (13) reduces the number of independent scalar functions required to describe the displacement ϵ from three to two. Equation (13) may be satisfied identically by choosing two functions, f and g , as

$$\epsilon = -\frac{1}{2} \nabla^2 f \hat{r} + \nabla f + \nabla \times (g \hat{r}). \quad (14)$$

Notice that $\nabla \times \epsilon$ involves only g , while the radial component of ϵ involves only f . Setting equation (14) equal to equation (2), we find $u = -(1/2) \nabla^2 f$. We choose to work with the scalar functions u and g .

The motion of the surrounding fluid is described by the Navier-Stokes equations and the continuity equation

$$\rho \partial \mathbf{v} / \partial t + \rho (\mathbf{v} \cdot \nabla) \mathbf{v} = \eta \nabla^2 \mathbf{v} - \nabla p, \quad (15a)$$

and

$$\nabla \cdot \mathbf{v} = 0, \quad (15b)$$

respectively. Here \mathbf{v} is the velocity of the fluid, ρ and η are, respectively, its density (1 gm/cm³) and viscosity (0.01 poise), and p is the hydrostatic pressure. Because the relaxation times are on the order of seconds and the decay lengths of the velocity fluctuations measured are on the order of the size of the vesicles (about 30 μm), the ratio of the inertial to the viscous forces is about 0.001. Consequently, the inertial term can be neglected in equation (4a) and the problem falls into the regime of low Reynolds number hydrodynamics [29].

The solution to the Navier-Stokes equations in the low Reynolds number limit in spherical coordinates is expressed by Lamb [30] in terms of three scalar functions: φ , χ and p where φ and χ are solutions to the homogeneous equation and p is the hydrostatic pressure. The expression for the curl of the velocity involves χ while the radial component of the velocity involves φ and p . The functions φ , χ and p are expressed in terms of solid spherical harmonics.

Brenner [29, 31] writes the boundary conditions on the velocity in terms of the vector identities

$$\hat{\mathbf{n}} \cdot \mathbf{v} |_{r=a} = \hat{\mathbf{n}} \cdot \mathbf{V}, \quad (16a)$$

$$(\hat{\mathbf{r}} \cdot \nabla) (\hat{\mathbf{r}} \cdot \mathbf{v}) - \nabla \cdot \mathbf{v} |_{r=a} = -\nabla \cdot \mathbf{V}, \quad (16b)$$

and

$$\hat{\mathbf{r}} \cdot \nabla \times \mathbf{v} |_{r=a} = \hat{\mathbf{r}} \cdot \nabla \times \mathbf{V}, \quad (16c)$$

where \mathbf{v} is the fluid velocity interior and exterior to the membrane and \mathbf{V} is given by equation (8). A similar identity holds for the forces. To obtain this replace \mathbf{V} by equation (12) and replace \mathbf{v} by the negative of the difference between the viscous forces inside and outside the membrane, $\hat{\mathbf{n}} \cdot (\hat{\boldsymbol{\sigma}}^i - \hat{\boldsymbol{\sigma}}^o)$, where $\hat{\boldsymbol{\sigma}}$ is the

viscous stress tensor [28]. Define $\Delta \boldsymbol{\sigma} = \hat{\mathbf{n}} \cdot (\hat{\boldsymbol{\sigma}}^i - \hat{\boldsymbol{\sigma}}^o)$, then

$$\hat{\mathbf{n}} \cdot (\Delta \boldsymbol{\sigma}) |_{r=a} = \hat{\mathbf{n}} \cdot \mathbf{F}, \quad (17a)$$

$$(\hat{\mathbf{r}} \cdot \nabla) [\hat{\mathbf{r}} \cdot (\Delta \boldsymbol{\sigma})] - \nabla \cdot (\Delta \boldsymbol{\sigma}) |_{r=a} = -\nabla \cdot \mathbf{F}, \quad (17b)$$

and

$$\hat{\mathbf{r}} \cdot \nabla \times (\Delta \boldsymbol{\sigma}) |_{r=a} = \hat{\mathbf{r}} \cdot \nabla \times \mathbf{F}. \quad (17c)$$

This simplifies the calculation because the terms involving $\nabla \times \mathbf{v}$ (or $\nabla \times \Delta \boldsymbol{\sigma}$) (only a function of χ) are coupled only to all the terms involving $\nabla \times \mathbf{V}$ (or $\nabla \times \mathbf{F}$) (only a function of g , Eqs. (12), (14)). These terms are of no interest to us because they have no radial displacement term and are not measurable.

The simplified problem involves two scalar functions describing the fluid inside, p^i , φ^i , two describing the fluid outside, p^o , φ^o and two functions describing the fluid membrane, u , the normal component of the membrane displacement and d , the two dimensional pressure. Writing $U_{nm}(t) = U_{nm} e^{-t/\tau_{nm}}$, in equation (4), and expanding the rest of the functions in terms of solid and surface spherical harmonics [24, 29], we have

$$p^i = \sum_{n,m} P_{nm}^i r^n Y_{nm}(\theta, \psi) e^{-t/\tau_{nm}}, \quad (18a)$$

$$\varphi^i = \sum_{n,m} \phi_{nm}^i r^n Y_{nm}(\theta, \psi) e^{-t/\tau_{nm}},$$

$$p^o = \sum_{n,m} P_{nm}^o r^{-n-1} Y_{nm}(\theta, \psi) e^{-t/\tau_{nm}}, \quad (18b)$$

$$\varphi^o = \sum_{n,m} \phi_{nm}^o r^{-n-1} Y_{nm}(\theta, \psi) e^{-t/\tau_{nm}},$$

and

$$d = \sum_{n,m} d_{nm} Y_{nm}(\theta, \psi) e^{-t/\tau_{nm}}. \quad (18c)$$

where τ_{nm} is the relaxation time associated with the normal mode indexed by n and m . Again, the sums begin at $n = 2$. The expressions on the right hand side of equation (16) involving the membrane velocity and on the right hand side of equation (17) involving the membrane restoring force are derived in section A.3 of the appendix. Brenner gives expressions for the left hand sides of equation (16) [29, 31] and equation (17) [31] in terms of the ϕ_{nm} and P_{nm} (see the appendix section A.4). Inserting equation (4) and 18 into equations (3), (8), (12) (16a, b), and (17a, b) as shown in section A.5 of the appendix, and solving for τ_{nm} , yields

$$\tau_n = \frac{\eta a^3 Z(n)}{K_C n(n+1) - d_0 a^2}, \quad (19)$$

where

$$Z(n) = \frac{(n^2 - 1)(2n + 3) + n(n + 2)(2n - 1) - [6(n + 2)(n - 1)(2n + 1)]/(n^2 + n + 4)}{[(n + 2)(n - 1)n^2(n + 1)^2]/(n^2 + n + 4)}. \quad (20)$$

Note that $Z(n) > 0$ for $n > 1$, which is the regime of interest. The subscript m has been dropped since τ_{nm} depends only on n . In equation (19), d_0 is the two-dimensional surface pressure in the membrane of the time-averaged equilibrium sphere.

To understand the significance of the denominator in equation (19), first consider the possible equilibrium shapes of a vesicle of given surface area at $T = 0$ K. Jenkins [1] has calculated the equilibrium shape, area-to-volume ratio, and surface pressure of a vesicle as a function of the external pressure excess, $\Delta P_0 = P_0^o - P_0^i$, across the membrane where $P_0^o(P_0^i)$ is the hydrostatic pressure of the fluid outside (inside) the vesicle. If ΔP_0 is negative, the only stable shape is the sphere and, since the external pressure excess for a sphere is related to d_0 by Laplace's formula, $\Delta P_0 = 2 d_0/a$. Here d_0 is negative; a membrane element is under a surface tension. Jenkins has found that the sphere is also stable for a small range of positive ΔP_0 's. The two dimensional stress, d_0 , is then compressive, and has a range,

$$0 \leq d_0 \leq 6 K_C/a^2. \quad (21)$$

If the volume is allowed to change as ΔP_0 is increased from zero, a shape « bifurcation » occurs at $\Delta P_0 = 12 K_C/a^3$ ($d_0 = 6 K_C/a^2$) when the equilibrium vesicle shape buckles and becomes ellipsoidal. The relaxation time in equation (19) recovers the bifurcation (at $n = 2$) found by Jenkins [1] and Deuling and Helfrich [13]. Before the bifurcation, as the external pressure is increased, there appears a positive two-dimensional stress d_0 in the membrane plane, but the vesicle shape remains spherical. After the bifurcation, all ellipsoids except for the sphere, have unique values of d_0 determined by their surface-to-volume ratio. This discussion neglects fluctuations, i.e. $T = 0$ K. Next we consider the effect of small thermal fluctuations on these states.

Our problem requires that we determine whether the bifurcation at positive pressure and $T = 0$ K is reflected in our fluctuation spectra for $T > 0$ K. Our strategy is to compare (a) the volume decrease (from that of a sphere) needed to accommodate all of the thermal fluctuations with (b) the decrease in volume associated with an ellipsoidal equilibrium shape at $T = 0$ K whose ellipticity is equal to the root mean square of that of the $n = 2$ mode measured in our experiment. We find that the change in volume needed to accommodate all of the thermal fluctuation modes is 25 times the corresponding volume change of an ellipsoid that accommodates the lowest mode, that is, one whose major and minor semi-axes are $a \pm \langle U_{2m}^2 \rangle^{1/2}$. This means that the surface to volume ratio of the fluctuating vesicle is quite large, so much so that at $T = 0$ K it would have a *much* larger ellipticity than is ever measured for the $n = 2$ modes in our experiments. For the thermal fluctuations, we conclude that the fluctuation modes are essentially independent because each mode requires

a small part of the available excess area to reach its equipartition amplitude. The amplitude of the deviations from spherical form are so small that each excited mode can be treated as if the restoring force would return the vesicle to a sphere, its time-averaged shape. Therefore, the vesicle fluctuations are unaffected by the bifurcation that occurs at higher ellipticities.

The picture we have is that, at finite temperatures, a vesicle fluctuates whenever its volume is below that of a sphere. The fluctuations take up the excess area and the membrane is not compressed (i.e. $d_0 = 0$) until the volume decrease is enough for the fluctuations to reach their equipartition amplitude. Further pressure decreases the volume until eventually the bifurcation occurs (at $d_0 = 6 K_C/a^2$). Thus, above $T = 0$ K, we have interpreted the equilibrium shapes calculated by Jenkins [1] as the time-averaged shapes about which the fluctuations occur and hence, they are the shapes to which the curvature forces act to restore the membrane. We have analysed here only the spherical time-averaged shapes.

In our experiments, we measure the time correlation function of the difference, $r1(t)$, of two perpendicular diameters of the fluctuating sphere (Fig. 1). The plane of the measurement is the $\theta = \pi/2$ plane, as shown in figure 2. To first order, the quantity measured is

$$r1 = u\left(\frac{\pi}{2}, 0\right) + u\left(\frac{\pi}{2}, \pi\right) - u\left(\frac{\pi}{2}, \frac{\pi}{2}\right) - u\left(\frac{\pi}{2}, 3\frac{\pi}{2}\right). \quad (22)$$

From equations (4), (19) and (20), the correlation function measured is

$$\frac{\langle r1(\tau) r1(0) \rangle}{a^2} = \sum_{\substack{m=2,6,\dots \\ n=2,4,\dots \\ n+m \text{ even}}}^{\infty} 32 \langle U_{nm}^2 \rangle \frac{(2n+1)(n-m)!}{4\pi^2(n+m)!} \times 2^{2m} \left[\frac{\Gamma\{(n+m+1)/2\}}{\Gamma\{(n-m+2)/2\}} \right]^2 e^{-\tau/\tau_n}, \quad (23)$$

where ! denotes factorial and $\Gamma\{x\}$ is the gamma function of argument x [32]. For our experiments (using Eqs. (19) and (20)) only the $n = 2$ terms in equation (23) are important for points after $\tau = 0$ since $Z(n) \sim 1/n$ so $\tau_n \sim 1/n^3$. Finally, from equations (6), (19), (20), (23), the quantity measured is

$$\frac{\langle r1(\tau) r1(0) \rangle}{a^2} = 0.3 \frac{k_b T}{K_C} e^{-\tau/\tau_2} + 0.02 \frac{k_b T}{K_C} \delta(\tau), \quad (24a)$$

where

$$\tau_2 = \frac{2.29 \eta a^3}{6 K_C - d_0 a^2}, \quad (24b)$$

and $\delta(\tau)$ is the Kroeckner delta [24].

The amplitude of the correlation function involves only the curvature elastic modulus, K_C , but the correlation time involves K_C and the two-dimensional pressure of the time-averaged equilibrium sphere, d_0 . The area of each membrane element remains constant during the fluctuation, so d does no net work and cannot appear in the energy. The value of d_0 is restricted (Eq. (21)); but it is not uniquely determined for these fluctuating spheres, so we can only do a consistency check on the theory. In our experiments K_C is determined by the amplitude of the correlation function; then d_0 is calculated from K_C and the correlation time.

3. Experiments.

3.1 PREPARATION OF SPHERICAL VESICLES. — Fluctuating spherical vesicles are most successfully prepared with the method described in reference [3] using the desalted stock solution containing 50 mg egg phosphotidylcholine (Applied Science, State College, Pa.) in 5 ml 2 : 1 v/v chloroform-methanol and 5×10^{-4} mole fraction 3,3'-dehexadecylindocarbocyanine iodide (diI) [33], a fluorescent lipid analogue (a generous gift of Dr. Alan S. Waggoner). Sometimes larger batches of vesicles are prepared by placing 0.5 ml of stock solution in a 10 ml flask, evaporating the solvent, gently adding 10 ml of deionized, doubly distilled water, and letting the vesicles swell for several days. For observations, a 50 μm path length microslide (Vitro Dynamics, Rockaway, New Jersey)

samples the solution about half a centimeter above the vesicle cloud.

We have found it to be extremely important to prevent the evaporation of water by closing the flask well and/or leaving the stoppered flask in a water saturated atmosphere. If this is not done, most of the spherical vesicles are attached to very long and thin tethers. Boroske *et al.* [23] have found that this structure is a result of osmotic shrinkage of the vesicle.

3.2 EXPERIMENTAL RESULTS. — We select vesicles for study whose average shape seems to be spherical and whose fluctuations are large. A video tape is made of the fluctuating vesicle as observed with fluorescence microscopy and the video image processing system described in reference [3] is used to store a video frame on the computer every Δt seconds. Each frame is analysed as shown in figure 1. For each time, we measure r_1 (Eq. (22)) of two independent modes whose axes are 45° apart. The number of bilayers in the membrane is estimated from the intensity profile across an edge of the sphere. The fluorescence intensity of thin multibilayer vesicles is observed to be approximately an integer multiple of that of single bilayer vesicles with the same diameter. The data for a single bilayer quasi-spherical vesicle is shown in figure 3a and the correlation function calculated from the data is shown in figure 3b. If N is the total number of consecutive video frames analysed, the correlation function is calculated from the formula

$$G(j \Delta t) = \frac{\sum_{i=1}^{N-j} r_1(i \Delta t) r_1(i \Delta t + j \Delta t)}{N - j}. \quad (25)$$

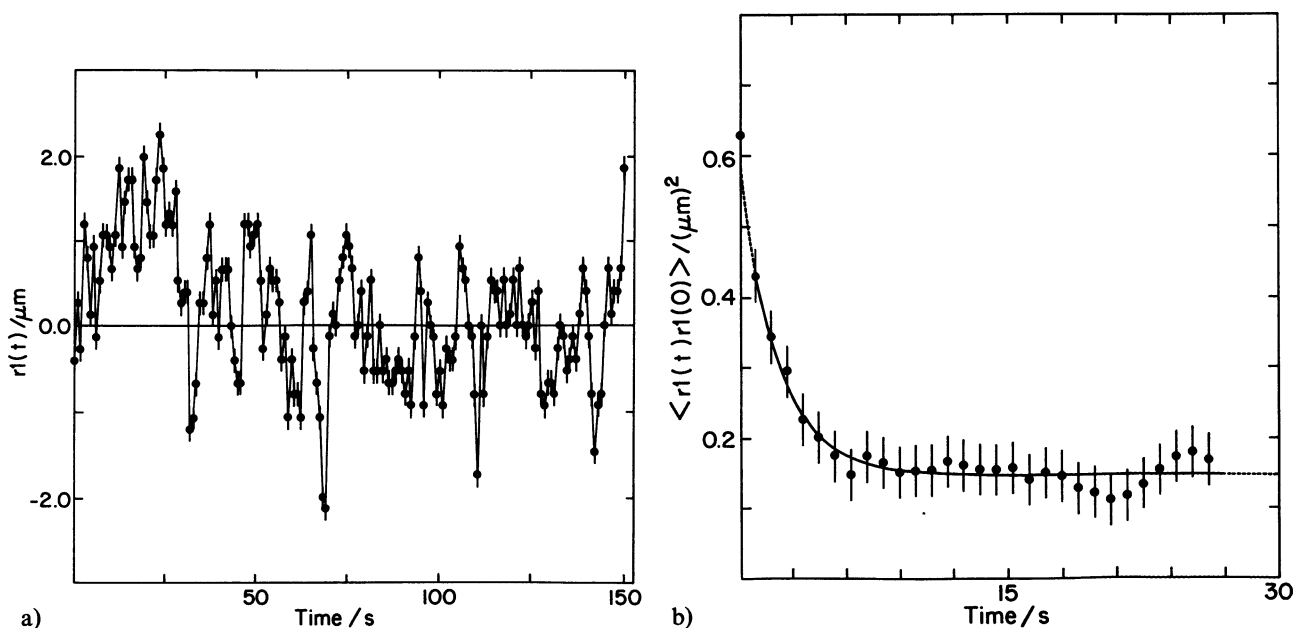


Fig. 3. — The data and calculated correlation function for a single bilayer quasi-spherical vesicle of radius 7.5 μm . (a) $r_1(t)$ versus t for one of the two independent r_1 values. (b) The calculated correlation function from both r_1 values.

The two independent correlation functions are averaged together. The error bars, $(\sigma^2)^{1/2}$, for $G(j \Delta t)$ are determined from the formula [34]

$$\sigma^2(j \Delta t) = \frac{\frac{2 N \Delta t}{\tau_c} - 1 + 2 e^{-N \Delta t / \tau_c} + \left[\left(\frac{2 j \Delta t}{\tau_c} + 1 \right) \left(\frac{2 N \Delta t}{\tau_c} - 1 \right) - 2 \left(\frac{j \Delta t}{\tau_c} \right)^2 \right] e^{-2 j \Delta t / \tau_c}}{2(N \Delta t / \tau_c)^2}, \quad (26)$$

where τ_c is the correlation time. Since $G(j \Delta t)$ is a random variable, the error in its measurement is expected to scale like $N^{-1/2}$; however, adjacent points in the sum used to calculate $G(j \Delta t)$ (Eq. (25)) are correlated over a time τ_c . Therefore, the total number of independent time intervals in equation (25) is $N \Delta t / \tau_c$ not N so the error should be proportional to $(N \Delta t / \tau_c)^{-1/2}$ roughly as equation (26) implies. Since the computer limits N to 175 points, or about 17 correlation times, the correlation amplitude and times are measurable to within a factor of 2.

A weighted linearized least-squares routine [35] is used to fit the correlation function to an amplitude multiplying an exponential decay plus a constant. The fit is also shown in figure 3b. The residual constant is due to the error in locating the centre of the sphere. The $\tau = 0$ point is omitted from the fit. Using equation (24), K_C is determined from the correlation amplitude and then d_0 (in units of K_C/a^2) is determined from the correlation time. The estimated error for a parameter of the fit correspond to one standard deviation. (The probability that the parameter falls within the indicated range of error is about 68 % [35].)

Although two additional fluctuation modes, $n = 4$, $m = \pm 4$ and $n = 3$, $m = \pm 3$ were measured, the resolution of the system was too low to analyse the data. This is consistent with the expected amplitude and correlation times of these modes compared to that of the $n = 2$, $m = \pm 2$ modes which were successfully analysed.

The data for eight spherical vesicles with radii varying from 5 to 16 μm , is shown in table I. We find $\langle u^2 \rangle^{1/2}$ to be about 0.05 a , so the fluctuations are small. The surprising result is that the value of K_C is independent of the number of bilayers in the wall of the spherical vesicle. (The data and correlation function for a multibilayer vesicle are shown in Fig. 4.) From the results on the cylindrical vesicles in reference [3], it appears that all of the quasi-spheres act as single bilayers in the measurements. We hypothesize that the inner layers are more flaccid than the outer layer and hence do not affect the measurements. We have often observed a sudden decrease in the fluctuation amplitude of a thin multibilayer vesicle, followed by the appearance of a small cylindrical « bud » on the inside of the vesicle. We take this « zipping » phenomena as confirmation of the flaccidness of the inner layers of the original vesicle. This process will be discussed in a future publication [36].

The average and standard deviation for the curvature elastic modulus from the mean values of the fitted amplitude of all the vesicles in table I is

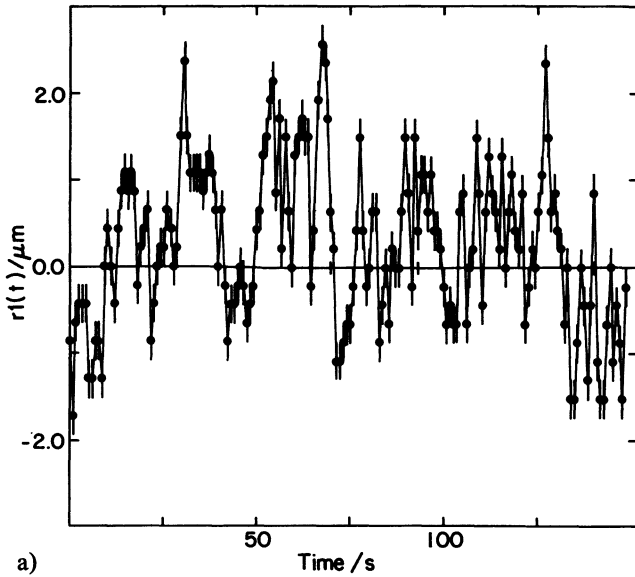
$$K_C = 1.5 \pm 0.5 \times 10^{-12} \text{ ergs}, \quad (27)$$

in agreement with previous values on artificial vesicles [2, 3]. The fitted values of d_0 are

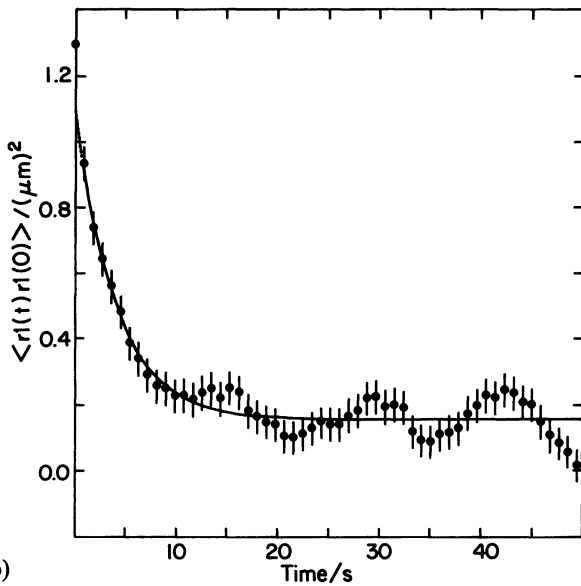
$$0.9 K_C/a^2 \leq d_0 \leq 5.4 K_C/a^2, \quad (28)$$

Table I. — Results on quasi-spherical vesicles

$a/(\mu\text{m})$	$\langle u^2 \rangle^{1/2}/a$	No. Layers	K_C (10^{-12} ergs)	d_0 ($K_C a^{-2}$)	$2.29 \eta a^3 / (\tau_2 K_C)$
9	0.05	4	0.85 ± 0.03	3.2 ± 0.3	2.8 ± 0.3
10	0.05	4-5	0.88 ± 0.03	1.0 ± 0.3	5.0 ± 0.3
7	0.05	1?	1.3	3.8	2.1
11	0.04	3-5	1.5 ± 0.1	1.0 ± 0.6	5.0 ± 0.6
7.5	0.04	1	1.6 ± 0.3	3.3 ± 0.7	2.7 ± 0.7
16.5	0.04		1.8 ± 0.1	1.3 ± 0.4	4.7 ± 0.4
5.5	0.04	1?	1.9 ± 0.2	5.2 ± 0.9	0.73 ± 0.14
12	0.03	1?	2.2 ± 0.1	5.4 ± 1	0.62 ± 0.09



a)



b)

Fig. 4. — The data and calculated correlation function for a quasi-spherical vesicle of radius 11 μm . The wall of the vesicle contains about 4 bilayers. (a) One set of $r1(t)$ values. (b) The calculated correlation function from six independent sets of $r1(t)$ values.

consistent with the expected range of d_0 values in equation (21). Note that if d_0 did not appear in the expression for the correlation time in equation (24), then $\eta a^3 / (\tau_2 K_C)$ would be equal to a constant. This is contradicted by the last column in table I where the value of this quantity is seen to vary by a factor of 7.

Since conservation of area and volume couples the (otherwise) normal modes of the thermal fluctuations, we selected distinctly flaccid vesicles with large excess areas in which no one mode absorbs a substantial fraction of the total excess area. Thus coupling of the spherical harmonic modes can be

neglected and they can be treated as normal fluctuation modes. However, the occurrence of the shape bifurcation to an average ellipsoidal shape limits the acceptable flaccidity. The measured mean values of $r1$, our measure of ellipticity, do reflect the bifurcation since the axis of mean ellipticity cannot rotate on the timescale of the experiment [37]. To avoid corrections to the fluctuation spectra for the bifurcation, we must limit the flaccidity to an excess curvature energy of the mean ellipsoid of less than $k_b T$. This limit is established by restricting the mean values of $r1$ to much less than the corresponding amplitude of the $n = 2$ fluctuation mode, i.e. $|\langle r1 \rangle| \ll 4 \langle U_{2m}^2 \rangle^{1/2}$.

4. Conclusions.

We conclude, from the results reported here and from our measurements on cylindrical vesicles [3], that $K_C \sim 1-2 \times 10^{-12}$ ergs. This implies a splay elastic constant for the corresponding smectic A liquid crystal of $K_1 \sim 2 \times 10^{-6}$ dynes if the repeat distance of the stack of bilayers is taken as 60 Å. For thermotropic smectic A liquid crystals with lamellar repeat distances of 20 Å, $K_1 \sim 10^{-6}$ dynes [17].

The new feature in our analysis of the quasi-spheres is the treatment of the membrane as a two-dimensional incompressible fluid. The addition of the Lagrange multiplier associated with this constraint, the two-dimensional pressure, d , allows us to satisfy boundary conditions on the three components of the fluid velocity and on the three components of the fluid forces. For the quasi-spheres, d_0 is not unique but is a function of the amount of infinitesimal volume removed from the perfect sphere. Since the value of d_0 is directly proportional to the excess hydrostatic pressure of the fluid inside the sphere by the formula of Laplace [1] it is theoretically possible to control d_0 by changing the osmotic pressure of the fluid. Changing d_0 by K_C/a^2 requires changing the hydrostatic pressure difference by $2 K_C/a^3$. If this were equated to an osmotic pressure, it would correspond to a change of the concentration difference across the membrane of $2 K_C/(a^3 k_b T)$ or roughly 0.1 nmolar for a 10 μm radius quasi-spherical vesicle. We have found this small concentration change extremely difficult to control.

Further studies on vesicles are desirable to determine the value of K_C as a function of temperature and composition of the vesicles. We have observed that the magnitude of the fluctuations seems to increase just above the liquid crystalline-to-gel phase transition as the temperature is lowered. The effect on K_C of the concentration of cholesterol in the membrane is of interest to biophysicists.

Brochard *et al.* [38] modified the original theory of the fluctuations of the red blood cell to allow for

normal and tangential motion of the membrane molecules, an anharmonic effect in which u appears in a fourth order term in the energy. They concluded that the equipartition theorem applied to the amplitude of the fluctuations was still valid. We concur. In the present theory, anharmonic effects can be ignored in both the statics and dynamics of the fluctuations as long as the excess area needed by each normal mode to reach its equipartition amplitude is a small fraction of the total excess area of the real vesicle above that of the time-averaged sphere. We think that a similar normal mode analysis of the fluctuations about the discoid shape of the red blood cell is needed.

The calculations for the red blood cell should also include a spontaneous curvature because the two monolayers of the red cell membrane are not identical [13]. We have examined the effect of a spontaneous curvature in the calculations for quasi-spherical vesicles; it does not appear in the expression for the correlation amplitude but it does appear in the correlation time. If spontaneous curvature is present, then d_0 in equation (19) is replaced by $d_0 - 2 K_C H_s/a - K_C H_s^2/2$ where H_s is the value of the spontaneous curvature (with the outward normal taken as positive). Since the values of d_0 and H_s for the discoid shape are unique [1, 13], it should be possible to determine K_C from the correlation time of the fluctuations of the red blood cell. An additional constraint should derive from the spectrin network [20-

21]. However, its effect on the cell's curvature modulus may be small since the connections between the lipid layer and the spectrin cortex are free to slip; thus the curvature moduli only add linearly. The curvature modulus of the cortex should not overwhelm the lipid layer because the spectrin layer is so thin. This conjecture has now been supported by recent work of Evans [39] who used large amplitude deformations of the red blood cell to determine a value for the cell's curvature modulus from a buckling instability. He also found $K_C \sim 1.8 \times 10^{-12}$ ergs, in agreement with our results. Thus the cortex apparently does not dominate the effective curvature modulus.

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Appendix.

This appendix gives the details of the calculations. The calculation of the energy is to second order and that of the forces is to first order. For the membrane forces, we follow Jenkins [1] with a modification of notation explained later. A good reference for the necessary differential geometry is Eisenhart [16].

The shape of the fluctuating vesicle is described in reference to the equilibrium sphere (with normalized radius = 1) by the displacement vector

$$\boldsymbol{\varepsilon} = u(\theta, \psi) \hat{\mathbf{r}} + \alpha(\theta, \psi) \boldsymbol{\theta} + \beta(\theta, \psi) \sin \theta \boldsymbol{\psi}. \quad (\text{A.1})$$

The fluctuating shape is, in spherical coordinates (r, θ, ψ) ,

$$\mathbf{r}(\theta, \psi) = \hat{\mathbf{r}} + \boldsymbol{\varepsilon}. \quad (\text{A.2})$$

A.1 INCOMPRESSIBILITY AND THE CALCULATION OF CURVATURE. — To find an expression for the curvature, $H = (1/R_1) + (1/R_2)$, the first and second fundamental forms of the surface [16] must be calculated.

The equation for the surface is a function of two parameters, θ and ψ . The tangent vector, \mathbf{a}_θ , parallel to the lines $\psi = \text{constant}$ on the surface is

$$\mathbf{a}_\theta \equiv \frac{\partial \mathbf{r}}{\partial \theta} = \hat{\mathbf{r}}(u_{,\theta} - \alpha) + \boldsymbol{\psi}(\beta_{,\theta} \sin \theta + \beta \cos \theta) + \boldsymbol{\theta}(1 + u + \alpha_{,\theta}), \quad (\text{A.3})$$

where $u_{,\theta} = \partial u / \partial \theta$, etc., and the following identities are used [40]:

$$\frac{\partial \hat{\mathbf{r}}}{\partial \theta} = \hat{\mathbf{r}}_{,\theta} = \boldsymbol{\theta}, \quad \boldsymbol{\theta}_{,\theta} = -\hat{\mathbf{r}}, \quad \boldsymbol{\psi}_{,\theta} = 0.$$

The tangent vector, \mathbf{a}_ψ , parallel to the lines $\theta = \text{constant}$ is

$$\mathbf{a}_\psi \equiv \mathbf{r}_{,\psi} = \hat{\mathbf{r}}(u_{,\psi} - \beta \sin^2 \theta) + \boldsymbol{\theta}(\alpha_{,\psi} - \beta \cos \theta \sin \theta) + \boldsymbol{\Psi}(\sin \theta + u \sin \theta + \alpha \cos \theta + \beta_{,\psi} \sin \theta), \quad (\text{A.4})$$

where $u_{,\psi} = \partial u / \partial \psi$ etc., and we have used

$$\hat{\mathbf{r}}_{,\psi} = \boldsymbol{\Psi} \sin \theta, \quad \boldsymbol{\theta}_{,\psi} = \boldsymbol{\Psi} \cos \theta, \quad \boldsymbol{\Psi}_{,\psi} = -\hat{\mathbf{r}} \sin \theta - \boldsymbol{\theta} \cos \theta.$$

The outward unit normal to the surface is

$$\hat{\mathbf{n}} = \frac{\mathbf{a}_\theta \times \mathbf{a}_\psi}{|\mathbf{a}_\theta \times \mathbf{a}_\psi|};$$

hence,

$$\hat{\mathbf{n}} = \hat{\mathbf{r}} + \boldsymbol{\theta}(\alpha - u_{,\theta}) + \boldsymbol{\Psi}(\beta \sin \theta - u_{,\psi} / \sin \theta). \quad (\text{A.5})$$

The tensor, a_{ij} , whose components are those of the first fundamental form of the surface, is

$$a_{ij} = \begin{vmatrix} a_{\theta\theta} & a_{\theta\psi} \\ a_{\psi\theta} & a_{\psi\psi} \end{vmatrix} = \begin{vmatrix} \mathbf{a}_\theta \cdot \mathbf{a}_\theta & \mathbf{a}_\theta \cdot \mathbf{a}_\psi \\ \mathbf{a}_\psi \cdot \mathbf{a}_\theta & \mathbf{a}_\psi \cdot \mathbf{a}_\psi \end{vmatrix}.$$

Using equations (A.3), (A.4)

$$a_{\theta\theta} = 1 + 2u + 2\alpha_{,\theta},$$

$$a_{\theta\psi} = \beta_{,\theta} \sin^2 \theta + \alpha_{,\psi},$$

and

$$a_{\psi\psi} = \sin^2 \theta + 2u \sin^2 \theta + 2\alpha \sin \theta \cos \theta + 2\beta_{,\psi} \sin^2 \theta. \quad (\text{A.6})$$

Define $a \equiv \det a_{ij} = a_{\theta\theta} a_{\psi\psi} - a_{\theta\psi}^2$ (since a_{ij} is symmetric). Then

$$a = \sin^2 \theta (1 + 4u + 2\alpha / \tan \theta + 2\alpha_{,\theta} + 2\beta_{,\psi}). \quad (\text{A.7})$$

The area element at the point on the membrane (θ, ψ) is $dA = a^{1/2} d\theta d\psi$. To keep the area element constant, we must have $dA = a^{1/2} d\theta d\psi = \sin \theta d\theta d\psi$ or, from (A.7)

$$2u + \alpha / \tan \theta + \alpha_{,\theta} + \beta_{,\psi} = 0. \quad (\text{A.8})$$

This is the incompressibility constraint, $\nabla \cdot \boldsymbol{\varepsilon} = 0$, where ∇ is the three dimensional nabla operator.

Because $a \neq 0$, a_{ij} possesses a unique inverse, a^{ij} , which is also symmetric

$$a^{ij} = \begin{vmatrix} a^{\theta\theta} & a^{\theta\psi} \\ a^{\psi\theta} & a^{\psi\psi} \end{vmatrix} = a^{-1} \begin{vmatrix} a_{\psi\psi} & -a_{\psi\theta} \\ -a_{\theta\psi} & a_{\theta\theta} \end{vmatrix}.$$

Using equations (A.6) and (A.7)

$$a^{\theta\theta} = 1 - 2u - 2\alpha_{,\theta},$$

$$a^{\theta\psi} = -(\beta_{,\theta} + \alpha_{,\psi} / \sin^2 \theta), \quad (\text{A.9})$$

and

$$a^{\psi\psi} = (1 / \sin^2 \theta) (1 - 2u - 2\alpha / \tan \theta - 2\beta_{,\psi}).$$

The reciprocal vectors, $\mathbf{a}^i = a^{ij} \mathbf{a}_j$ (with $i, j = \theta, \psi$ and repeated indices summed) are also tangent to the surface, but \mathbf{a}^θ is perpendicular to the lines $\psi = \text{constant}$ and \mathbf{a}^ψ is perpendicular to the lines $\theta = \text{constant}$.

$$\mathbf{a}^\theta = \hat{\mathbf{r}}(u_{,\theta} - \alpha) + \boldsymbol{\theta}(1 - u - \alpha_{,\theta}) + \boldsymbol{\Psi}(\beta \cos \theta - \alpha_{,\psi} / \sin \theta), \quad (\text{A.10})$$

and

$$\mathbf{a}^\psi = \hat{\mathbf{r}}(u_{,\psi} / \sin^2 \theta - \beta) + \boldsymbol{\theta}(-\beta_{,\theta} - \beta / \tan \theta) + \boldsymbol{\Psi}(1 / \sin \theta) (1 - u - \alpha / \tan \theta - \beta_{,\psi}). \quad (\text{A.11})$$

The second fundamental form, b_{ij} , of the surface is $b_{ij} = \hat{\mathbf{n}} \cdot \mathbf{r}_{,ij}$:

$$b_{ij} = \begin{vmatrix} b_{\theta\theta} & b_{\theta\psi} \\ b_{\psi\theta} & b_{\psi\psi} \end{vmatrix} = \begin{vmatrix} \hat{\mathbf{n}} \cdot \mathbf{r}_{,\theta\theta} & \hat{\mathbf{n}} \cdot \mathbf{r}_{,\theta\psi} \\ \hat{\mathbf{n}} \cdot \mathbf{r}_{,\psi\theta} & \hat{\mathbf{n}} \cdot \mathbf{r}_{,\psi\psi} \end{vmatrix},$$

where $\mathbf{r}_{,\theta\theta} = \partial \mathbf{r}_{,\theta} / \partial \theta$ etc.

From equations (A.3-5),

$$\mathbf{r}_{,\theta\theta} = \hat{\mathbf{r}}(-1 - u - 2\alpha_{,\theta} + u_{,\theta\theta}) + \Theta(2u_{,\theta} - \alpha + \alpha_{,\theta\theta}) + \Psi(\beta_{,\theta\theta} \sin \theta + 2\beta_{,\theta} \cos \theta - \beta \sin \theta),$$

$$\mathbf{r}_{,\theta\psi} = \hat{\mathbf{r}}(u_{,\theta\psi} - \alpha_{,\psi} - \beta_{,\theta} \sin^2 \theta - \beta \sin \theta \cos \theta) + \Theta(u_{,\psi} + \alpha_{,\theta\psi} - \beta \cos^2 \theta - \beta_{,\psi} \sin \theta \cos \theta) + \\ + \Psi(\cos \theta + u \cos \theta + u_{,\theta} \sin \theta - \alpha \sin \theta + \alpha_{,\theta} \cos \theta + \beta_{,\psi} \cos \theta + \beta_{,\theta\psi} \sin \theta),$$

and

$$\mathbf{r}_{,\psi\psi} = \hat{\mathbf{r}}(-\sin^2 \theta - u \sin^2 \theta + u_{,\psi\psi} - \alpha \cos \theta \sin \theta - 2\beta_{,\psi} \sin^2 \theta) + \\ + \Theta(-\sin \theta \cos \theta - u \sin \theta \cos \theta - \alpha \cos^2 \theta - 2\beta_{,\psi} \sin \theta \cos \theta + \alpha_{,\psi\psi}) \\ + \Psi(2u_{,\psi} \sin \theta + 2\alpha_{,\psi} \cos \theta - \beta \sin \theta + \beta_{,\psi\psi} \sin \theta).$$

The components of the second fundamental form are

$$b_{\theta\theta} = -1 - u - 2\alpha_{,\theta} + u_{,\theta\theta}, \\ b_{\theta\psi} = u_{,\theta\psi} - u_{,\psi}/\tan \theta - \alpha_{,\psi} - \beta_{,\theta} \sin^2 \theta \quad (\text{A.12})$$

and

$$b_{\psi\psi} = \sin^2 \theta (-1 - u + u_{,\theta}/\tan \theta + u_{,\psi\psi}/\sin^2 \theta - 2\alpha/\tan \theta - 2\beta_{,\psi}).$$

The sum, H , of the radii of curvature is given by $H = (1/R_1) + (1/R_2) = b_i^i = a^{ij} b_{ji}$. From (A.9), (A.12), this is

$$H = -2 + 2u + \nabla^2 u \quad (\text{A.13})$$

which is (3).

A.2 CALCULATION OF THE MEMBRANE FORCES. — The membrane forces are derived in Jenkins [1]. The notation here differs from his. His $c = 2K_C$ and his $h = H/2$.

The tangential force equation (Eq. (2.32) in Jenkins) is

$$- \mathbf{F}_{\text{tan}} = d_{,i} \mathbf{a}^i$$

where d is the two dimensional pressure in the plane of the membrane.

Using (A.10-11) this becomes, to first order,

$$- \mathbf{F}_{\text{tan}} = \nabla d = \Theta d_{,\theta} + \Psi d_{,\psi}/\sin \theta \quad (\text{A.14})$$

which is (12b).

The normal force equation is (2.35) in Jenkins,

$$- \mathbf{F}_{\text{norm}} = K_C(1/a^{1/2})(a^{1/2} a^{ij} H_{,j})_{,i} + H[d + 2K_C((1/4)H^2 - k)]$$

where $k = \det b_j^i = \det a^{im} b_{mj} = 1 - 2u - \nabla^2 u$ from (A.9, A.12). From the expression for H in (A.13), $[(1/4)H^2 - k] = 0$. Using $(1/a^{1/2})(a^{1/2} a^{ij} H_{,j})_{,i} = \nabla^2 H$ (page 113 in Ref. [16]), the normal force is

$$- \mathbf{F}_{\text{norm}} = (K_C \nabla^2 H + dH) \hat{\mathbf{n}} \quad (\text{A.15})$$

which is (12a).

A.3 MEMBRANE EXPRESSIONS FOR VELOCITY AND FORCE USED IN THE BOUNDARY CONDITIONS. — The velocity boundary conditions, the right hand side of (16), are found from (8, 2 and 14)

$$\hat{\mathbf{n}} \cdot \mathbf{V} = \partial u / \partial t, \\ - \nabla \cdot \mathbf{V} = 0,$$

and

$$\hat{\mathbf{r}} \cdot \nabla \times \mathbf{V} = - \nabla^2 (\partial g / \partial t).$$

Using the expression for u in (4), with $U_{nm}(t) = U_{nm} e^{-t/\tau_{nm}}$, these boundary conditions become

$$\hat{\mathbf{n}} \cdot \mathbf{V} = \sum_{n,m} (-1/\tau_{nm}) U_{nm} Y_{nm}(\theta, \psi) e^{-t/\tau_{nm}}, \tag{A.16a}$$

$$-\nabla \cdot \mathbf{V} = 0, \tag{A.16b}$$

and

$$\hat{\mathbf{r}} \cdot \nabla \times \mathbf{V} = -\nabla^2(\partial g/\partial t). \tag{A.16c}$$

Using in the membrane force (A.14-15), the expressions for H (Eq. A.13), and the unit normal, $\hat{\mathbf{n}}$ (Eq. A.5), the membrane force $\mathbf{F} = \mathbf{F}_{\text{tan}} + \mathbf{F}_{\text{norm}}$ is

$$\mathbf{F} = \hat{\mathbf{r}}[-K_C(\nabla^4 u + 2\nabla^2 u) + 2d - d_0(\nabla^2 u + 2u)] + \theta[-d_\theta + 2d_0(\alpha - u_\theta)] + \psi[-d_\psi/\sin\theta + 2d_0(\beta \sin\theta - u_\psi/\sin\theta)] \tag{A.17}$$

where d_0 is the two dimensional pressure in the equilibrium sphere.

Equations (2) and (14) imply $u = -(1/2)\nabla^2 f$. The boundary conditions on the membrane forces, given by the right hand side of equation (17), are

$$\begin{aligned} \hat{\mathbf{n}} \cdot \mathbf{F} &= 2d - K_C(\nabla^4 u + 2\nabla^2 u) - d_0(\nabla^2 u + 2u), \\ -r\nabla \cdot \mathbf{F} &= \nabla^2 d - 4d + 2K_C(\nabla^4 u + 2\nabla^2 u) + 4d_0(\nabla^2 u + 2u), \end{aligned}$$

and

$$\hat{\mathbf{r}} \cdot \nabla \times \mathbf{F} = -2d_0 \nabla^2 g.$$

Using the expansions for u and d , equations (4) and (18c), the boundary conditions on the membrane forces are

$$\hat{\mathbf{n}} \cdot \mathbf{F} = 2d_0 + \sum_{n,m} [2d_{nm} - \{K_C n(n+1)(n+2)(n-1) - d_0(n+2)(n-1)\} U_{nm}] Y_{nm}(\theta, \psi) e^{-t/\tau_{nm}}, \tag{A.18a}$$

$$\begin{aligned} -r\nabla \cdot \mathbf{F} &= -4d_0 + \sum_{n,m} [- (n^2 + n + 4)d_{nm} + \{2K_C n(n+1)(n+2)(n-1) - \\ &\quad - 4d_0(n+2)(n-1)\} U_{nm}] Y_{nm}(\theta, \psi) e^{-t/\tau_{nm}}, \end{aligned} \tag{A.18b}$$

and

$$\hat{\mathbf{r}} \cdot \nabla \times \mathbf{F} = -2d_0 \nabla^2 g. \tag{A.18c}$$

A.4 FLUID VELOCITY AND STRESS BOUNDARY CONDITIONS. — Lamb's [29, 31] solution for the fluid velocity, \mathbf{v} , in spherical coordinates at the low Reynolds number limit, is expressed in terms of three scalar functions, p , ϕ , and χ . If we put $\chi = \sum_{n,m} \chi_{nm}(t) Y_{nm}(\theta, \psi)$, and use the expression in (18a, b) for p and ϕ , then the fluid velocity inside the spherical vesicle ($r < 1$) is

$$\begin{aligned} \mathbf{v}^i &= \sum_{n,m} [\nabla \times (\mathbf{r}\chi_{nm}^i r^n Y_{nm}(\theta, \psi))] + [\nabla(\phi_{nm}^i r^n Y_{nm}(\theta, \psi)) + \\ &\quad + (n+3)/\{2(n-1)(2n+3)\eta\} r^2 \nabla(P_{nm}^i r^n Y_{nm}(\theta, \psi)) - \mathbf{r}n/\{(n+1)(2n+3)\eta\} P_{nm}^i r^n Y_{nm}(\theta, \psi)] e^{-t/\tau_{nm}}. \end{aligned}$$

The expression for the velocity outside the spherical vesicle, $r > 1$, is obtained by substituting $-n-1$ for n in the above expression:

$$\begin{aligned} \mathbf{v}^o &= \sum_{n,m} [\nabla \times (\mathbf{r}\chi_{nm}^o r^{-n-1} Y_{nm}(\theta, \psi))] + [\nabla(\phi_{nm}^o r^{-n-1} Y_{nm}(\theta, \psi)) - \\ &\quad - (n-2)/\{2n(2n-1)\eta\} r^2 \nabla(P_{nm}^o r^{-n-1} Y_{nm}(\theta, \psi)) + \mathbf{r}(n+1)/\{n(2n-1)\eta\} P_{nm}^o r^{-n-1} Y_{nm}(\theta, \psi)] e^{-t/\tau_{nm}}. \end{aligned}$$

The boundary conditions on the fluid velocity (left hand side of Eq. (16)) are given by Brenner [29, 31]. For $r < 1$:

$$\hat{\mathbf{n}} \cdot \mathbf{v}^i |_{r=1} = \sum_{n,m} [n/\{2\eta(2n+3)\} P_{nm}^i + n\phi_{nm}^i] Y_{nm}(\theta, \psi) e^{-t/\tau_{nm}}, \tag{A.19a}$$

$$(\hat{\mathbf{r}} \cdot \nabla)(\hat{\mathbf{r}} \cdot \mathbf{v}^i) - \nabla \cdot \mathbf{v}^i |_{r=1} = \sum_{n,m} [n(n+1)/\{2\eta(2n+3)\} P_{nm}^i + n(n-1)\phi_{nm}^i] Y_{nm}(\theta, \psi) e^{-t/\tau_{nm}}, \quad (\text{A.19b})$$

and

$$\hat{\mathbf{r}} \cdot \nabla \times \mathbf{v}^i |_{r=1} = \sum_{n,m} n(n+1) \chi_{nm}^i Y_{nm}(\theta, \psi). \quad (\text{A.19c})$$

For $r > 1$, substitute $n-1$ for n ,

$$\hat{\mathbf{n}} \cdot \mathbf{v}^o |_{r=1} = \sum_{n,m} [(n+1)/\{2\eta(2n-1)\} P_{nm}^o - (n+1)\phi_{nm}^o] Y_{nm}(\theta, \psi) e^{-t/\tau_{nm}}, \quad (\text{A.20a})$$

$$(\hat{\mathbf{r}} \cdot \nabla)(\hat{\mathbf{r}} \cdot \mathbf{v}^o) - \nabla \cdot \mathbf{v}^o |_{r=1} = \sum_{n,m} [-(n+1)n/\{2\eta(2n-1)\} P_{nm}^o + (n+1)(n+2)\phi_{nm}^o] Y_{nm}(\theta, \psi) e^{-t/\tau_{nm}}, \quad (\text{A.20b})$$

and

$$\hat{\mathbf{r}} \cdot \nabla \times \mathbf{v}^o |_{r=1} = \sum_{n,m} (n+1) n \chi_{nm}^o Y_{nm}(\theta, \psi). \quad (\text{A.20c})$$

Setting the coefficients of the $Y_{nm}(\theta, \psi)$'s in equation (A.19) and (A.20) equal to those in (A.16) as prescribed in (16), we find

$$\begin{aligned} P_{nm}^i &= (1/\tau_{nm}) \eta(n-1)(2n+3)/n U_{nm}, \\ \phi_{nm}^i &= -(1/\tau_{nm}) (n+1)/(2n) U_{nm}, \\ P_{nm}^o &= -(1/\tau_{nm}) \eta(2n-1)(n+2)/(n+1) U_{nm}, \end{aligned} \quad (\text{A.21})$$

and

$$\phi_{nm}^o = -(1/\tau_{nm}) n/\{2(n+1)\} U_{nm}.$$

Notice that the terms involving g and χ separate out. This will be true in the force boundary conditions as well, so these terms can be ignored.

The force, \mathbf{P}^i , that the inner fluid exerts on the membrane is given by $-\hat{\mathbf{n}} \cdot \tilde{\boldsymbol{\sigma}}^i$ where $\tilde{\boldsymbol{\sigma}}^i$ is the stress tensor of the inner fluid,

$$-\tilde{\boldsymbol{\sigma}}^i = -\sigma_{rr}^i \hat{\mathbf{r}}\hat{\mathbf{r}} - \sigma_{r\theta}^i \hat{\mathbf{r}}\boldsymbol{\theta} - \sigma_{r\psi}^i \hat{\mathbf{r}}\boldsymbol{\psi} - \sigma_{\theta r}^i \boldsymbol{\theta}\hat{\mathbf{r}} - \sigma_{\theta\theta}^i \boldsymbol{\theta}\boldsymbol{\theta} - \sigma_{\theta\psi}^i \boldsymbol{\theta}\boldsymbol{\psi} - \sigma_{\psi r}^i \boldsymbol{\psi}\hat{\mathbf{r}} - \sigma_{\psi\theta}^i \boldsymbol{\psi}\boldsymbol{\theta} - \sigma_{\psi\psi}^i \boldsymbol{\psi}\boldsymbol{\psi},$$

where the σ_{jk}^i are given in any fluid mechanics book (see Ref. [28], page 52). To first order, $\mathbf{P}^i = -\hat{\mathbf{n}} \cdot \tilde{\boldsymbol{\sigma}}^i = \mathbf{P}_r^i + \mathbf{P}_{ex}^i$ where \mathbf{P}_r^i is the fluid force if the membrane normal is in the radial direction and \mathbf{P}_{ex}^i is the extra force because the normal vector is not radial. Using equation (A.5),

$$\mathbf{P}_{ex}^i = P_0^i [(\alpha - u_{,\theta}) \boldsymbol{\theta} + (\beta \sin \theta - u_{,\psi}/\sin \theta) \boldsymbol{\psi}],$$

where P_0^i is the equilibrium hydrostatic pressure of the inner fluid at the membrane. The force expressions for the outer fluid are similar, but with a change in sign.

Substituting for α, β in terms of u and g (Eq. (14)), using the incompressibility condition (Eq. (A.8)), and defining $\Delta\boldsymbol{\sigma}_e = -(\mathbf{P}_{ex}^i - \mathbf{P}_{ex}^o)$, the difference in the extra traction of the fluid outside and inside the membrane, the left hand side of equation (17), for $\Delta\boldsymbol{\sigma}_e$, may be written as

$$\hat{\mathbf{n}} \cdot \Delta\boldsymbol{\sigma}_e |_{r=1} = 0, \quad (\text{A.22a})$$

$$\begin{aligned} (\hat{\mathbf{r}} \cdot \nabla)(\hat{\mathbf{r}} \cdot \Delta\boldsymbol{\sigma}_e) - \nabla \cdot \Delta\boldsymbol{\sigma}_e |_{r=1} &= -(P_0^i - P_0^o) (\nabla^2 u + 2u) \\ &= \sum_{n,m} (P_0^i - P_0^o) (n+2)(n-1) U_{nm} Y_{nm}(\theta, \psi) e^{-t/\tau_{nm}}, \end{aligned} \quad (\text{A.22b})$$

and

$$\hat{\mathbf{r}} \cdot \nabla \times \Delta\boldsymbol{\sigma}_e |_{r=1} = + (P_0^i - P_0^o) \nabla^2 g. \quad (\text{A.22c})$$

Using the expression in Brenner [31] for \mathbf{P}_r^i and \mathbf{P}_r^o , and for the left hand side of equation (17) (letting $n \rightarrow n-1$ to obtain the expressions for $r > 1$), and defining $\Delta\boldsymbol{\sigma}_r = -(\mathbf{P}_r^i - \mathbf{P}_r^o)$, we find

$$\hat{n} \cdot \Delta \sigma_r |_{r=1} = - (P_0^i - P_0^o) + \eta \sum_{n,m} [-2(n+1)(n+2) \phi_{nm}^o + (n^2 + 3n - 1) / \{ \eta(2n - 1) \} P_{nm}^o + 2n(n-1) \phi_{nm}^i + (n^2 - n - 3) / \{ \eta(2n + 3) \} P_{nm}^i] Y_{nm}(\theta, \psi) e^{-t/\tau_{nm}}, \quad (\text{A.23a})$$

$$(\hat{r} \cdot \nabla) (\hat{r} \cdot \Delta \sigma_r) - \nabla \cdot \Delta \sigma_r |_{r=1} = 2(P_0^i - P_0^o) + \eta \sum_{n,m} [2(n+1)(n+2)^2 \phi_{nm}^o - (n^3 + 3n^2 + 5n - 3) / \{ \eta(2n - 1) \} P_{nm}^o + 2n(n-1)^2 \phi_{nm}^i + (n^3 + 2n + 6) / \{ \eta(2n + 3) \} P_{nm}^i] Y_{nm}(\theta, \psi) e^{-t/\tau_{nm}}, \quad (\text{A.23b})$$

and

$$\hat{r} \cdot \nabla \times \Delta \sigma_r |_{r=1} = \text{function of } \chi_{nm}^i, \chi_{nm}^o. \quad (\text{A.23c})$$

A.5 FINAL RESULT. — We insert equations (A.21) into (A.23), add (A.22) to (A.23), and set the coefficients of $Y_{nm}(\theta, \psi)$ equal to those in (A.18) as prescribed in (17). The resulting eigenvalue equations are

$$0 = 2d_{nm} + U_{nm}[d_0(n+2)(n-1) - K_C n(n+1)(n+2)(n-1) + \eta(1/\tau_{nm}) \{ (n-1)(2n+3)/n + (n+2)(2n-1)/(n+1) \}], \quad (\text{A.24a})$$

and

$$0 = - (n^2 + n + 4) d_{nm} - U_{nm}[2d_0(n+2)(n-1) - 2K_C n(n+1)(n+2)(n-1) + \eta(1/\tau_{nm}) 3(n+2)(n-1)(2n+1) / \{ n(n+1) \}]. \quad (\text{A.24b})$$

Solving the above equations for τ_{nm} , we obtain equations (19) and (20).

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