ANALYSIS OF THE TORUS SURROUNDING PLANAR LIPID BILAYER MEMBRANES

STEPHEN H. WHITE

From the Department of Biochemistry, School of Medicine, University of Virginia, Charlottesville, Virginia 22901. Dr. White's present address is the Department of Physiology, California College of Medicine, University of California, Irvine, California 92664.

ABSTRACT The characteristics and behavior of the torus (annulus) surrounding planar lipid bilayer membranes formed across a cylindrical aperture are analyzed using equations for the shape and volume of the annulus derived by the methods of variational calculus. The analysis leads to the following results: (*a*) Design criteria for the aperture can be established. (*b*) The transition region between thin film and thick annulus can be defined quantitatively and its effect on the measurement of specific capacitance determined. (*c*) At fixed annulus volume the diameter of the thin membrane is a function of the thin film–annulus contact angle. This suggests a new method for examining changes in free energy of the thin film, and explains why the area of thin film increases reversibly when potentials are present across the film. (*d*) In the absence of buoyant forces, the equations for the shape and volume of the annulus consist of incomplete elliptic integrals of the first and second kinds; however, the shape of the annulus in the transition region can be described with good accuracy by an approximate equation of greater simplicity.

INTRODUCTION

Thin lipid films approximately 50 A thick can be formed by spreading a solution of phospholipid dissolved in an alkane solvent across an aperture immersed in an aqueous phase (see reviews by Tien and Diana, 1968; Henn and Thompson, 1969; Goldup et al., 1970). These films, first described by Mueller et al. (1963), are equilibrium structures that consist of a planar lipid bilayer membrane surrounded by a thick annulus of parent lipid solution called the Plateau-Gibbs border (Tien, 1968). Since the annulus, or torus, has an average thickness much greater than that of the bilayer, it is universally assumed that the electrical and permeability characteristics of the system are determined by the bilayer portion of the film. As a result of this assumption, which is usually valid, the annulus has received little attention. Tien (1968) has described the effects of the Plateau-Gibbs border on the formation of bilayer films but has only partially analyzed the equilibrium which exists between bilayer and annulus.

The purpose of this paper is to examine in detail the characteristics of the annulus.

It will be shown that the behavior and properties of the total film system can be significantly affected by these characteristics. For example, the view is widely held (Goldup et al., 1970) that the dimensions of the aperture and septum for supporting the films are largely unimportant as long as the aperture is not "too large." The analysis presented here shows that in fact these dimensions can be critical.

Wobschall (1971) has shown that the dynamic characteristics of lipid bilayer membranes depend in part upon the annulus. There are, in addition, several other observations which indicate that the properties of the annulus are important. The size of the bilayer region depends in part upon the diameter of the aperture and the volume of lipid solution applied. The thin film area decreases as volume increases (Vreeman, 1966). If an electric field is placed across the membrane, the area reversibly increases (Babakov et al., 1966). C. Huang and T. E. Thompson (personal communication) have observed that stable films for certain types of lipid solution can be obtained only for special configurations of the aperture. These observations appear to be related to the behavior of the annulus. They could be readily examined if analytical expressions for the shape and volume of the annulus were available. Wobschall (1971) calculated the equilibrium annulus shape approximately by finding arcs of circles which intersect the aperture and film at the proper points and angles. In the present paper exact equations are derived using the methods of variational calculus and assuming the absence of buoyant forces. The equations are used to discuss the phenomena mentioned above.

The complete equation for the contour of the annulus consists of incomplete elliptic integrals of the first and second kinds. For the transition region between thin membrane and thick annulus, however, an approximate solution of greater simplicity can be derived. This equation should be useful for estimating with good precision the effect of the transition region on permeability phenomena usually attributed solely to the bilayer region.

THEORY

The Equation for the Annulus

The surface free energy F_A of an annulus of volume V must be a minimum at equilibrium. If S represents the surface area of the annulus and γ_{AW} the interfacial tension at the annulus-water interface, then

$$F_A = \gamma_{AW} S. \tag{1}$$

Since γ_{AW} may be considered to be constant, the requirement that F_A be minimum is achieved by requiring S to be a minimum. The problem is thus an isoperimetric one: find the equation of the surface of minimum area enclosing the volume V. In addition to this requirement certain boundary conditions must be satisfied. At equilibrium the contact angles between thin film and annulus and between annulus and aperture must remain constant for a given lipid solution, aperture material, aqueous phase, and temperature.

The following assumptions are made:

(*a*) The film is formed across an aperture which is a right circular cylinder of radius R and height T such that $T \gg R$.

(*b*) A fixed volume V of parent lipid solution is applied to the aperture. With great precision it can be assumed that the volume of the thin film is negligible compared with that of the annulus. Therefore, take the volume of the annulus as V.

(*c*) Buoyancy effects are neglected. This is unrealistic, of course, since the density of the lipid solution is smaller than that of water; however, the gross behavior of the system should be unaffected. This assumption means that the annulus will have cylindrical symmetry and may thus be treated as a figure of revolution.

(*d*) The interfacial tensions γ_F of the thin film and γ_{AW} of the annulus are taken as constant which is true at equilibrium in the absence of temperature or electrolyte variations.

Let the thin membrane be circular with radius r_0. The cross-section of the membrane will then appear as in Fig. 1 where the Z axis coincides with the center line of the aperture. Also shown in Fig. 1 are the contact angles α and β which are related to the interfacial tensions (Davies and Rideal, 1963). At the junction of thin film and annulus $\gamma_F = 2\gamma_{AW} \cos \alpha$ while at the junction of the annulus and support $\gamma_{SW} = \gamma_{AS} + \gamma_{AW} \cos \beta$ where γ_{AS} = annulus-support interfacial tension.

The following dimensionless coordinates are introduced for mathematical convenience:

$$\mathcal{Z} = \frac{Z}{R} \text{ and } \rho = \frac{r}{R}. \tag{2}$$

The problem of finding the shape of the annulus may now be correctly and completely stated. Determine the function $\mathcal{Z}(\rho)$ which causes the surface area S enclosing the fixed volume V to be at a minimum subject to the boundary conditions:

$$\mathcal{Z}(\rho_0) = 0, \tag{3}$$

$$\left.\frac{d\mathcal{Z}}{d\rho}\right|_{\rho=\rho_0} = \tan \alpha, \tag{4}$$

$$\left.\frac{d\mathcal{Z}}{d\rho}\right|_{\rho=1} = \cot \beta, \tag{5}$$

where $\rho_0 = r_0/R$. Because the annulus is a figure of revolution, the following expressions represent its surface area and volume:

$$S = 4\pi R^2 \int_{\rho_0}^{1} \rho\left[1 + \left(\frac{d\mathcal{Z}}{d\rho}\right)^2\right]^{1/2} d\rho, \tag{6}$$

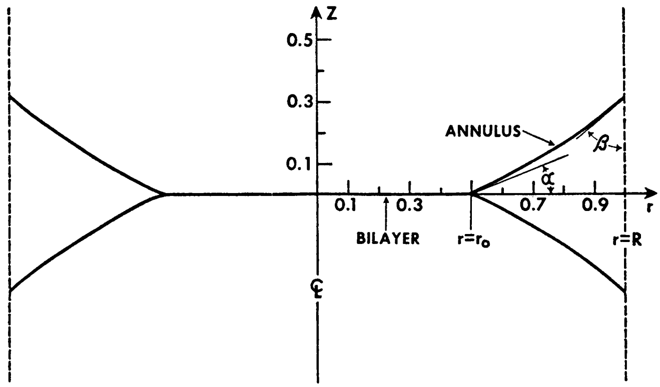

FIGURE 1 Cross-sectional view of lipid bilayer membrane. The coordinate system and important parameters are shown. The dashed lines represent the boundary of the aperture. The Z coordinate coincides with the center line (CL) of the aperture. To simplify the equations, the coordinate system is transformed to a dimensionless form such that $\hat{Z} = Z/R$ and $\rho = r/R$. α and β are the thin film to annulus and annulus to aperture contact angles respectively. The drawing represents the computer evaluation of equation 18 for $r_0 = 0.5$ mm, $R = 1$ mm, $\alpha = 24°$, and $\beta = 50°$. The absence of buoyant forces is assumed so that the membrane-annulus system has cylindrical symmetry. Equation 18 gives the shape in the first quadrant only.

$$V = 4\pi R^3 \int_{\rho_0}^{1} \hat{Z}\rho \mathrm{d}\rho = \text{constant}, \tag{7}$$

where $\hat{Z}(\rho)$ represents the equation for the shape in the first quadrant.

The necessary condition which $\hat{Z}(\rho)$ must satisfy in order for S to be a minimum is the Euler equation (Courant and Hilbert, 1953):

$$\frac{\partial f}{\partial \hat{Z}} - \frac{\mathrm{d}}{\mathrm{d}\rho}\left(\frac{\partial f}{\partial \hat{Z}'}\right) = 0, \tag{8}$$

where

$$f \equiv \rho\left[1 + \left(\frac{\mathrm{d}\hat{Z}}{\mathrm{d}\rho}\right)^2\right]^{1/2} + 2a\hat{Z}\rho, \tag{9}$$

and $2a$ is a constant to be determined.

Equations 8 and 9 yield

$$\frac{\mathrm{d}}{\mathrm{d}\rho}\left\{\frac{\rho\left(\frac{\mathrm{d}\hat{Z}}{\mathrm{d}\rho}\right)}{\left[1 + \left(\frac{\mathrm{d}\hat{Z}}{\mathrm{d}\rho}\right)^2\right]^{1/2}}\right\} = 2a\rho. \tag{10}$$

Equation 10 is easily integrated to yield a first-order differential equation for $\hat{Z}$:

$$\frac{\mathrm{d}\hat{Z}}{\mathrm{d}\rho} = \frac{a\rho^2 + b}{[\rho^2 - (a\rho^2 + b)^2]^{1/2}}, \tag{11}$$

where b is the constant of integration. Both a and b may be evaluated at this point using the boundary conditions 4 and 5:

$$a = \frac{\cos \beta - \rho_0 \sin \alpha}{(1 - \rho_0^2)}, \tag{12}$$

$$b = \frac{\rho_0 \sin \alpha - \rho_0^2 \cos \beta}{(1 - \rho_0^2)}. \tag{13}$$

It is useful to note that $a + b = \cos \beta$. $\mathcal{Z}(\rho)$ may now be written from equation 11 as

$$\mathcal{Z}(\rho) = \int_{\rho_0}^{\rho} \frac{(a\xi^2 + b)\, d\xi}{[\xi^2 - (a\xi^2 + b)^2]^{1/2}}. \tag{14}$$

This integral is of the elliptic type and may be integrated with the help of tables (Bird and Friedman, 1954). It is convertible to a standard form by setting the radicand in equation 14 equal to $(B^2 - \xi^2)(\xi^2 - A^2)$. This procedure converts equation 14 to

$$\mathcal{Z}(\rho) = \int_{\rho_0}^{\rho} \frac{(\xi^2 \pm AB)\, d\xi}{[(B^2 - \xi^2)(\xi^2 - A^2)]^{1/2}}, \tag{15}$$

where

$$A^2 = \frac{(1 - 2ab) - (1 - 4ab)^{1/2}}{2a^2}, \tag{16}$$

$$B^2 = \frac{2b^2}{(1 - 2ab) - (1 - 4ab)^{1/2}}. \tag{17}$$

The $\pm$ appears in front of the AB since $AB = |b/a|$ and b/a may be positive or negative depending upon the value of ρ_0 for particular α and β. It is expected that for most physically significant problems that $\sin \alpha \leq \cos \beta$ and the solutions in this paper will assume that this condition holds. In this case a must always be positive while b will be negative when $\rho_0 > \sin \alpha/\cos \beta$ and positive when $\rho_0 < \sin \alpha/\cos \beta$. The sign in front of AB is thus chosen to be the same as that of b.

The integration of equation 17 yields the following expression for $\mathcal{Z}(\rho)$ in the first quadrant:

$$\mathcal{Z}(\rho) = C_{\pm} - [BE(\varphi, k) \pm AF(\varphi, k)],$$

with

$$C_{\pm} = BE(\varphi_0, k) \pm AF(\varphi_0, k), \tag{18}$$

where the sign corresponds to that of b. E, F, φ φ_0, and k are defined as follows:

$$F(\varphi, k) = \int_0^{\varphi} \frac{d\theta}{(1 - k^2 \sin^2 \theta)^{1/2}} \equiv \text{incomplete elliptic integral of the first kind,} \quad (19)$$

$$E(\varphi, k) = \int_0^{\varphi} (1 - k^2 \sin^2 \theta)^{1/2}\, d\theta \equiv \text{incomplete elliptic integral of the second kind,} \quad (20)$$

$$k^2 = \frac{B^2 - A^2}{B^2},$$

$$\varphi = \sin^{-1}\left[\frac{B^2 - \rho^2}{B^2 - A^2}\right]^{1/2},$$

$$\varphi_0 = \sin^{-1}\left[\frac{B^2 - \rho_0^2}{B^2 - A^2}\right]^{1/2}. \quad (21)$$

Examination of equations 16 and 17 reveals that when $a = 0$ or $b = 0$, A and B are undefined. a becomes zero when $\rho_0 = (\cos \beta/\sin \alpha)$ and b becomes zero when $\rho_0 = (\sin \alpha/\cos \beta)$. Equation 18 is undefined when ρ_0 assumes these values. There are still solutions to the problem, however, as can be seen from equation 11 by setting a or b equal to zero. For these cases the integration is straightforward and the solutions are:

$$a = 0: Z(\rho) = b \ln [\rho + (\rho^2 - b^2)^{1/2}] - b \ln [\rho_0 + (\rho_0^2 - b^2)^{1/2}, \quad (22)$$

$$b = 0: Z(\rho) = \left[\frac{1}{a^2} - \rho_0^2\right]^{1/2} - \left[\frac{1}{a^2} - \rho^2\right]^{1/2}. \quad (23)$$

Approximate Solution

The portion of the annulus that is likely to have the greatest effect on physical measurements of lipid bilayer membranes is that near ρ_0, i.e., the thinner segment of the annulus. An approximate solution in this region can be obtained from equation 11. Using equations 12 and 13 and allowing $\rho \to \rho_0$ and $\beta \to 0$, equation 11 becomes

$$\frac{dZ}{d\rho} \approx \frac{a\rho}{\cos \alpha} + \frac{b}{\cos \alpha} \frac{1}{\rho}. \quad (24)$$

This is easily integrated to give for $\rho \sim \rho_0$ and $\beta \sim 0$:

$$Z(\rho) = \frac{1}{2} \frac{a}{\cos \alpha} \rho^2 + \frac{b}{\cos \alpha} \ln \rho - D, \quad (25)$$

where

$$D = \frac{1}{2} \frac{a}{\cos \alpha} \rho_0^2 + \frac{b}{\cos \alpha} \ln \rho_0.$$

The Volume of the Annulus

The volume of the annulus for a given α, β, and ρ_0 can be found by integrating equation 7 after substituting equation 18. The integration is laborious but straightforward with the help of Bird and Friedman (1954). The result is

$$\frac{V}{4\pi R^3} = M[E(\varphi_0, k) - E(\varphi_1, k)] + N[F(\varphi_0, k) - F(\varphi_1, k)]$$
$$- \frac{B^3k^2}{6}[\sin\varphi_0 \cos\varphi_0(1 - k^2\sin^2\varphi_0)^{1/2} - \sin\varphi_1\cos\varphi_1(1 - k^2\sin^2\varphi_1)^{1/2}],$$

where

$$M = \frac{B^3}{6}(2k^2 - 1) - \frac{B^3k^2}{6}\sin^2\varphi_1 \pm \left(-\frac{B^2A}{2}\right),$$
$$N = \pm\frac{B^2A}{2}(1 - k^2\sin^2\varphi_1) + \frac{(1 - k^2)B^3}{6}, \quad (26)$$

and the sign is chosen as that of b.

DISCUSSION

Graphs of $\tilde{Z}(\rho)$ representing the intersection of the annulus with the $\tilde{Z}$-ρ plane in the first quadrant are shown in Figs. 2–5. In each case two of the parameters α, β,

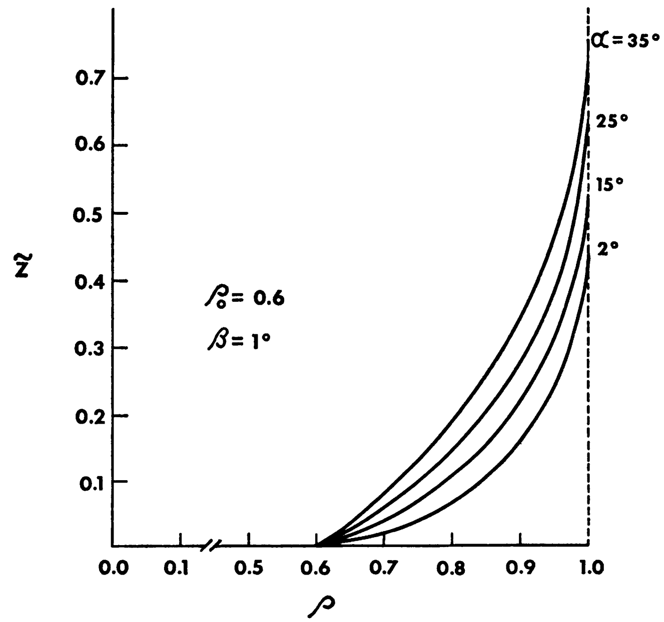

FIGURE 2 Shape of annulus in first quadrant with α as a parameter (see Fig. 1). The curves represent the computer evaluation of equation 18 with $\rho_0 = 0.6$ and $\beta = 1°$. $\tilde{Z} = Z/R$ and $\rho = r/R$ where R is the radius of the aperture. Notice that as α increases $\tilde{Z}(1)$ increases.

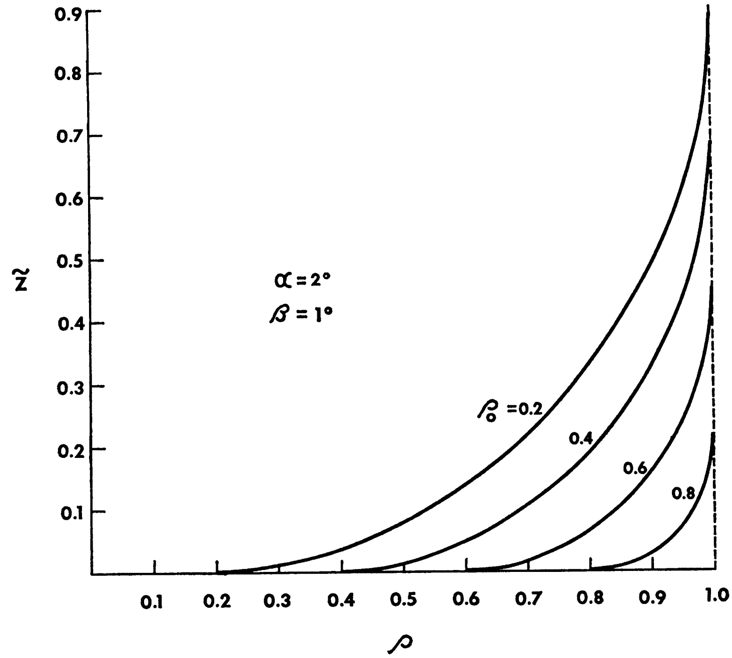

FIGURE 3 Shape of the annulus in the first quadrant with ρ_0 as a parameter (see Fig. 1). The curves represent the computer evaluation of equation 18 with $\alpha = 2°$ and $\beta = 1°$. $\tilde{Z} = Z/R$ and $\rho = r/R$ where R is the aperture radius. Notice that as ρ_0 decreases $\tilde{Z}(1)$ increases. This means that as annulus volume increases, ρ_0 will decrease. The shape of the curves is largely determined by the contact angles α and β.

and ρ_0 are fixed while the third is allowed to take on a range of values. The graphs were obtained from equation 18 with the aid of a Burroughs Corp. (Detroit, Mich.) B5500 Computer. The library for this machine conveniently contained a program for evaluating elliptic integrals based on equations and algorithms developed by Hofsommer and Van De Riet (1963).

The graphs show that α and β determine the shape of the annulus while volume of applied lipid determines ρ_0. In most cases α will probably be 20° or less. Haydon and Taylor (1968) find $\alpha = 1°53'$ for glyceryl monooleate in decane while Pagano (1968) determined that $\alpha \approx 20°$ for spherical films of lecithin in chloroform-methanol-tetradecane. A value of 80° was reported by Moran and Ilani (1970) for lecithin and cholesterol in methyl oleate but this is probably an exceptional case. The values β might obtain are not known but they are expected to be reasonably small since lipid solutions usually "wet" the plastic surface of the aperture well.

Fig. 3 illustrates a practical problem encountered in the design of apertures for lipid bilayer membrane experiments. It shows that if the geometry of the annulus described by equation 18 is to be maintained for some range of ρ_0's that there is a minimum septum thickness determined by the smallest ρ_0 to be used. For example,

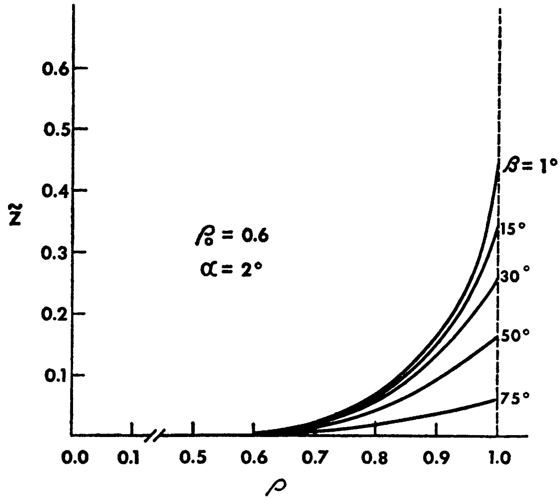

FIGURE 4 Shape of the annulus in the first quadrant with β as a parameter (see Fig. 1). The curves represent the computer evaluation of equation 18 with $\rho_0 = 0.6$ and $\alpha = 2°$. $\tilde{Z} = Z/R$ and $\rho = r/R$ where R is the aperture radius. Notice that as β increases, $\tilde{Z}(1)$ decreases.

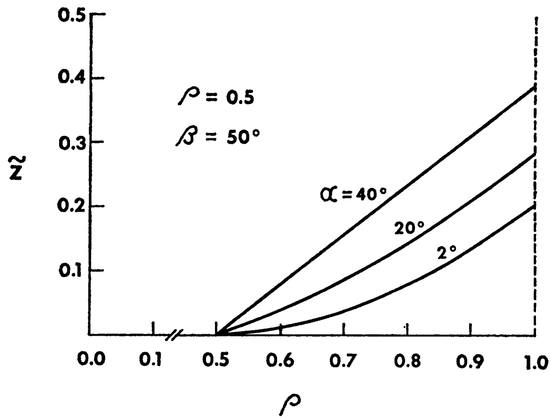

FIGURE 5 Shape of the annulus in the first quadrant with α as a parameter (see Fig. 1). This set of curves is similar to Fig. 2 except that $\beta = 50°$ rather than 1° and $\rho_0 = 0.5$. Note that for $\alpha + \beta = 90°$ the curve degenerates into a straight line.

if the minimum membrane ρ_0 was to be 0.4 for $\alpha = 2°$ and $\beta = 1°$, then the minimum septum thickness would have to be about $2R\,\tilde{Z}(1) \approx 1.4R$. If a thinner partition was used the lipid solution would need to pass over the edge of the aperture in order for β to have the correct value. This would lead to (*a*) drainage of the annulus until a suitable ρ_0 was reached, (*b*) "bending" of the annulus around the edge of the aperture if the lipid solution were very viscous, or (*c*) an instability in the system which would cause breakage of the film. All three of these phenomena

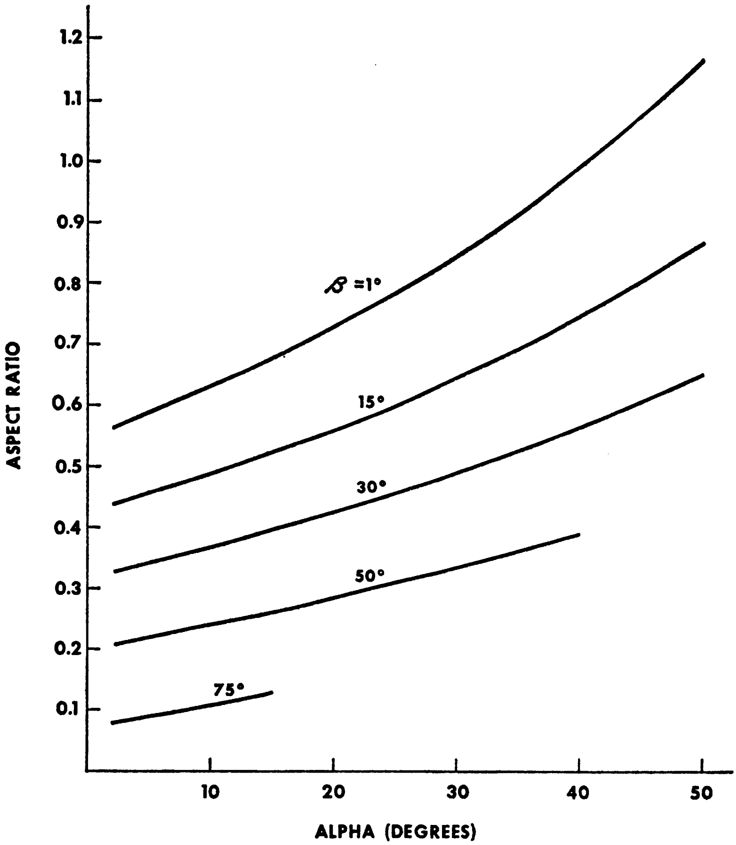

FIGURE 6 Aspect ratio as a function of α with β as a parameter. Aspect ratio is defined from equation 18 as $\hat{Z}(1)$ with $\rho_0 = 0.5$ which is the ratio of minimum septum thickness to aperture diameter for a membrane whose diameter equals 0.5 times the aperture diameter. The figure shows that the aspect ratio increases with α but decreases with increasing β. For practical purposes, an aperture with an aspect ratio of 1 should allow stable membranes to be formed from most lipid solutions.

have been observed in this laboratory and elsewhere. Drainage and instability problems can be circumvented by using a long cylindrical aperture, an aperture with an appropriate edge geometry, or by using a very thin septum so that in effect the wall of the aperture would be rotated through an angle of 90° in order to satisfy β. In this latter case, however, drainage would probably still present some problems. These findings suggest that it will be difficult to form films of large diameter unless the septum is sufficiently thick. As long as the septum thickness to aperture diameter ratio is kept constant for a given α, β, and ρ_0, it should be possible to form planar films of large area (disregarding buoyancy effects).

Design criteria for cylindrical apertures can be specified by the *aspect ratio* for the aperture-membrane system. The aspect ratio is defined here arbitrarily as the ratio of minimum septum thickness to aperture diameter for $\rho_0 = 0.5$. Since $\tilde{Z}(1) = (\frac{1}{2}$ minimum thickness$/R)$ for a given ρ_0, the aspect ratio is given by $\tilde{Z}(1)$ for $\rho_0 = 0.5$. Fig. 6 shows the aspect ratio plotted as a function of α with β as a parameter. The aspect ratio increases with increasing α but decreases for increasing β. It can be seen that if an aperture has an aspect ratio of about 1, then almost any film would be stable as far as the annulus is concerned. The 50° and 75° curves are truncated in this figure because of the requirement placed on equation 18 that $\sin \alpha \leq \cos \beta$. For the ρ_0 given, this condition is violated when $\alpha + \beta > 90°$. Fig. 5 demonstrates that when $\alpha + \beta = 90°$ the curve of the annulus degenerates into a straight line. It should be emphasized, however, that the condition $\sin \alpha < \cos \beta$ was set in order to simplify the equations. For a given ρ_0, α, and β a solution still exists for $\sin \alpha > \cos \beta$ if ρ_0 is in an allowable range and the sign of AB is properly chosen in equation 15.

The region of the annulus near the thin film is of considerable interest because of the contribution it might make to physical measurements on the membrane system. Fig. 7 shows the contour of the annulus in the transition region near ρ_0 for $\alpha = 2°$ and $\beta = 0°$. Marked on the figure is the point at which $\tilde{Z}$ would equal 5000 A for $R = 0.1$ cm. This point will arbitrarily be referred to as the transition region termination for two reasons: (*a*) At this point the annulus will be about 200 times thicker than the thin film and beyond this point the annulus should have a negligible effect on the electrical and permeability characteristics of the total system. (*b*) Since the annulus will be about two wavelengths of light thick at this point, beyond it the system will no longer be optically black. Note that in Fig. 7 for $R = 0.1$ cm and $\alpha = 2°$ the transition region is about 10 μ wide which under most circumstances will be the maximum expected since as α increases the transition width will decrease.

Also illustrated by Fig. 7 is the comparison of the approximate solution of equation 25 with the exact solution. Values of $\tilde{Z}(\rho)$ calculated from equation 25 are shown by the cross marks (+). The two solutions agree to within 3% over the range shown. If α and β are both small as in this example, the approximation will be off by over 50% when $\rho = 1$; however, for small α (~2°) and large β(~75°) the two solutions can agree to within 0.3% over the entire range $\rho_0 \leq \rho \leq 1$.

The effect of the transition region on measurements of bilayer specific capacitance are of particular interest in this laboratory. The precise determination of specific capacitance depends upon how accurately total membrane capacitance and membrane area can be determined. The transition region affects both of these determinations. The area determination is affected because of the difficulty of visually determining the beginning of the thin film. The uncertainty can be assumed to be roughly equal to the width Δr of the transition region. Apertures for bilayer work typically have a diameter of 0.2 cm. In this case from Fig. 7 Δr will be about 10^{-3} cm and roughly independent of r_0. The ratio of the transition area ΔA to the thin film area $A = \pi r_0^2$ will be approximately $(2\Delta r/r_0)$. If $r_0 = 0.01$ cm then $\Delta A/A =$

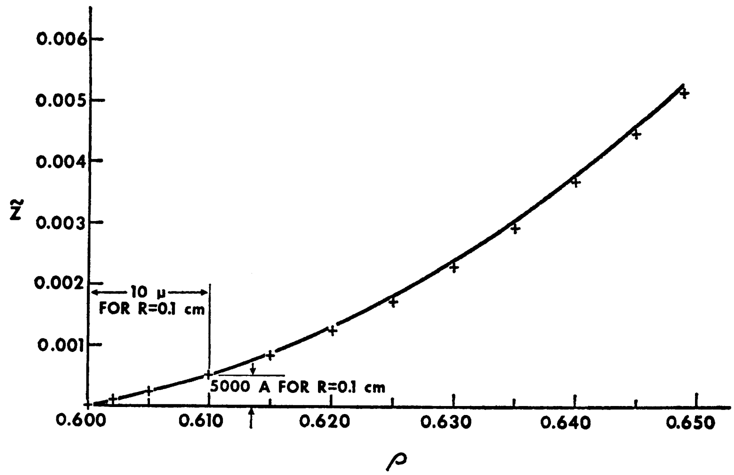

FIGURE 7 Shape of the annulus in the region near $\rho_0 = 0.6$ for $\alpha = 2°$ and $\beta = 0$. The curve represents the computer evaluation of equation 18. The cross marks (+) indicate values of $\tilde{Z}$ obtained using the approximate solution of equation 25. The two solutions agree to within 3% over the range of ρ shown. Also shown on the figure is the extent of the transition region for $R = 0.1$ cm. The $\tilde{Z}$ axis is magnified × 20 with respect to the ρ axis for clarity.

0.2 while if $r_0 = 0.09$ cm $\Delta A/A = 0.022$. Thus, the accuracy is best for large ρ_0. Most investigators use light reflected from the bilayer at an acute angle to measure the membrane r_0 with a microscope reticle in order to calculate A. The accuracy of this method may be limited to about 2% disregarding reticle inaccuracies and non-uniform membrane boundaries. The accuracy can probably be improved using the principles developed by White (1970) or Haydon and Taylor (1968).

Let ΔC be the capacitance of the transition region and C the capacitance of the thin film. An expression for $\Delta C/C$ can be obtained by assuming the capacitance of a subregion $d\rho$ of the transition region $\Delta\rho$ is given by $dC = (\pi R\epsilon\epsilon_0\rho d\rho)/\tilde{Z}(\rho)$ where ϵ is the dielectric coefficient and $\epsilon_0 = 8.85 \times 10^{-12}$ farads/m. $\tilde{Z}(\rho)$ may be taken as linear in the transition region. By integrating from ρ_0 to $\rho_0 + \Delta\rho$ and neglecting second-order term,

$$\frac{\Delta C}{C} \approx \frac{\delta}{t} \cdot \frac{\Delta\rho}{\rho_0^2}, \tag{27}$$

where δ is the thin film thickness and t is the transition termination thickness. Since $\delta/t = 5 \times 10^{-3}$ and $\Delta\rho$ is approximately constant and equal to 0.01 for $\alpha = 2°$, $\Delta C/C$ is found to be 5×10^{-3} if $\rho_0 = 0.1$. As ρ_0 approaches 1, $\Delta C/C$ will approach 5×10^{-5}. Under the worse conditions then the measurement of C will be off by 0.5% while for large ρ_0 the error will approach 0.005%. For larger α the error will be smaller so these values may be taken as upper limits.

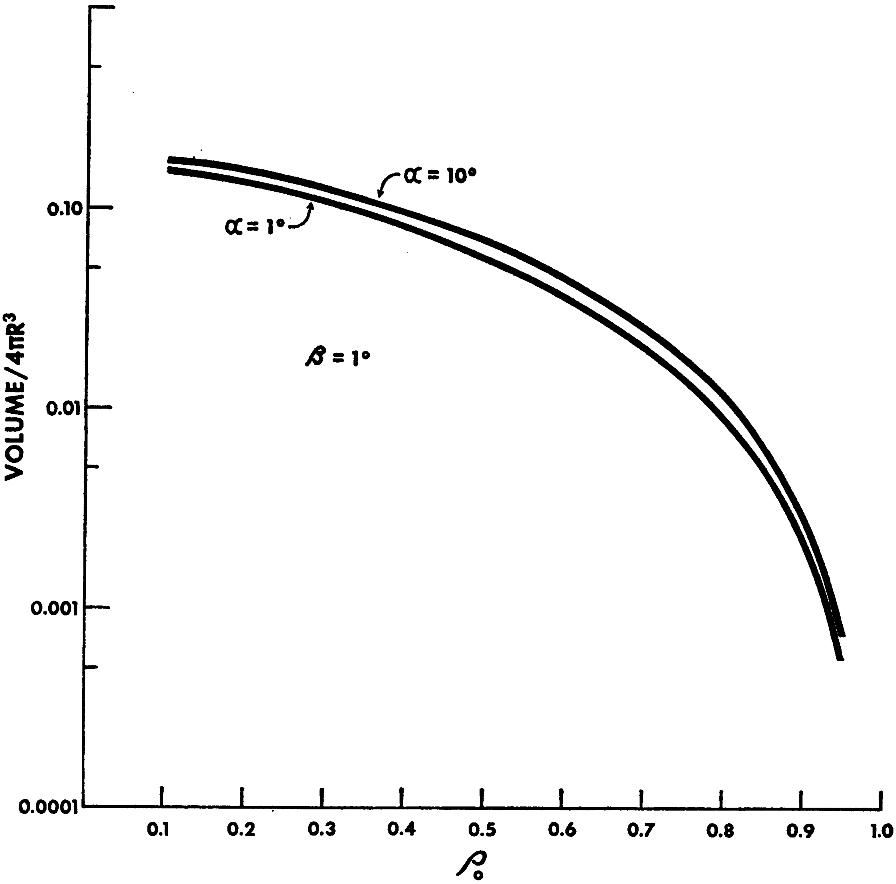

FIGURE 8 Annulus volume as a function membrane radius. Volume is scaled by $4\pi R^3$ and membrane radius r_0 is scaled by R where R is the aperture radius. The curves represent the computer evaluation of equation 26 with $\beta = 1°$. Membrane radius will decrease as the annulus volume decreases. If α increases, as it does when an electric field is placed across the membrane, membrane radius will increase if the annulus volume remains constant.

The behavior of the annulus volume at constant β as ρ_0 and α are changed is shown in Fig. 8 which represents the evaluation of equation 26 on the computer. As ρ_0 is increased from 0.1 to 0.95, $V/4\pi R^3$ decreases by almost three powers of 10. For fixed β and ρ_0, volume increases with increasing α. Suppose a fixed volume of lipid were applied to the aperture. Then if α were to change the figure shows that ρ_0 would also change. This explains why the radius of thin film increases in the presence of an applied electric field. It has been shown elsewhere (White, 1970) that when a potential is present across a lipid bilayer membrane the free energy decreases. Such a decrease will lead to an increase in α and therefore an increase in ρ_0. The changes in ρ_0 accompanying changes in α may be useful for measuring changes in α, i.e., changes in free energy of the thin film. This phenomenon may also be a partial explanation for the breakage of films in the presence of large voltages (~100 mv or greater). For films with contact angles of the appropriate size the change in α accompanying the potential might cause the thickness of the annulus at $\rho = 1$ to exceed the aspect ratio for the system and therefore lead to an instability. The contact angle for lecithin in chloroform-methanol-tetradecane is about 20°. If a 100 mv potential were applied the free energy could decrease by as much as a factor of 4 which means α would increase to about 40°. If β were small, Fig. 6 demonstrates that the aspect ratio would need to increase from 0.73 to 0.95 if the geometry were to be maintained. If α were closer to 2° the effect would not be nearly as large.

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