Biochimica et Biophysica Acta, 776 (1984) 169–175
Elsevier

BBA 72220

FISSION OF THE BILAYER LIPID TUBE

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(Received December 6th, 1983)

Key words: Membrane fusion; Lipid tube fission; Mechanical stability; Hydrostatic pressure; Membrane tension; Lipid bilayer

The fusion of two black lipid membranes results in the formation of peculiar bilayer lipid tubes ('cylindrical' membranes (Neher, E. (1974) Biochim. Biophys. Acta 373, 328–336 and Melikyan, G.B., Abidor, I.G., Chernomordik, L.V. and Chailakhyan, L.M. (1983) Biochim. Biophys. Acta 730, 395–398)). The mechanical stability of such tubes has been investigated experimentally and theoretically. With increasing hydrostatic pressure on the outside of the tube the radius of its middle part decreases. After this radius has reached a critical value, which constitutes 0.55 of the radius of the tube base, there occurs a collapse of the tube and its disintegration into two planar bilayers (fission). Expressions are obtained which relate the transmembrane difference of the hydrostatic pressure, causing the collapse, to the geometrical characteristics of the tube (its length and the radius of its base) and to the tension of the lipid bilayer. A method for measuring the membrane tension is proposed on the basis of the phenomenon considered.

Introduction

The mechanism of fusion of biological membranes is being examined using various model systems [1], among which the model of two interacting bilayer lipid membranes, as proposed by Liberman and Nenashev [2], holds a special place. This model, as has been shown in Ref. 5, enables one to observe single acts of the fusion of bilayers, which result in the formation of a membrane tube. It has been established that the complete fusion is preceded by the appearance of a trilaminar structure, that is to say, the fusion of the external monolayers of the interacting membranes with the formation in the contact region of one bilayer common to both membranes [3–5]. The trilaminar structure is quite stable: its lifetime is comparable to that of a planar lipid bilayer [6]. This structure can be disrupted by an electrical voltage with the resulting formation of a membrane tube. By compressing the tube externally with hydrostatic pressure, it is possible to cause its collapse and fission into two planar membranes [5,6]. Thus, in the system described, one can repeat several times the cycle involving the fusion of two bilayers with the subsequent fission of the membrane tube. Obviously, the fusion and fission are similar: in either case, the need is for disintegration of the contact region of two membranes and for connection of the neighbouring sections of the contacting membranes.

The electrostimulated and Ca^{2+}-stimulated fusion of bilayers was described previously [5–7]. In the present paper, we describe the mechanism of fission of a membrane tube.

Formulation of the problem. Analysis of the shape of the membrane tube

Consider the membrane resulting from the fusion of two bilayers. It is shaped like a tube interconnecting two holes on which menisci of the

0005-2736/84/$03.00

initial membranes are located (Fig. 1, inset). We shall suppose that the internal volume, V, of the system under consideration is limited by the membrane tube itself and by the planes of the holes on which the menisci are located. The tube length will be denoted by L and the radius of the holes limiting the tube, by r (Fig. 1, inset). Applied to the tube is a transmembrane difference of hydrostatic pressure, ΔP, which will be considered positive if the pressure in the environment is greater than that in the internal volume. It is our task to find the conditions for the existence of the membrane tube in the equilibrium state.

To equilibrium of the system at constant temperature and pressure there corresponds a minimum of the Gibbs free energy [8] which can be written as

$$\Phi = \sigma A + \Delta P V + c \tag{1}$$

where σ is the membrane tension, A is the membrane area, and c (a constant) involves all the terms which are independent of the system configuration, so it will be dropped subsequently.

Let the curve $y(x)$ represent a contour, the rotation of which about the axis x gives the surface of the membrane tube (Fig. 1, inset). In this case, the free energy is described as

$$\Phi = 2\pi\sigma \int_{-L/2}^{L/2} y\sqrt{1+\left(\frac{\mathrm{d}y}{\mathrm{d}x}\right)^2}\,\mathrm{d}x + \pi\Delta P \int_{-L/2}^{L/2} y^2\,\mathrm{d}x \tag{2}$$

For finding the shape of the membrane tube, which corresponds to the minimum of the free energy [2], it is necessary to solve Euler's equation [9]

$$y\left(\frac{\mathrm{d}^2y}{\mathrm{d}x^2}\right) - \left(\frac{\mathrm{d}y}{\mathrm{d}x}\right)^2 - 1 + \frac{\Delta P}{\sigma} y\left(1+\left(\frac{\mathrm{d}y}{\mathrm{d}x}\right)^2\right)^{3/2} = 0 \tag{3}$$

Let us assume that the value of the transmembrane pressure difference is small. $(\Delta P/\sigma)\cdot R < 1$, then the solution of Eqn. 3 is represented as:

$$y = \rho\,\mathrm{ch}\frac{x}{\rho} + \frac{\Delta P \rho^2}{4\sigma}\mathrm{ch}^3\frac{x}{\rho} \tag{4}$$

where ρ is the constant determined from the boundary condition

$$\rho\,\mathrm{ch}\frac{L}{2\rho} + \frac{\Delta P\rho^2}{4\sigma}\mathrm{ch}^3\frac{L}{2\rho} = R \tag{5}$$

It is seen from Eqn. 4 that the shape of the membrane tube is symmetrical (Fig. 1, inset), and in the middle is its narrowest section; the radius of the cross-section of the tube at this point, according to Eqn. 4, is:

$$r = \rho + \frac{\Delta PR}{4\sigma}\rho^2 \tag{6}$$

The solution of Eqn. 5 for the constant ρ defines, taking into account Eqn. 4, the shape of the membrane tube. The ρ value obtained is a function of the system parameters, namely the transmembrane

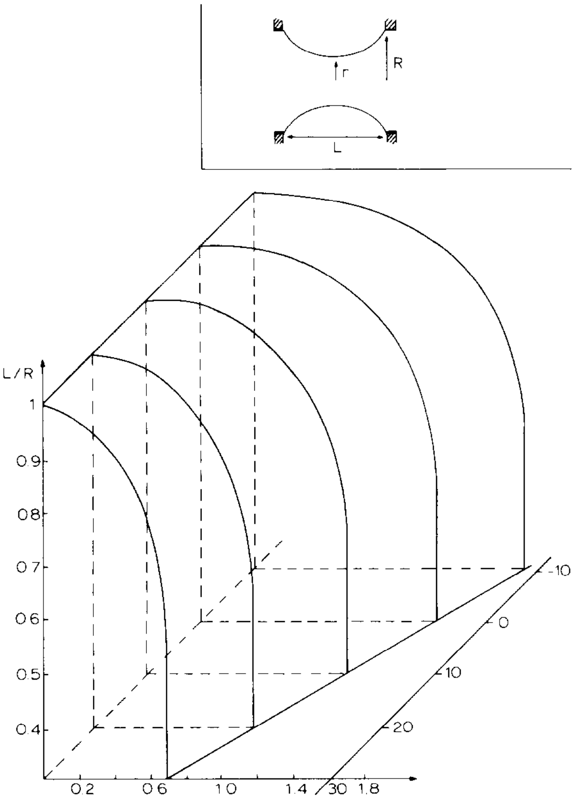

Fig. 1. The dependence of the radius of the narrowest section of the membrane tube, r, on the tube length and the transmembrane pressure difference ΔP. The quantities L and ΔP are referred to $R = 0.065$ cm. The tension of membranes was assumed to be 1.7 dyn/cm. (inset) The cross-section of the membrane tube along the axis of rotation. L, length; r, radius of the narrowest section of the tube; R, the radius of the base of the membrane tube.

pressure difference ΔP, the tube length L and the radius R. However, there is a range of values of the cited parameters at which Eqn. 5 has no solution. The cases where Eqn. 5 has no solution, that is to say, the boundary condition cannot be satisfied, correspond to the absence of an equilibrium shape of the tube, in which case its collapse occurs.

Inspection of Eqn. 5 indicates that collapse of the tube occurs if the transmembrane pressure difference is greater than the critical value ΔP^* given by the expression

$$\Delta P^* = \frac{2.2\sigma}{R}(1-0.754L/R) \tag{7}$$

The same result can be expressed in another form: collapse of the membrane tube occurs at a given value of the transmembrane pressure difference ΔP if its length is in excess of the critical value L^* represented as

$$L^*/R = 1.325(1-\Delta PR/2.2\sigma) \tag{8}$$

Fig. 1 gives the values of the radius of tube narrow section, r, as functions of the tube length L (both quantities are referred to R) and of the pressure difference ΔP. The range of the quantities L and ΔP, in which the values of r are not determined, corresponds to collapse of the tube and is determined by Eqns. 7 or 8.

It follows from Eqn. 8 that in the case where the transmembrane pressure difference is equal to zero, $\Delta P = 0$, the membrane tube becomes unstable if its length is in excess of the value of $L^* = 1.325R$. At a positive value of ΔP, shorter tubes suffer collapse (Fig. 1) while at a negative value of ΔP, for the collapse to occur the membrane tube must be of a greater length.

Fig. 2 displays the cross-sections of Fig. 1, which represent the values of the radius of the narrowest section of the membrane tube as functions of its length, the transmembrane pressure difference ΔP being the parameter on the curves. One can see that r cannot be less than $0.553R$, i.e., the collapse with respect to this parameter is of the threshold character.

Of interest is the dependence of the value of $0.455 \cdot \Delta P^* \cdot R$ on L/R. This dependence is represented, according to Eqn. 7, by straight lines intersecting at one point corresponding to $\Delta P^* = 0$. In addition, when this dependence is extrapolated up to the point $L/R = 0$, the value of $0.455 \cdot \Delta P^* \cdot R$ coincides with the membrane tension σ.

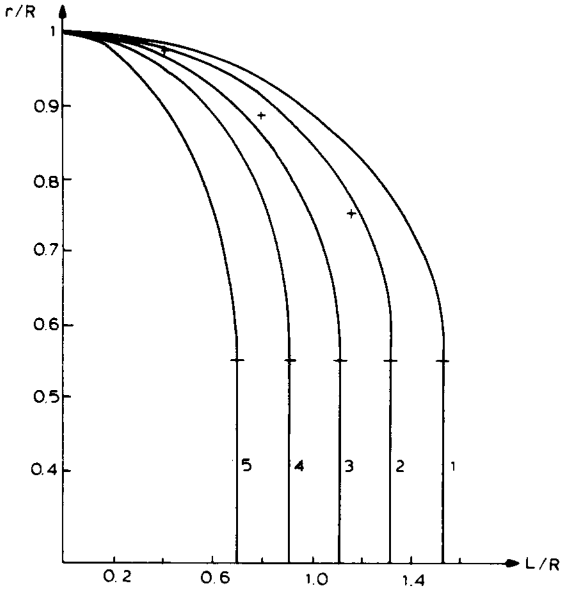

Fig. 2. The dependence of the radius of the narrowest section of the tube, r, on its length. The parameter on the curves is represented by the transmembrane pressure difference, the value of which for curve 1 is -10 dyn/cm^2; for curve 2, 0; for curve 3, 10 dyn/cm^2; for curve 4, 20 dyn/cm^2; for curve 5, 30 dyn/cm^2. It was assumed that $R = 0.065$ cm and $\sigma = 1.7$ dyn/cm. Experimental points are given for curve 2 in the case of azolectin in decane.

Materials and Methods

Membranes were formed in the usual manner from solutions of azolectin (azolectin soybean phosphatidylcholine, fraction II, Sigma) and phosphatidylethanolamine (PE, Sigma) in n-decane (30 and 20 mg/ml, respectively) on holes 1.5 mm in diameter. Solvent-free bilayers were obtained from solutions of the same lipids in squalene (Merck) (10 mg/ml azolectin and 7 mg/ml PE) by modifying the technique of White [10] as applied to our lipids.

The temperature in the measuring cell was maintained at 30°C. The experiments were carried out in the 0.1 M KCl solution (chemically pure) at pH of about 6.0.

For the optical and electrical monitoring of the interaction between two lipid membranes, a cell of special construction was produced (Fig. 3). The bilayers were formed on holes 1 and 3 in the walls of special Teflon discs. The third compartment of the cell can smoothly move within the second compartment without changing the overall volume of the latter. One can thus vary the distance between the membranes in the course of an experiment. The visual observation of the bilayers in the direction both perpendicular and parallel to the plane of the membranes was carried out using two binocular microscopes. The error in measurement of the diameter of the planar membranes, and also of the diameter and length of the membrane tube, was 5%.

In measuring the electrical characteristics of the bilayers, a voltage of ±20 mV as a sawtooth waveform was applied from a PAR-175 generator to the membranes at a rate of 100 V/s. The capacitive currents of the membranes were recorded with the aid of operational current amplifiers (Keithley-427) and a dual-beam storage oscillograph (Tektronix-5103). The circuit diagram and the principle of the capacitance monitoring of the interaction between membranes are described in detail in Refs. 4 and 6.

Teflon pivots 3 and 4 (Fig. 3), which are calibrated and connected to the micrometric screws, make it possible to control the hydrostatic pressures in the side compartments of the cell. The zero difference of the hydrostatic pressures $\Delta P = 0$ is obtained by minimising the capacitances of the two membranes.

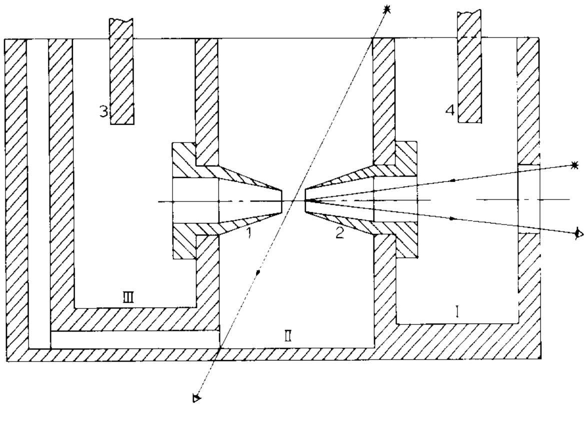

Fig. 3. Construction of the measuring cell. 1 and 2, holes in the walls of special Teflon discs; 3 and 4, calibrated Teflon pivots to control the hydrostatic pressure; I, II, III, compartments of the cell. The movement of the third compartment allows the distance between holes 1 and 2 to be varied. The visual observation was carried out through glass windows in the cell walls in both transmitted and reflected light.

The specific capacitance for the membranes of azolectin in *n*-decane and squalene is 3.9 ± 0.2 and 7.8 ± 0.4 mF/m^2 and that for the membranes of PE in *n*-decane and squalene is 4.4 ± 0.2 and 6.9 ± 0.3 mF/m^2, respectively.

The tension of the bilayers was determined with the method described in Ref. 11. The membranes were inflated up to a hemisphere. On measuring the radius of the hemisphere and the difference of hydrostatic pressures, we have calculated the tension from the formula

$$\sigma = \Delta P \cdot R/2$$

Results

The experiment illustrated in Fig. 4 is fairly simple. By gradually increasing the hydrostatic pressure in the side compartments of the cell, the membranes were inflated (Fig. 4a) until they were brought into contact. After the formation of a trilaminar structure (which was checked by the capacitance of the contact region), a square voltage pulse of 500 mV amplitude and 40 ms duration, breaking down the bilayer in the contact region, was applied to the bilayers (Fig. 4b). The form of the resulting membrane structure (Fig. 4c) was dependent on the parameters of the system (the tube length L and the transmembrane pressure difference ΔP). Then the pressures were equalized by balancing out the hydrostatic pressure difference created in order to bring the bilayers into contact. As a result, the membrane tube turns out to be somewhat narrowed in its middle part. The narrowing radius r was measured with the binocular microscope. The values of r at $\Delta P = 0$ and at different L/R are presented in Fig. 2 and well fit the corresponding theoretical curve. With increasing pressure on the outside of the membrane tube, the radius r decreased. When the transmembrane pressure difference reached some critical value of ΔP^*, the radius abruptly went to zero. This was accompanied by collapse of the tube and by its disintegration into two membranes.

The dependences of the value of $0.455 \cdot \Delta P^* \cdot R$

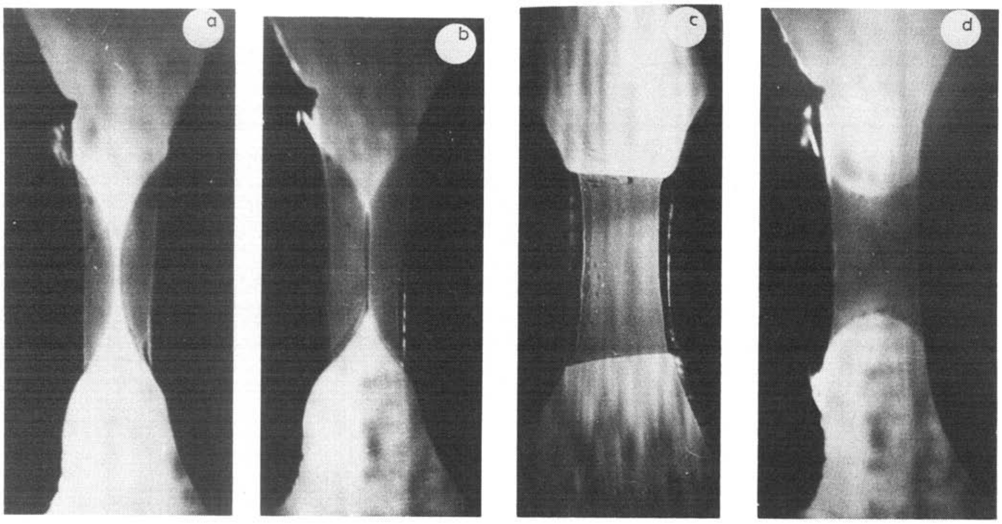

Fig. 4. The stages of interaction of bilayers. (a) Inflated membranes (they were squeezed out towards each other until they were brought into contact). The membranes are visible in the form of semitransparent spherical segments on the truncated cones of special Teflon discs; (b) the formation of a trilaminar structure (the bilayer in the contact region is visible as a thin line between the oblate hemispheres of membranes); (c) the membrane tube interconnecting the holes in the two Teflon discs at a negative difference of hydrostatic pressures; (d) the membrane tube at a positive transmembrane pressure difference. In making photographs, the solutions in compartments I and III were coloured with neutral red dye, owing to which one can observe in transmitted light the form of aqueous volumes limited by the bilayers.

on the ratio L/R for the membranes of azolectin and PE in decane and squalene are presented in Fig. 5a and b. The chosen coordinate system, as has been demonstrated above, is convenient for determining the tension of bilayers. The experimental points well fit the theoretical curves plotted for different σ and passing, according to calculations, through the point $L/R = 1.325$. The tension of the azolectin and PE membranes, which is equal to the segments intercepted on the Y-axis by the corresponding curves, is: 1.7 ± 0.2 and 3.0 ± 0.3 dyn/cm in decane, and 2.1 ± 0.2 and 2.3 ± 0.2 dyn/cm in squalene, respectively.

For examining the kinetics of the collapse, we have measured the current flowing along the axis of the membrane tube with the application of a small constant voltage of 30 mV. At the instant of collapse, one can observe a rapid fall (approx. 50 ms) of the conduction current to zero, which is reflective of the decrease in the radius r and of the fission of the tube with the formation of two bilayers (Fig. 6). It appears from the figure that the curve showing the time variation of current has two inflections (the arrows in the figure): the first corresponds to the onset of collapse and the second is likely to reflect the slowing down of the process directly before the fission.

Discussion

In the preceding sections we have presented the results obtained from the experimental and theoretical investigation of the fission of the membrane tube. It is shown that the stability of the tube is determined by its length and radius, by the transmembrane pressure difference and the membrane tension. Therefore, the fission of the membrane tube can be caused by increasing the external pressure at a given length or by increasing the length at a fixed pressure, as was done in Ref. 2. The relationship between the transmembrane pressure difference causing the collapse and the ratio of the tube length to the radius of the tube base depends on the bilayer tension σ (see Eqn. 8). Owing to this, an analysis of the experimental curves in Fig. 5 makes it possible to obtain such an important characteristic of the bilayer as σ. The tension values, obtained by the method described

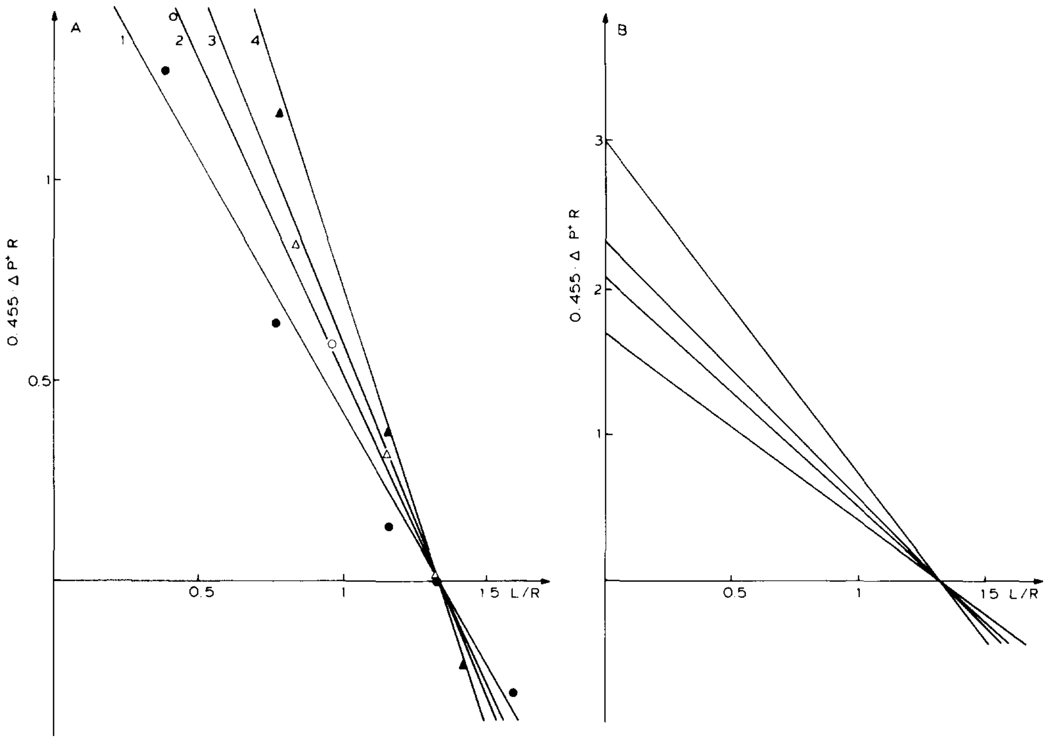

Fig. 5 (a, b). The dependence of the hydrostatic pressure causing fission of the tube on its length. ●, azolectin in decane; ○, azolectin in squalene; ▲, PE in decane; △, PE in squalene. The experimental points well fit the theoretical curves for the corresponding tension values. At $L/R = 1.325$, the ΔP^* equals zero for all membranes. The segments intercepted by the curves on the Y-axis are equal in the chosen coordinate system to the tension of lipid bilayers.

above and by the method used in Ref. 11 for membranes of azolectin in *n*-decane, turned out to be sufficiently similar: 1.7 ± 0.2 and 2.2 ± 0.5 dyn/cm.

Although the proposed method of measuring the tension of bilayers involves the use of a special cell, it has some advantages. The possibility of realization of the fusion-fission cycle enables one to make measurements repeatedly and average them on one 'pair' of membranes. The accuracy of the method is approx. 10%. Based on the coincidence of the points of intersection with the x-axis for the curves obtained on membranes of different composition, one can measure the ΔP^* at only one fixed L and calculate the σ directly from Eqn. 8. The important advantage of the method proposed in our paper is the possibility of measuring the σ for solvent-free membranes. The most accurate familiar method of determining the tension of

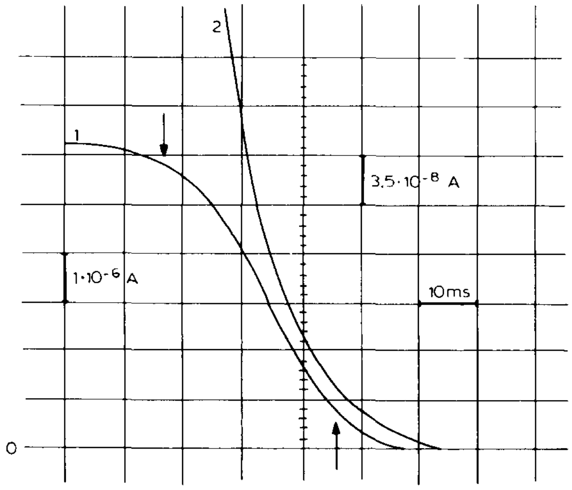

Fig. 6. Oscillograms of current along the axis of the membrane tube in the process of collapse, taken with different sensitivity (curves 1 and 2).

bilayers, based on the measurement of contact angles in the boundary zone between the bulk phase (lens or meniscus) and the bilayer [12], presupposes the presence of a solvent in the bilayer while the abovementioned method used in Ref. 11 gives in the case of squalene membranes a large spread of values because of the irregular shape of the meniscus (in the upper part of the squeezed-out membrane, the whole of excess squalene is accumulated in the form of a drop).

The described phenomenon of fission of the membrane tube, which occurs stepwise when the radius of its narrowest section reaches a certain value ($r = 0.553R$), may also have biological analogues. In particular, it may be anticipated that similar processes are realized during the cell division and in such rearrangements of biological membranes as pinocytosis, erythropoiesis and the like, as well as when a membrane vesicle is obtained using the method of Neher and co-workers [13].

Acknowledgements

The authors wish to thank Yu.A. Chizmadzhev and I.G. Abidor for encouragement throughout and for valuable advice and comments. We are also grateful to L.M. Chailakhyan and V.N. Churayev for helpful discussions and to A.A. Pereverzev for technical assistance.

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