On the Theory of Membrane Fusion.
The Adhesion-Condensation Mechanism

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Abstract. The primary act of the adhesion-condensation mechanism underlying membrane fusion is considered. This act involves the formation of a close dehydrated contact between membranes and the subsequent crystallization of molecules of the external monolayers in the contact region. Crystallization associated with a decrease in the area per molecule gives rise to elastic stresses which cause a disruption of the external monolayer of the membrane in the contact region. This disruption results in the formation of a trilaminar structure (a monolayer fusion occurs). It has been shown that for the formation of a trilaminar structure between liposomes with a radius of 20 nm the contact area must be at least 22 % of that external monolayer. Moreover, the membrane has to overcome an energy barriers; according to estimates for 20 nm liposomes, the maximum value of the barrier is approximately 20 kT. The height of the disruption barrier decreases with increasing area of the contact region. Estimates have been obtained for the minimum area of a "hole" in the contracting monolayers, which arises from their disruption for 20 nm liposomes, this area is approximately 2×10^2 nm².

The developed theory explains the data obtained by the Papahadjopoulos group in their experiments on the fusion of phosphatidylserine liposomes (Portis et al. 1979; Wilshut et al. 1980; Düzgünes et al. 1981); in addition, it enables the description of the mechanism underlying the disruption of a liposome as a result of expansion of its membrane. The process of disruption was studied by Kwok and Evans (1981).

Key words: Membrane fusion — Adhesion mechanisms — Condensation mechanism

Introduction

In previous papers (Markin and Kozlov 1983a; Kozlov and Markin 1983) a classification of the membrane fusion mechanisms has been introduced based on the primary act which the structural rearrangement of bilayers originates from. The primary act characterizes the fusion of both biological and artificial membranes,

and it does not include the removal of various proteins from the contact region of membranes, which occurs at earlier stages of cell fusion (Lucy 1978; Markin 1981).

Two main fusion mechanisms have been distinguished: the stalk mechanism, which starts with the formation of a bridge between the interacting membranes and the adhesion mechanism at the initial stage of which a close contact between the membranes is established. In the latter case a partial interpenetration of the membranes can not be ruled out. The adhesion mechanism includes adhesion-micellar and adhesion-condensation mechanisms, according to the way of transformation of the membrane structure following the establishment of a contact.

The adhesion-micellar mechanism involves the formation in the contact region of inverted micelles which disrupt the integrity of the interacting membranes and lead to fusion (Markin and Kozlov 1983a; Kozlov and Markin 1983; Cullis et al. 1980).

The adhesion-condensation mechanism is based on phase transition from liquid to the crystalline state of lipid molecules in the contact region of the external monolayers of the interacting membranes. The transition from the liquid to the crystalline state is accompanied by a decrease in the area per molecule, i. e. it actually is a condensation of molecules (Chapman et al. 1967).

Crystallization of molecules of the external monolayer may cause stresses leading to disruption of the external monolayers of the contacting liposomes. As a result, the internal volumes of liposomes become separated by a single bilayer, i.e. a trilaminar structure is formed (in other words a monolayer fusion occurs) (Markin and Kozlov 1983a; Kozlov and Markin 1983). Further evolution of the trilaminar structure may lead to disruption of the bilayer separating the internal volumes of cells (liposomes), i.e. to complete fusion.

The possible existence of the adhesion-condensation mechanism of fusion has been indicated by results obtained in recent years by the Papahadjopoulos group in experiments on fusion of negatively charged phosphatidylserine (PS) liposomes under the action of calcium ions.

These studies yielded following main results:

(1) Fusion of PS liposomes occurs only if Ca^{2+} cations are present in the liposome bathing solution;

(2) at low Ca^{2+} concentration (1 mmol/l) no fusion occurs; the fusion of small liposomes with a diameter of about 25 nm requires concentrations of Ca^{2+} of minimum 1.2×10^{-3} mol . l^{-1} and the fusion of large liposomes (about 100 nm in diameter) minimum 2.4×10^{-3} mol . l^{-1}, the ratio of adsorbed Ca^{2+} ions to PS molecule number being close to $1:2$;

(3) in a system of PS liposomes fusion is accompanied by aggregation of PS liposomes, liberation of heat due to crystallization of lipid molecules, release of the liposome content and by the formation of structures called cochlear cylinders;

(4) the most rapid processes are aggregation, liberation of heat and fusion all of

them occuring approximately within the same time interval; release of the liposome contents and the formation of cochlear cylinders both occur with a delay;

(5) in the presence of Ca^{2+} ions the membrane contact region is practically entirely dehydrated whereas in the presence of Mg^{2+} ions or in absence of divalent cations there is a considerable amount of water between the lamellae;

(6) in the presence of calcium, the lamellar distance corresponding to the distance between the centers of the contacting bilayers is by 0.7 nm smaller than the bilayer thickness measured separately;

(7) in the presence of Ca^{2+} ions the phase transition temperature T_C is by more than 100° higher as compared to that observed in the absence of divalent cations.

The above authors have put forward a hypothesis claiming that each Ca^{2+} ion forms a "trans" -complex with two PS molecules of the adjacent membranes; as a result, a close contact is established involving no molecules of water; it is these "trans" - complexes which are believed by the authors to upset the stability of membranes, thereby promoting their fusion. In a previous paper (Markin and Kozlov 1983b) it has been showed that if a close dehydrated contact is formed between charged PS liposomes in the presence of calcium, the phase transition temperature in the contact region must exceed by more than 160° the transition temperature in a membrane contacting electrolyte solution. This results from the fact that charges of the external monolayer with adsorbed Ca^{2+} ions form a lattice of alternating opposite charges of the chess-board type, which tends to be compressed. In the region of the dehydrated contact with a low dielectric constant the electric attraction between molecules turns out to be very strong, this leading to a considerable increase in the transition temperature as compared to that in the remaining part of the bilayer. In this connection, it may be assumed that if initially the membranes of PS liposomes were in the liquid state at too high temperature then, after the formation of a dehydrated contact between them in the presence of calcium, the molecules of the external monolayers sited in the contact region will go over into the crystalline state.

A theory of stresses and ruptures in a membrane as a result of molecule crystallization in the external monolayer is developed below. Based on the theory, we shall explain the data obtained by the Papahadjopoulos group in experiments on fusion of PS liposomes (Portis et al. 1979; Wilshut et al. 1980; Düzgünes et al. 1981). We shall show in particular that stresses arising from crystallization of molecules in the contact region can result in membrane disruption in this region, triggering the adhesion-condensation mechanism of membrane fusion.

Formulation of problem. Let us consider two unilamellar liposomes composed of negatively charged molecules. Let us assume that the external monolayers of both liposomes are neutralized by calcium ions and are in mutual contact, the contact region including $2N^c$ molecules (N^c from each of the interacting monolayers) being

Fig. 1. Two liposomes in contact. The heads of the contacting negatively charged membranes in the presence of Ca^{2+} form a chess-board-type lattice of alternating charges.

fully dehydrated (Fig. 1). In accordance with the results of Markin and Kozlov (1983b) we shall suppose that $2N^c$ molecules in the contact region have all gone over to the crystaline state while the remaining molecules of both membranes have remained in the liquid state.

Let us assume for the sake of simplicity that all the contacting liposomes have the same radii, and we shall refer all subsequent reasoning to one of the liposomes (without any loss of generality).

We shall suppose, that an equilibrium area b_λ or b_γ is characteristic of an undeformed lipid molecule in the liquid and in the crystalline state, respectively, whereby $b_\lambda > b_\gamma$. If the actual area per molecule, a, differs from that under equilibrium, then respective strain energy per molecule in the liquid and in the crystalline state is

$$w_\lambda = \Gamma \frac{(a_\lambda - b_\lambda)^2}{b_\lambda}; \quad w_\gamma = \Gamma \frac{(a_\gamma - b_\gamma)^2}{b_\gamma} \tag{1}$$

where a_λ and a_γ are the actual areas per molecule in the liquid and crystalline state, respectively and Γ is the modulus of elasticity, the value of which for artificial bilayer membranes is close to 5×10^{-2} N/m (Israelachvili et al. 1977) (the values of the equilibrium areas for dipalmitoyllecithin are $b_\lambda \approx 0.65$ nm^2 and $b_\gamma \approx 0.48$ nm^2 (Traüble and Eibl 1974)).

The areas of the monolayers of a closed liposome cannot change independently. It is easy to consider the case of liposomes of an arbitrary radius; however, to avoid tedious expressions we shall assume that the radii of the liposomes are well larger than the membrane thickness and, hence, it may be inferred that both the areas of the monolayers and the corresponding total numbers of molecules in the monolayers are equal. Let us denote the number of molecules in one monolayer N. If the
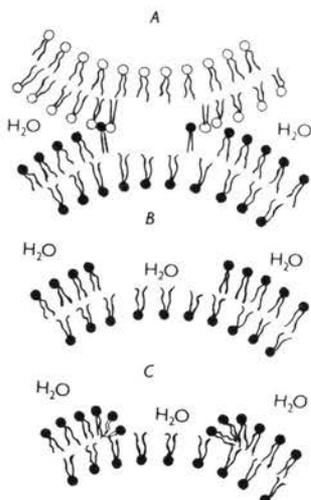
Fig. 2. Disruption of the external monolayer. A: A "hole" in the contact region of the membranes. B: A hypothetical "hole" outside the contact region of the membranes; the "hole" edges are hydrophobic. C: A hypothetical "hole" outside the contact region of the membranes; the "hole" edges are covered with polar heads.

membrane molecules are all in the same phase state, the areas of the monolayers equal the equilibrium areas and the bilayer is not stressed. In our case, N^c molecules of the external monolayer are in the crystalline state and all the remaining membrane molecules are in the liquid state; thus the equilibrium area of the external monolayer is smaller than that of the internal one. In view of the equality of the actual areas of the monolayers the external monolayer becomes expanded relative to its equilibrium area while the internal one is compressed. This may lead to disruption of the external monolayer, occuring in both the contact region and the part of the membrane, adjoining the electrolyte solution (Fig. 2A, 2B). We shall determine conditions under which disruption of either type may occur.

Basic equations. To the thermodynamic equilibrium of the liposome at a constant pressure, P, and a constant temperature, T, there corresponds a minimum of its Gibbs free energy, Φ (Landau and Lifshitz 1976). Therefore, to solve the above problem the knowledge of conditions under which disruption of the external monolayer causes Φ to decrease, is essential.

The expression for Φ can be written as

$$\Phi = N^c \left(\varepsilon_\gamma^c - Ts_\gamma^c + Pv_\gamma^c \right) +$$

$$+ (N - N^c)(\varepsilon_\lambda^o - Ts_\lambda^o + Pv_\lambda^o) + N \left(\varepsilon_\lambda^i - Ts_\lambda^i + Pv_\lambda^i \right) + U(A), \qquad (2)$$

where $\varepsilon_\gamma^c, v_\gamma^c$ and s_γ^c are the energy, volume and entropy per molecule in the contact region of the membranes respectively; $\varepsilon_\lambda^o, v_\lambda^o$ and s_λ^o are the corresponding variables for molecules sited in the external monolayer outside the contact region; and ε_λ^i, v_λ^i and s_λ^i are variables characterizing molecules in the internal monolayer.

The variables $\varepsilon_\gamma^c, \varepsilon_\lambda^o$ and ε_λ^i include all kinds of energy, namely, elastic energy (1), which changes with varying area per molecule; electric energy of the interaction of molecules; and the energy per one neutralized molecule in its underformed state. $U(A)$ is the energy of a "hole" of the area A, which arises from disruption of the external membrane monolayer. Further we shall suppose that the "hole" is circular, corresponding to the minimum of its energy (the Gibbs free energy of both contacting liposomes is obtained by doubling the right-hand member of Eq.2).

As for the entropy per molecule, we shall assume it to be independent of the area per molecule, a (provided that the shift of a from the equilibrium area b is not too extensive); the entropy per molecule in the liquid and crystalline state is $s_{\lambda 0}$ and $s_{\gamma 0}$, respectively.

At a pressure $P = 1$ atm the dependence of the volume of a molecule on its area can be neglected because the corresponding contribution to the Gibbs free energy is small compared with the elastic energy (Markin and Kozlov 1983b).

Finally, according to the results of Markin and Kozlov (1983b) there is a strong mutual attraction between the molecules sited in the region of dehydrated contact in the presence of adsorbed calcium. As a result, a molecule within the contact region reaches the limit of deformability; this means that changes in the elastic energy, arising from deformations of the molecules, increase much more drastically than it might be expected from (1). As far as the molecules outside the contact region are concerned, their areas are close to those equilibrium (provided the area of the contact region is much smaller than that of the monolayer); changes in the elastic energy are defined by formula (1). It follows that in deformations of the membrane due to the formation of a "hole" the change in the energy per molecule in the contact regions is less than that of the remaining molecules. Therefore, the area per molecule in the contact region will further be assumed to be invariable and its value will be taken for estimates.

In the above assumptions the Gibbs free energy (2) of the liposome is written as

$$\Phi = (N - N^c)\,\varepsilon_\lambda^o\,(a_\lambda^o) + N\varepsilon_\lambda^i\,(a_\lambda^i) + U(A) + \text{const} \qquad (3)$$

Here, $\varepsilon_\lambda^o\,(a_\lambda^o)$ and $\varepsilon_\lambda^i\,(a_\lambda^i)$ are the energies per molecule in the external (with the exception of the contact region) and the internal monolayer as functions of the area per the molecule (respective), const incorporates all terms independent of the areas of the monolayers and remaining unchanged with the varying area of the "hole".

It follows from Eq. (3) that the parameters defining Φ are a_λ^o, a_λ^i and A. An additional condition of the approximate equality of the areas of the monolayers is imposed on these parameters:

$$N^c b_\gamma + (N - N^c)\, a_\lambda^o + A = N a_\lambda^i \tag{4}$$

Eq. (4) indicates that only two of the three parameters mentioned above are independent.

We shall suppose that it is the elastic energy (1) that is the main contribution to changes in $\varepsilon_\lambda^o(a_\lambda^o)$ and $\varepsilon_\lambda^i(a_\lambda^i)$ as a result of deformation. Here, we shall neglect changes in the electric energy of molecules which according to the estimates of Markin and Kozlov (1983b) are much smaller than changes in the elastic energy.

Disruption in the liposome contact region. Let us consider under what conditions the external monolayers of the liposomes may disrupt in the region of their contact (Fig. 2A). What is the energy of a "hole" formed as a result of such a disruption? The contact is dehydrated and the intact monolayers limiting the internal volumes of the liposomes protect the "hole" against the penetration of water; thus no energetically disadvantageous contact of the hydrophobic parts of the membrane with water as a result of the disruption may occur. The "hole" has an excessive energy from a different reason. As mentioned above, the polar heads of the external monolayer of the liposome with adsorbed Ca^{2+} ions form a lattice of alternating opposite charges (or the chess-board type) (Fig. 1). The molecules forming the lattice are effectively attracted to each other (Markin and Kozlov 1983b). As a result of disruption of the monolayer, some of the molecules become sited at the edge of the "hole". A calculation using the modified cut-off disc method (Cullis et al. 1980) shows that the electric energy of a molecule sited deep in the lattice is smaller than that of a molecule at the edge of the "hole" (Appendix A). The excessive electric energy per molecule at the edge of the "hole" is

$$w = 7 \times 10^{-3}\, \frac{e_o^2}{\varepsilon \varepsilon_o}\, \frac{1}{\sqrt{b_\gamma}} \tag{5}$$

Here, e_o is the charge of the polar head, ε is the dielectric constant of the medium surrounding the polar heads and ε_o is the dielectric permeability of the vacuum. The Gibbs free energy of one liposome (3), taking into account Eqs. (5) and (1), is

$$\Phi = \Gamma\,(N - N^c)\,\frac{(a_\lambda^o - b_\lambda)^2}{b_\lambda} + \Gamma N\,\frac{(a_\lambda^i - b_\lambda)^2}{b_\lambda} + \beta \sqrt{A} + \text{const} \tag{6}$$

where $\beta \equiv 10^{-2}\, \dfrac{e_o^2}{\varepsilon \varepsilon_o}\, \dfrac{1}{b_\gamma}$; and A is the area of the hole.

The first terms on the right side of Eq. (6) represent the membrane elastic energy which, as shall be shown does not disappear even if the membrane ruptures (the "hole" area $A \neq 0$). The third term is the "hole" energy per liposome, determined by the appearance of an excessive electric energy in the molecules sited at the edge of the "hole". This term determines the linear tension of the "hole" perimeter. It follows from Eq. 6 that the magnitude of the linear tension of the hole is a function

of the dielectric constant ε of the medium surrounding the polar heads. Further estimates will show that if a dehydrated contact with a low ε is established, the linear tension is large enough to counteract membrane disruption.

The constant in Eq. (6) incorporates all contributions to Φ, which remain unchanged with varying A.

Let us consider how the Gibbs free energy Φ depends on the "hole" area A. It should however be stressed that at a fixed value of A the areas per lipid molecule outside the contact region, a_λ^o and a_λ^i, must satisfy the condition of mechanical equilibrium, which requires the occurrence of a minimum Φ, i.e. $\dfrac{\partial \Phi}{\partial a_\lambda^o}=0$ and $\dfrac{\partial \Phi}{\partial a_\lambda^i}=0$. These conditions in conjunction with Eq. (4), which determines the relationship between the areas of the monolayers, lead to following expressions for a_λ^o and a_λ^i

$$a_\lambda^o = \frac{2Nb_\lambda - N^c b_\gamma - A}{2N - N^c},\tag{7}$$

and

$$a_\lambda^i = \frac{2\,(N - N^c)b_\lambda + N^c b_\gamma + A}{2N - N^c}\tag{8}$$

The substitution of Eqs. (7) and (8) into Eq. (6) yields an expression for the Gibbs free energy

$$\Phi = \Gamma\,\frac{(2N - N^c)}{b_\lambda}\left[\frac{\dfrac{N^c}{N}\,(b_\lambda - b_\gamma) - \dfrac{A}{N}}{2 - \dfrac{N^c}{N}}\right]^2 + \beta\sqrt{A} + \text{const}\tag{9}$$

An analysis of expressions (7) and (8) indicates that, with the appearance of a "hole", the area per molecule in the external monolayer decreases and that in the internal monolayer increases, both of them approximating their equilibrium values. This results in a decrease in the membrane elastic energy represented by the first term in Eq. (9). However, a "hole" energy given by the second term in Eq. (9) appears. These two trends compete; as a result the disruption of the external monolayer can be energetically gainful under certain conditions only.

So far, the "hole" area A has been assumed to be fixed. In order to extablish the conditions of disruption we shall investigate the dependence of the Gibbs free energy Φ on A. This dependence (for a doubled value of Φ, corresponding to the Gibbs free energy of both liposomes) is shown in Fig. 3 (curves 1—4).

The parameter characterizing the curves in Fig. 3 is the area of the membranes contact region (the number of lipid molecules is N^c) in each of the liposomes in the
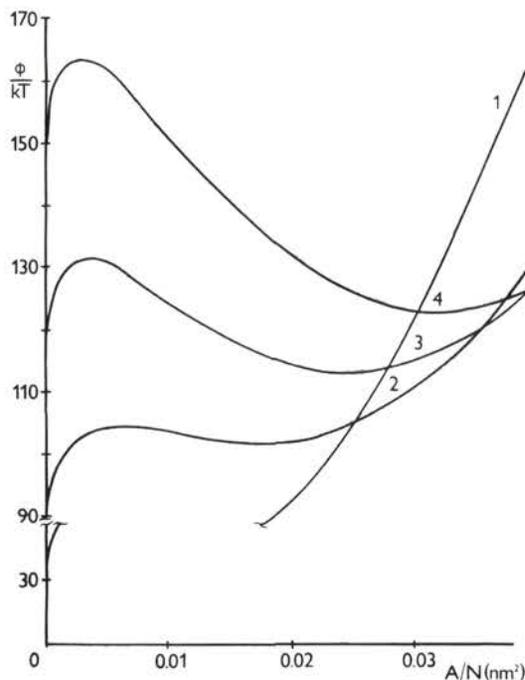
Fig. 3. The dependence of the Gibbs free energy of the liposome on the "hole" area in the contact region of the external monolayer. Value of Φ is substracted from the value corresponding to $A = 0$ and the zero contact area of the membranes. Abscissa: the contact area of the liposomes (the number of molecules sited in the contact region). Eq. (9) was used to obtain values $b_\lambda = 0.65$ nm^2; $b_\gamma = 0.48$ nm^2; and $N = 7700$. $1: N^c/N = 0.1$. $2: N^c/N = 0.19$. $3: N^c/N = 0.22$. $4: N^c/N = 0.25$.

contact region. The curve $\Phi(A)$ can increase monotonically (Fig. 3, curve 1). This means that, with the formation of a "hole", the appearance of excessive energy of the membrane cannot be compensated for by a decrease in the elastic energy: thus the disruption is thermodynamically not gainful. If the contact area of the membranes is sufficiently large the dependence $\Phi(A)$ may reach a minimum (Fig. 3, curves (2—4)).

An analysis of expression (9) shows that the minimum contact area at which a minimum of the Gibbs free energy appears for the first time includes $\tilde{N}^c$ molecules of the external monolayer in each of the liposomes; this is determined to within $(\tilde{N}^c/2N)^3$ from the formula

$$
\frac{N^c}{N} = 3 \frac{\left(\dfrac{\beta}{4\Gamma \sqrt{b_\lambda N}}\right)^{2/3}}{\dfrac{b_\lambda - b_\gamma}{b_\lambda} + \left(\dfrac{\beta}{4\Gamma \sqrt{b_\lambda N}}\right)^{2/3}}
\tag{10}
$$

Taking into account the above values of variables in Eq. (10) for the 20 nm liposome we obtain $\tilde{N}^c/N \approx 0.18$. If the value of the Gibbs free energy is greater at the minimum than that at the point $A = 0$ (Fig. 3, curve 2) the appearance of a "hole" is not gainful. Otherwise, if Φ is less at the minimum than or equal to its value at the point $A = 0$ (Fig. 3, curves 3—4), disruption may occur, since in such a situation, the total effect due to both decrease in elastic stresses and the appearance of a "hole" leads to a decrease in the Gibbs free energy. Thus, the condition of disruption of the external monolayer is the extention of the contact area (or of the number of molecules of each of the liposomes in the contact region N^{c*}) to a value at which the equality of the values of the Gibbs free energy Φ at the minimum point and at the point $A = 0$ begins to hold.

With a further increase in the area of the contact region the Φ value becomes smaller at the minimum point than that at the point $A = 0$ (Fig. 3, curve 4). This means that the appearance of a "hole" becomes energetically even more gainful.

The number N^{c*}, at which disruption may occur and the area A^* of a "hole" arising in the external monolayer can be found using following equations obtained from requirements $\partial \Phi / \partial A = 0$ and $\Phi(A^*) = \Phi(0)$

$$\frac{N^c (b_\lambda - b_\gamma) - A}{2N - N^c} - \frac{\beta b_\lambda}{4\Gamma} \frac{1}{\sqrt{A}} = 0, \tag{11}$$

$$\frac{2N^c (b_\lambda - b_\gamma) - A}{2N - N^c} - \frac{\beta b_\lambda}{\Gamma} \frac{1}{\sqrt{A}} = 0. \tag{12}$$

Assuming that $N^c \ll 2N$, the solution to Eqs. (11) and (12) gives to within $(N^c/2N)^3$:

$$\frac{N^{c*}}{N} = \frac{3}{2} \frac{\left(\frac{\beta}{\Gamma \sqrt{N b_\lambda}}\right)^{2/3}}{\frac{b_\lambda - b_\gamma}{b_\lambda} + \frac{1}{2} \left(\frac{\beta}{\Gamma \sqrt{N b_\lambda}}\right)^{2/3}} \tag{13}$$

$$A^* = \left[\frac{\beta b_\lambda N}{\Gamma} \cdot \frac{1 - \frac{1}{4} \frac{b_\lambda}{b_\lambda - b_\gamma} \left(\frac{\beta}{\Gamma \sqrt{N b_\lambda}}\right)^{2/3}}{1 + \frac{1}{2} \frac{b_\lambda}{b_\lambda - b_\gamma} \left(\frac{\beta}{\Gamma \sqrt{N b_\lambda}}\right)^{2/3}} \right]^{2/3} \tag{14}$$

Considering the 20 nm liposome and assuming $\varepsilon = 2$, $b_\lambda = 0.65$ nm² and $b_\gamma = 0.48$ nm², the second term in the denominators of expressions (13) and (14) and in the numerator of Eq. (14) can be neglected and these expressions can be rewritten with the exception of a small percentage as

$$A^* = \left(\frac{\beta b_\lambda N}{\Gamma}\right)^{2/3}, \tag{15}$$
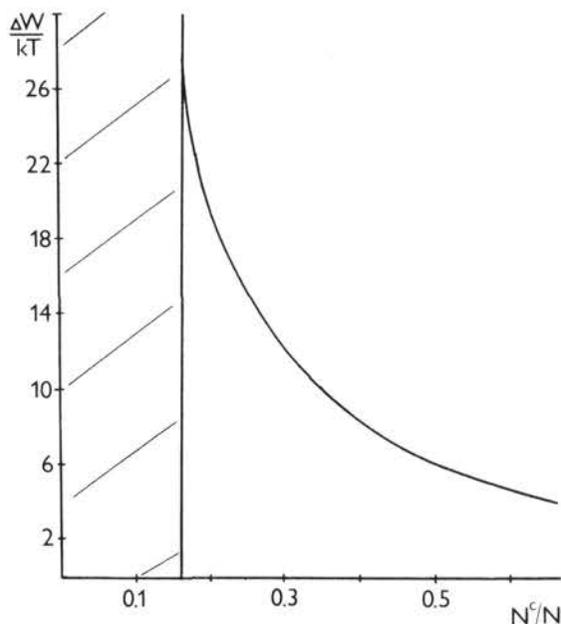
Fig. 4. The dependence of the energy barrier value on the contact region area for a disruption of the external monolayers in the contact region of the liposomes. The plot was constructed for to occur values $b_\lambda = 0.65$ nm^2; $b_\gamma = 0.48$ nm^2; $N = 7700$; and $\Gamma = 5 \times 10^{-2}$ N/m. In the shaded region there is no barried as such since a monotonic increase in energy as a function of the "hole" area corresponds this region. The asterisk denotes the contact region at which disruption is in principle possible $\Phi(A^*) = \Phi(0)$.

$$N^{c*} = \frac{3}{2}N \left(\frac{\beta}{\Gamma \sqrt{b_\lambda N}} \right)^{2/3} \frac{b_\lambda}{b_\lambda - b_\gamma} \tag{16}$$

Estimates by Eqs. (15) and (16) yield $N^{c*}/N \approx 0.22$ and $A^* \approx 2 \times 10^2$ nm^2. This means that disruption in the contact region becomes possible as soon as the contact area has covered more than 22 % of the monolayer molecules; in this case, the minimum area of the "hole" formed is approximately equal to the area per 300 lipid molecules.

It is evident from Fig. 3 (curves 2—4) that for a "hole" to be formed, the membranes have to overcome an energy barrier associated with the linear tension of the "hole" perimeter. The value of the barrier can be analytically calculated only for the case $\Phi(A^*) = \Phi(0)$:

$$\Delta W = \frac{N}{b_\lambda} \left(\frac{5 \times 10^{-3}}{\sqrt{N}} \frac{1}{\Gamma} \frac{e_o^2}{\varepsilon \varepsilon_o} \frac{b_\lambda}{b_\gamma} \right)^{4/3} \tag{17}$$

Taking into account the above values of the variables in Eq. (17) an estimate gives $\Delta W \approx 40kT$. With increasing contact area the value of the barrier decreases (Fig. 4). However, the barrier cannot disappear entirely what the tact area extent might be: this follows from the existence of the linear tension of the hole perimeter.

Let us estimate the characteristic time within which the external monolayer in the contact region may disrupt if the area of this region equals, say, 22 % of the membrane area. We shall make use of expression for the rate of formation of pores in a membrane, as described in Weaver and Mintzer (1981);

$$v = v_o \, V_B \exp\left(-\frac{\Delta W}{kT}\right) \tag{18}$$

where V_B is the total volume of the lipid molecules in the membrane contact region; and v_o is the characteristic frequency $(2 \times 10^{38} \, s^{-1} \, m^{-3})$. For the 20nm liposomes an estimate of the characteristic time for the formation of a trilaminar structure, $\tau = v^{-1}$, considering that the energy barrier in the case considered is 40 kT, gives $\tau \approx 2 \times 10^{-6}$ s. This means that the energy barrier does not limit the process of formation of a trilaminar structure, and the main condition is that the contact area of the liposomes amounts to 22 % of the membrane area (for the 20 nm liposomes).

Discussion

The above theory considered the possibility of disruption of the external monolayer of a negatively charged liposome membrane in contact with another liposome in the presence of Ca^{2+} ions. The lipid molecules in the contact region go over from the liquid to the crystalline state (12) and become compressed; as a result, the membrane develops elastic stresses which lead to disruption of the external monolayer in the contact region of the liposomes (Fig. 2).

Expression (13) gives the minimum area of the contact region at which disruption can occur. An estimate by Eq. (13) for liposomes of radius 20 nm has shown that a rupture in the contact region can occur if the area of the contact region constitutes approximately a fifth part of the total area of the external monolayer.

Expression (14) has been constructed for the minimum area of "holes" arising from the disruption of the external monolayer. Estimates have shown that if the liposome radius is 20 nm the minimum area of a "hole" is approximately $2 \times 10^2 \, nm^2$ as a result of a rupture in the contact region.

Finally, expression (17) has been obtained for the energy barrier which the liposome has to overcome for a rupture to occur when the formation of a "hole" becomes energetically gainful. The value of the barrier for disruption in the contact region was found to be approximately 20 kT.

An estimate of the characteristic time for the formation of a "hole", τ at the
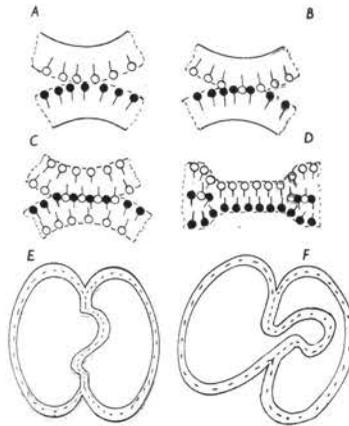
Fig. 5. The primary act of membrane fusion A—C: the formation and growth of the contact region (crystalline "spot"); D: disruption of the external monolayer and the formation of a trilaminar structure; E: a hypothetical disruption of the trilaminar structure to the occurrence of an excessive area of the internal monolayers during the expansion of the contact region; F: a hypothetical disruption of the trilaminar structure due to the considerable bending of the monolayer separating the internal volumes of the liposomes.

above value of the energy barrier has shown that $\tau \approx 2 \times 10^{-6}$ s, i.e. the layer must disrupt practically instantaneously as soon as the required conditions are met.

An increase in the contact area leads to a decrease in the energy barrier and, hence, to a decrease in the time for the formation of a "hole".

The above theory accounts for the experimental results of the Papahadjopoulos group (Portis et al. 1979; Düzgünes et al. 1981; Wilshut et al. 1980) mentioned in Introduction. Since the most important phenomenon studied in these works has been membrane fusion we shall start with the description of a possible mechanism underlying this process. The primary act of the adhesion-condensation mechanism of fusion under consideration is the establishment of a dehydrated contact between the membranes; this may apparently occur only in the presence of Ca^{2+} ions. However, establishing of the contact alone, is insufficient; for the fusion to take place, the integrity of the bilayer must be disrupted (Markin and Kozlov 1983a; Kozlov and Markin 1983). Here, the second aspect of the action of Ca^{2+} ions comes to expression. Being adsorbed by charges of the lipid molecules and neutralizing the total membrane charge, they form a lattice of alternating charges of the chess-board type. The heads of lipid molecules are strongly attracted to each other in a nonpolar medium represented by the dehydrated contact of the membranes. As a result, the phase transition temperature of the lipid in the contact region is by more than 160° higher than that in the remaining parts of the membrane (Markin

and Kozlov 1983b) i.e. according to the data of Newton et al. (1978), approximately 8°C for charged and 18°C for neutralized membrane. Hence, at temperatures of about 20° to 40°C, the external monolayer molecules in the contact region can go over to the crystalline state, whereas other molecules of the bilayer remain in their liquid state. This gives rise to stresses in the external monolayer of the liposome, leading to its disruption.

However, the disrupturing does not occur immediately. First, a small contact region is formed and molecules in this region began crystallizing, a crystalline "spot" appears in the external monolayer of each of the interacting liposomes. This process should be experimentally manifested as adhesion of the liposomes and start of phase transition. An analysis has indicated that the membranes polar groups of which form a lattice of alternating charges are electrically attracted to each other (Appendix B.) At small intermembrane distances, the attraction becomes very strong (B2), and the contact between the membranes must practically be irreversible. After a contact had been formed and a crystalline "spot" had appeared in the external monolayer, the events further proceed as follows. The parts of the membranes, adjacent to the contact region, are mutually attracted due to the interaction between the lattices of alternating charges (Appendix B). Due to this, the contact region becomes enlarged (Fig. 5B, C), i.e. it acts as a sort of a "zipper" "clasping" the liposomes to each other. When the contact area reaches approximately a fifth part of the area of the external monolayer, the external monolayers of the interacting membranes disrupt, the material from the contact region, is partially removed and a trilaminar structure is formed between the liposomes (Fig. 5); in other words, the internal volumes of the liposomes become separated by a simple instead of two bilayers.

Thus, in the above system of negatively charged liposomes the primary act of fusion (by the adhesion-condensation mechanism), may start in the presence of Ca^{2+}. In this case the primary act is completed by the formation of a trilaminar structure (Markin and Kozlov 1983a) or, put another way, by a monolayer fusion (Fig. 5D). Physical processes which occur at the subsequent stages of fusion leading to mixing of the contents of the liposomes, i.e. to complete fusion have not been considered in the present paper. We shall formulate several hypotheses on the nature of these processes. Further enlargement of the contact region should lead to an appreciable distorsion of the liposome shape and give rise to some excessive area of the internal monolayers, which in turn may cause disruption of the bilayer separating the internal volumes (Fig. 5E). Another possible variant is that the trilaminar structure becomes disrupted at later stages of the zipper clasping when in virtue of the geometric limitations the edges of the "hole" become overlapped and the bilayer separating the internal volumes has to be strongly deformed (Fig. 5F). Separate communications will be devoted to a theoretical analysis of stages following the primary act.

Efflux of the liposome contents. The Papahadjopoulos group (Portis et al. 1979; Düzgünes et al. 1981; Wilshut et al. 1980) has noted that, even at the initial stages of the process of interaction between PS liposomes, with the aggregation and fusion playing dominant role, an efflux of the liposome contents may be observed, although to a small degree. This suggests that, along with a rupture within the contact region, resulting in membrane fusion, in some cases a rupture occurs outside the contact region; as a result, the liposome contents is released into the external solution. It may be assumed that a rupture of this type has similar underlying mechanism as that occurring within the contact region (Appendix C). First, the external monolayer outside the contact region becomes disrupted, giving rise to a hole with a hydrophobic "bottom" and a perimeter covered with polar heads due to tilting of the lipid molecules (Fig. 2C). To the energy of the "hole bottom" corresponds to the surface tension σ, and the energy of the perimeter corresponds to the linear tension γ. Further evolution of such a "hole" should consist in disruption of the second monolayer of the membrane; in this case, the membrane hydrophobic part loses contact with the solution and the liposome content is released. In Appendix C, an expression shall be given for the area of the contact region of the membranes, required for the disruption outside this region to occur. An estimate using this expression and assuming the surface tension of the "hole", σ to be the same as that at the heptane-water interface, $\sigma \approx 5 \times 10^{-2}$ N/m (Adamson 1975) and the linear tension per monolayer $\gamma \approx 5 \times 10^{-12}$ N (Helfrich 1974; Litster 1975) shows that no disruption of the external monolayer of the given type will occur outside the contact region even if the contact region extends over entire external monolayer. For such a disruption to occur a relation $\sigma \leqslant 2 \times 10^{-2}$ N/m should in principle hold.

In the above mechanism stresses are eliminated due to the appearance of a "hole" in the external monolayer; as a result the total area of this monolayer becomes enlarged. However, another variant may be proposed, consisting in the formation of a through pore of a small radius outside the contact region with its perimeter covered with polar heads of lipid molecules; such a pore is termed inverted (Abidor et al. 1979). Material can get through the pore from the internal to the external monolayer, eliminating stresses in the membrane (Fig. 6). Let us estimate what the area of the contact region must be for such a mechanism to be feasible. The inverted pore possesses an energy associated with the tilting of lipid molecules on its perimeter. As follows from the paper by Kozlov and Markin (1983) the energy of the pore is

$$W = 2\pi D \left\{ 2 \frac{(\varrho + 2)^2}{\sqrt{(\varrho + 2)^2 - 1}} \; \text{arctg} \sqrt{\frac{\varrho + 3}{\varrho + 1}} - 4 \right\} \tag{19}$$

where $\varrho \equiv 2r/h$; r is the pore radius (the radius of the channel formed by the pore,
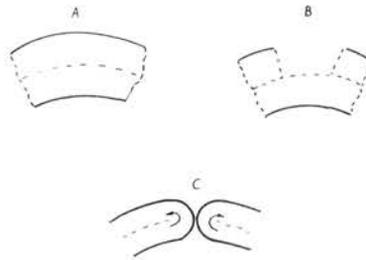
Fig. 6. A hypothetical mechanism of stress elimination in the membrane with the formation of an inverted pore and with the overflowing of the lipid from the internal into the external monolayer. A: the intact membrane; B: the intermediate stage of the process with the membrane hydrophobic part being uncovered C: the formation of an inverted pore and redistribution of the material.

Fig. 6); h is the monolayer thickness; and D is the coefficient of bending rigidity of the monolayer.

Let us assume that the pore has a zero radius, $\varrho = 0$. A pore can arise if its energy is less than or equal to the elastic energy accumulated in the membrane prior to the formation of the pore and elimination of stresses. From Eq. (7) and (19) it is easy to find the form of the question for the equation for the contact area (the number of particles of one membrane, contained in this region, required for the formation of such a pore):

$$\frac{\Gamma}{2} \frac{(N^c)^2}{N} \frac{(b_\lambda - b_\gamma)^2}{b_\lambda} = 5.3\ D \qquad (20)$$

It thus appears that

$$\frac{N^c}{N} = \sqrt{10.6\ \frac{D}{\Gamma N} \cdot \frac{b_\lambda}{(b_\lambda - b_\gamma)^2}} \qquad (21)$$

Eq. (21) includes the coefficient of bending rigidity D. For a bilayer $D \approx 10^{-19}$ J and this coefficient is proportional to the membrane thickness cubed, $D \sim h^3$ (Landau and Lifshitz 1965); thus for a monolayer $D \approx 1.2 \times 10^{-20}$ J. Using the above values of the contants occurring in Eq. (21) we obtain for 20 nm liposomes.

$$\frac{N^c}{N} \approx 0.1$$

According to the estimate, the inverted "pore" eliminating stresses in the membrane becomes gainful if the contact area includes more than 10 % of the membrane area. However, at the intermediate stage of the formation of a "pore", hydrophobic parts of the membrane must be uncovered (Fig. 6) giving rise to an energy barrier

$$\Delta W = 2\pi\gamma_G h + \pi h^2 \sigma - 5.3D, \tag{22}$$

where γ_G is the linear tension of the noninverted perimeter of the pore in the external monolayer at the first stage of the pore formation (Fig. 6). Adamson (1975) reports values of $\gamma_G \approx 10^{-11}$ N. Using the above values of the variables appearing in Eq. (22) we get $\Delta W \approx 1000$ kT. An energy barrier of the extent makes the mechanism under consideration unlikely. The inverted pore may be formed by some other mechanism, independent on: the uncovering of the hydrophobic parts. However this also seems unlikely because such a mechanism should be associated with an additional compression of the internal monolayer.

In conclusion of this section is should be pointed out that the problem of membrane disruption with the liposome contents being related remains obscure and calls for further investigations.

The effect of the liposome size on fusion. Wilshut et al. (1980) have shown that the liposome size plays a role in the process of fusion. Small liposomes fuse more readily than large ones. This is reflected by higher rates of fusion observed in suspensions of small liposomes.

This may be explained, first, by the fact that disruption of the external monolayer leading to fusion, necessitates the establishment of a contact region between the liposomes, with the area of this region being the larger the greater the liposome size. If this stage is limiting, then the characteristic fusion time must also increase with the liposome size.

Moreover, the rate of fusion must be strongly dependent upon the energy barrier ΔW which the membranes have to overcome for a "hole" to be formed in the external monolayer (the rate of the process is proportional to $\exp{(-\Delta W/kT)}$). The value of this barrier, as indicated by Eq. (17) is the higher the larger the liposome size, $\Delta W \sim N^3$; thus the larger the liposome the lower the rate of fusion.

It should be noted that all our calculations have been made under approximation of equality of the areas of both liposome monolayers. A more accurate calculation would not lead to a qualitative modification of the results, namely, the above conclusion concerning an increase of the energy barrier for the occurrence of a disruption with the liposome size, remains valid.

A simplified method of calculating the disruption in the membrane contact region. Another to illustrate the theory in a clear-cut manner we shall show how conditions of the external monolayer disruption can be obtained by a simpler through approximate method. Let us consider a membrane with N^c molecules in the crystalline state in the external monolayer and with the remaining molecules composing the membrane in the liquid state. If there is no "hole" in the external
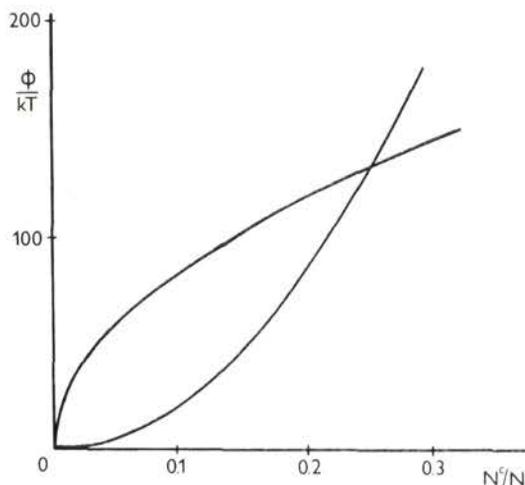
Fig. 7. The dependence of the Gibbs free energy of the liposomes, Φ, on the contact region area (the number of lipid molecules in the contact region). Φ_1: no disruption; Φ_2: disruption of the external monolayer eliminating elastic stresses. $\Gamma = 5 \times 10^{-2}$ N/m; $b_\lambda = 0.65$ nm^2; $b_y = 0.48$ nm^2; $N \approx 7700$; and $\varepsilon = 2$.

monolayer, the membrane is stressed. In this case, the Gibbs free energy of the membrane is

$$\Phi_1 = \Gamma \frac{N^{c2}}{2N} \frac{(b_\lambda - b_y)^2}{b_\lambda} + \text{const} \tag{23}$$

Let us assume that the external monolayer of the membrane has failed to withstand the expansion and disrupted, the area of the "hole" formed being such that elastic stresses in the membrane have totally disappeared. In this case, the Gibbs free energy will be

$$\Phi_2 = \beta \sqrt{b_\lambda - b_y} \sqrt{N^c} + \text{const} \tag{24}$$

Plots of Φ_1 and Φ_2 versus the contact area of the liposomes (the number of particles in the crystalline state N^C) are shown in Fig. 7 (curves 1 and 2). As the contact area increases, the system "shifts" from left to right along the lower curve. First, the system is represented by curve 1, corresponding to the absence of rupture; after a certain contact area has been established, it "passes" over to curve 2, which corresponds to the presence of a "hole". It is easy to find from Eqs. (23) and (24) that the contact area at which disruption becomes gainful comprises $\tilde{N}^c b_y$ where

$$\tilde{N}^c = \left[2N \frac{\beta}{\Gamma} \frac{b_\lambda}{(b_\lambda - b_y)^{3/2}} \right]^{2/3}$$

A numerical estimate for a 20 nm liposome on the condition that $b_\lambda \approx 0.65$ nm^2 and $b_\gamma \approx 0.48$ nm^2 gives $\tilde{N}^c/N \approx 0.25$. This value of the required contact area is somewhat overestimated since we have used an approximate condition of complete elimination of elastic stresses. The more rigorous theory outlined above allows for the fact that elastic stresses are not entirely eliminated even after disruption; the remaining stresses counterbalance the linear tension of the hole.

Application to the problem of liposome lysis. Based on the above theoretical approach the mechanism underlying liposomes lysis may be described as a result of expansion of the membranes, as investigated by Kwok and Evans (1981). Assuming that the lysis is due to the appearance of an inverted pore in the membrane, it may be shown that this process is associated with the membrane overcoming an energy barrier which is the lower the greater the expansion of the membrane. In our future communication we will show that such a mechanism describes well the experimental results obtained by Kwok and Evans (1981).

Appendix A

Calculation of excessive electric energy of a molecule at the edge of a "hole" in the external monolayer

Heads of lipid molecules of a negatively charged membrane with adsorbed Ca^{2+}ions form a lattice of alternating charges of the chess-board type (Markin and Kozlov 1983b). The electric energy of a molecule sited deep in the lattice, can be determined by a modified cut-off disc method (Kozlov and Markin 1982)

$$w_1 = -0.07 \frac{e_o^2}{\varepsilon\varepsilon_o} \frac{1}{\sqrt{b_\gamma}} \tag{A1}$$

where b_γ is the area per molecule; e_o is the elementary charge; ε_o is the dielectric permeability of vacuum; and ε is the dielectric constant of the medium surrounding the polar heads. (Markin and Kozlov 1983b) Expression (A1) indicates that there is attraction between the molecules in this lattice.

A molecule sited at the edge of a "hole" in the lattice possesses an excessive energy as compared to molecules sited deep in the lattice. This is due to the fact that the molecule at the edge of the "hole" has neighbours in only one half-space. To calculate the excessive energy, first, the energy of a particle sited in a linear chain of alternating charges (Fig. 8) must be known:

$$w_2 = -\frac{1}{8\pi} \frac{e_o^2}{\varepsilon\varepsilon_o} \frac{1}{\sqrt{b_\gamma}} \left(-1 + \frac{1}{2} - \frac{1}{3} + \ldots\right) = \frac{\ln 2}{4\pi} \frac{e_o^2}{\varepsilon\varepsilon_o} \frac{1}{\sqrt{b_\gamma}} \tag{A2}$$

$$\oplus \quad \ominus \quad \oplus \quad \ominus \underset{\vdash\sqrt{b_\gamma}\dashv}{\oplus} \ominus \quad \oplus$$
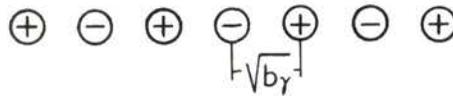
Fig. 8. A linear chain of charges alternating in sign. (see Appendix A).

Hence, the excessive energy of a molecule at the edge of the "hole" is

$$w = \frac{w_2 - w_1}{2} = 7 \times 10^{-3} \frac{e_o^2}{\varepsilon\varepsilon_o} \frac{1}{\sqrt{b_\gamma}}$$

Appendix B

The interaction between two chess-board-type lattices

Let us consider two opposing square lattices with staggered positive and negative charges, equal in modules to e_o. A stable relative position of such lattices is given when the negative charges of one of the lattices face the positive charges of the otherone. The energy of interaction of these lattices per unit area can be calculated by the modified cut-off disc method (Kozlov and Markin 1982)

$$W = -\frac{e_o^2}{4\pi\varepsilon\varepsilon_o\varrho^2} \left(\frac{1}{d} - \frac{4}{\sqrt{d^2 + \varrho^2}} + \frac{4}{\sqrt{d^2 + 4\varrho^2}} \right) -$$

$$-\frac{e_o^2}{4\pi\varepsilon\varepsilon_o\varrho^2} \left(\sqrt{d^2 + \frac{8\varrho^2}{\pi}} - \sqrt{d^2 + \frac{10\varrho^2}{\pi}} \right), \qquad (B1)$$

where d is the distance between the planes of the lattices and ϱ is the distance between neighbouring atoms in the lattices (the lattices are assumed to be identical). Expression (B1) suggests that if the planes of the lattices are at a sufficient distance from each other, $d \gg \varrho$, the interaction energy asymptotically tends to zero, and the strength of interaction also tends to zero (this is natural because each lattice as a whole is neutral). If the lattices are close to each other, $d < \varrho$, the interaction energy per unit area will be

$$W = -\frac{q^2}{4\pi\varepsilon\varepsilon_o\varrho^2} \frac{1}{d}, \qquad (B2)$$

i.e. there is a strong mutual attraction.

Note that if for some reason the lattices cannot be oriented in an optimum manner relative to one another the interaction of opposite charges will still turn them so that there will be attraction between them, if weaker than given by Eq. (B1).

Appendix C

Disruption of the external monolayer outside the contact region of the membranes

Let us assume that the external monolayer outside the contact region of the membranes has been disrupted (Fig. 2B, C). In this case, the part of the hydrophobic surface of the internal monolayer, forming the "hole bottom", becomes uncovered. The contact of this part of the membrane with the electrolyte solution gives rise to an excessive energy. In addition, the hydrophobic chains of the lipid molecules of the external monolayer, arranged along the perimeter of a "hole", may be uncovered (Fig. 2B). It is known (Petrov et al. 1980) however, that in most cases the "hole" edges become covered with the polar heads of lipid molecules (Fig. 2C) similarly as it is the case in inverted pores in a membrane. The perimeter of such an "inverted hole" possesses an energy associated with the tilting of lipid molecules sited on it.

From Eq. (3) an expression for the Gibbs free energy of the liposome can be derived:

$$\Phi = \Gamma \, (N - N^c) \frac{(a_\lambda^o - b_\lambda)^2}{b_\lambda} + \Gamma N \frac{(a_\lambda^i - b_\lambda)^2}{b_\lambda} + \sigma A +$$

$$+ 2\sqrt{\pi}\gamma\sqrt{A} + \text{const}, \tag{C1}$$

where σ is the energy of interaction of the unit area of the membrane hydrophobic part with the electrolyte solution, γ is the energy of the unit perimeter of "hole", and h is the length of the acyl chain of a lipid molecule. The first two terms on the right side of Eq. (C1) represent the elastic energy of the membrane; the third term represents the energy of interaction of the hydrophobic "hole bottom" with the electrolyte solution (Fig. 2B), i.e. virtually the surface tension of the "hole"; the fourth term represents the interaction energy of the acyl chains of molecules, arranged along the "hole" perimeter (it has the meaning of the linear tension of the "hole" edge; and *const* incorporates all the terms which remain unchanged with varying "hole" area A).

To analyze the dependence of the Gibbs free energy of the liposome on the "hole" area A, we shall write this expression considering that the areas per molecule, which correspond to the conditions of mechanical equilibrium, are given by expressions (7) and (8). As a result, we get

$$\Phi = \Gamma \frac{2N - N^c}{b_\lambda} \left(\frac{\frac{N^c}{N}(b_\lambda - b_\gamma) - \frac{A}{N}}{2 - \frac{N^c}{N}} \right)^2 + \sigma A + 2\sqrt{\pi}\gamma\sqrt{A} + \text{const} \tag{C2}$$

The contact area, required for a minimum to appear in the curve Φ (A), must include the number of molecules of the external layer $\tilde{N}^c$ given to within $(\hat{N}^c/2N)^3$ by the expression

$$\frac{\tilde{N}^c}{N} = \frac{\dfrac{\sigma}{\Gamma} + 3\left(\dfrac{\sqrt{\pi\gamma}}{2\Gamma\sqrt{b_\lambda N}}\right)^{2/3}}{\dfrac{b_\lambda - b_\gamma}{b_\lambda} + \dfrac{\sigma}{2\Gamma} + \left(\dfrac{\sqrt{\pi\gamma}}{2\Gamma\sqrt{b_\lambda N}}\right)^{2/3}} \tag{C3}$$

The area of the contact region (or the number of molecules in the contact region N^{c*}), required for a disruption to occur, and the minimum "hole" area A are determined from equations

$$\frac{N^c (b_\lambda - b_\gamma) - A}{2N - N^c} - \frac{\sigma b_\lambda}{2\Gamma} - \frac{\sqrt{\pi\gamma} b_\lambda}{2\Gamma\sqrt{A}} = 0 \tag{C4}$$

$$\frac{2N^c (b_\lambda - b_\gamma)}{2N - N^c} - \frac{\sigma b_\lambda}{2\Gamma} - \frac{2\sqrt{\pi\gamma} b_\lambda}{\Gamma\sqrt{A}} = 0 \tag{C5}$$

analogous in their meaning to Eqs. (12) and (13). From the solution of Eqs. (B4)—(B5) subject to the condition $N^c \ll 2N$ we find to within $(N^c/2N)^3$ that for a disruption to occur outside the contact region of the membranes this region must include

$$N^{c*} = N \frac{\dfrac{\sigma}{\Gamma} + \dfrac{3}{2}\left(\dfrac{2\sqrt{\pi\gamma}}{\Gamma\sqrt{b_\lambda N}}\right)^{2/3}}{\dfrac{b_\lambda - b_\gamma}{b_\lambda} + \dfrac{\sigma}{2\Gamma} + \dfrac{1}{2}\left(\dfrac{2\sqrt{\pi\gamma}}{\Gamma\sqrt{N b_\lambda}}\right)^{2/3}} \tag{C6}$$

molecules of the external monolayer, and the minimum area of the "hole" formed is

$$A^* = \left[\frac{2\sqrt{\pi\gamma} b_\lambda N \left(1 - \dfrac{1}{4}\left(\dfrac{2\sqrt{\pi\gamma}}{\Gamma\sqrt{b_\lambda N}}\right)^{2/3}\right)}{\Gamma\left(1 + \dfrac{b_\lambda}{(b_\lambda - b_\gamma)}\dfrac{\sigma}{2\Gamma} + \dfrac{1}{2}\dfrac{b_\lambda}{b_\lambda - b_\gamma}\left(\dfrac{2\sqrt{\pi\gamma}}{\Gamma\sqrt{b_\lambda N}}\right)^{2/3}\right)}\right]^{3/2} \tag{C7}$$

Calculation of the energy barrier, to be overcome by the membranes for a disruption to occur outside the contact region if the value of the Gibbs free energy at the minimum equals its value at $A = 0$, yields the expression

$$\Delta W = 0.5 \; N\Gamma b_\lambda \left(\frac{1}{\Gamma} \sqrt{\frac{\pi}{N}} \frac{\gamma}{\sqrt{b_\lambda}} \right)^{4/3} \tag{C8}$$

References

Abidor I. G., Arakelian V. B., Chernomordik L. V., Chizmadzhev Yu. A., Pastushenko V. F., Tarasevich M. R. (1979): Electric breakdown of bilayer lipid membranes: I. The main experimental facts and their qualitative discussion. Bioelectrochem. Bioenerg. **6**, 37—52

Adamson A. W. (1975): Physical Chemistry of Surfaces, Wiley, Interscience Publ., New York

Chapman D., Williams R. M., Ladbrooke B. D. (1967): Physical studies of phospholipids. VI. Thermotropic and lyotropic mesomorphism of some 1, 2 diacylphosphatidilcholines (lecithins). Chem. Phys. Lip. **1**, 445—475

Cullis P. R., de Kruiff B., Hope M. J., Nayar R., Schmid S. L. (1980): Phospholipid and membrane transport. Can. J. Biochem. **58**, 1091—1100

Düzgünes N., Wilshut J., Fraley R., Papahadjopoulos D. (1981): Studies on the mechanism of membrane fusion. Role of head-group composition in calcium- and magnesium-induced fusion of mixed phospholipid vesicles. Biochim. Biophys. Acta **642**, 182—195

Helfrich W. (1974): The size of bilayer vesicles generated by sonication. Phys. Let. **50**A, 115—116

Israelachvili J. N., Mitchell D. J., Ninham B. W. (1977): Theory of self-assembly of lipid bilayers and vesicles. Biochim. Biophys. Acta **470**, 185—201

Kozlov M. M., Markin V. S. (1982): Effect of discrete charge on potential distribution in bilayer lipid membranes. Biofizika **27**, 629—634 (in Russian)

Kozlov M. M., Markin V. S. (1983) Possible mechanism of membrane fusion. Biofizika **28**, 242—247 (in Russian)

Kwok R., Evans E. (1981): Thermoelasticity of large lecithin bilayer vesicles. Biophys. J. **35**, 637—652

Landau L. D., Lifshitz E. M. (1965): The Theory of Elasticity. Nauka, Moscow (in Russian)

Landau L. D., Lifshitz E. M. (1976): Statistical Physics, Nauka, Moscow (in Russian)

Litster J. D. (1975): Edge energy of lipid bilayers. Phys. Let. **53**A, 193—194

Lucy J. A. (1978) Mechanism of chemically induced cell fusion. In: Membrane Fusion (Ed. G. Poste, G. L. Nicolson), Elsevier/North-Holland Biomedical Press, Amsterdam

Markin V. S. (1981): Lateral organization of membranes and cell shapes. Biophys. J. **36**, 1—19

Markin V. S., Kozlov M. M. (1983a) On primary event in the course of membrane fusion. Biofizika **28**, 72—77 (in Russian)

Markin V. S., Kozlov M. M. (1983b): Inter- and intramembrane interactions and phase transitions. Gen. Physiol. Biophys. **2**, 201—215

Newton C., Pangborn W. Nir S., Papahadjopoulos D. (1978): Specificity of Ca^{2+} and Mg^{2+} binding to phosphatidylserine vesicles and resultant phase changes of bilayer membrane structure. Biochim. Biophys. Acta **506**, 281—287

Petrov A. G., Mitov M. D., Derzhanski A. I. (1980): Edge energy and pore stability in bilayer lipid membranes. Adv. Liq. Cryst. Res. Appl. **1**, 605—626

Portis A., Newton S., Pangborn W., Papahadjopoulos D. (1979): Studies of the mechanism of membrane fusion: Evidence for an intramembrane Ca^{2+} — phospholipid complex, synergism with Mg^{2+} and inhibition by spectrin. Biochemistry **18**, 780—790

Traüble H., Eibl H. (1974): Electrostatic effect on lipid phase transitions: membrane structure and ionic environment. Proc. Nat. Acad. Sci. USA, **71**, 214—219

Weaver J. C., Mintzer R. A. (1981): Decreased bilayer stability due to transmembrane potentials. Phys. Let. **86** A, 57—59

Wilshut J., Düzgünes N., Fraley R., Papahadjopoulos D. (1980): Studies of the mechanism of membrane fusion: kinetics of calcium ion induced fusion of phosphatidylserine vesicles followed by a new assay for mixing of aqueous vesicles contents. Biochemistry **19**, 6011—6021