On the Theory of Membrane Fusion.
The Stalk Mechanism

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Abstract. Based on literary data, conditions necessary for membrane fusion are discussed. It is proposed that fusion mechanisms should be classified according to the primary act involving a change in the membrane structure. Two principal fusion mechanisms are identified: the stalk mechanism, starting with the appearance of a stalk between approaching membranes, and the adhesion mechanism which involves bilayer reorganization as a result of a tight junction of the membranes. The origin and evolution of the monolayer and bilayer stalks between membranes are analysed. Using the expression for the elastic energy of the stalk it was possible to find the value of the spontaneous curvature of its membrane, K_s, at which the existence of a stalk is in principle possible. It is shown that, within the framework of the stalk mechanism, there exists a possibility of either the formation of a stalk of a finite radius, or complete fusion. The K_s values have been determined at which one of the variants occur. The energy barrier of the hydrophobic interaction and the elastic energy barrier, which have to be overcome by the membranes to form the stalk are analysed. The theoretical analysis of the stalk formation mechanism is supported by experimental data. It has been shown by freeze-fracture electrone microscopy that the addition of Ca^{+2}, Mg^{+2}, Mn^{+2} or Cd^{+2} to suspensions of egg phosphatidylcholine and cardiolipin (1:1 or 3:1) leads to the formation of numerous intramembrane particles (imp's) and crater-like (stalk) structures.

Key words: Membrane fusion mechanisms — Monolayer and bilayer stalks — Elastic energy — Egg phosphatidylcholine

Introduction

The interest in the problem of membrane fusion arises from the importance of this phenomenon for numerous biological processes (Poste and Nicolson 1978). Fusion is being studied on both natural and artificial membranes. Experiments with biological membranes have been made on a broad spectrum of models, although

the most popular ones obviously are erythrocytes and their ghosts (Lucy 1978). Model systems investigated involve the fusion of two planar lipid membranes (BLM) (Liberman and Nenashev 1968; Badzhinyan et al. 1971; Neher 1974), of BLM with artificial liposomes (Grishin et al. 1979; Razin and Ginsburg 1980; Zimmerberg et al. 1980; Yegorova et al. 1981), the mutual fusion of liposomes (Papahadjopoulos 1978), and, finally, the fusion of liposomes with cells (Gad et al. 1979).

Fusion of cellular membranes is apparently preceded by the removal of membrane proteins from the contact region and by the formation of a pure lipid bilayer (Lucy 1978; Papahadjopoulos 1978; Gingell and Ginsberg 1978). We are not going to examine this preliminary stage or forces operating here (Markin and Glaser 1980). We shall be interested in direct fusion of lipid bilayers. The purpose of the work is to analyse the possible fusion mechanism of lipid membranes from the viewpoint of the primary act in this process.

The authors tried to establish conditions necessary for fusion, i.e. properties of the membranes and external triggering effects produced by the environment.

Triggering effects can be mechanical, electrical or chemical. The first class of triggering effects involves mechanical compression of two BLM by hydrostatic pressure (Liberman and Nenashev 1968; Badzhinyan et al. 1971; Neher 1974). Electric effects involve superimposition of an electric field from an external source onto mutually approaching membranes (Senda et al. 1979; Zimmermann and Scheurich 1981). Finally, the third class comprises the effect of chemical agents known as fusogens (Lucy 1978).

Three particularly important properties of membranes affecting fusion can be identified: the membrane charge, the phase state of the lipid bilayer and the geometric characteristics, namely curvature.

If the fusion is initiated by fusogens it is, as a rule, necessary that at least a portion of lipid molecules in the membrane are charged. For example, in the case of calcium-initiated fusion of liposomes consisting of a mixture of a neutral lipid (phosphatidylethanolamine) and charged phosphatidylserine (PS) the PS content has to be at least 50 % (Papahadjopoulos et al. 1974). However, it is also possible to bring about the fusion of neutral membranes with the aid of alkylbromides and lysolecithin (Mason et al. 1979; Lucy 1970) or by hydrostatic pressure (Neher 1974).

As for the phase state of the bilayer, most authors assume that it should be liquid; thus,the fusion of spherical membranes begins at temperatures significantly exceeding that of phase transition (Breisblatt and Ohki 1975). The membrane in the liquid state has a high fluidity which tends to disturb the bilayer stability (Ahkong et al. 1975). Accordingly, the effect of substances such as glycerol-monooleate or oleic acid is explained by their capacity to change membrane fluidity (Ahkong et al. 1975). The above fusogenic lipids possess relatively long hydrocar-
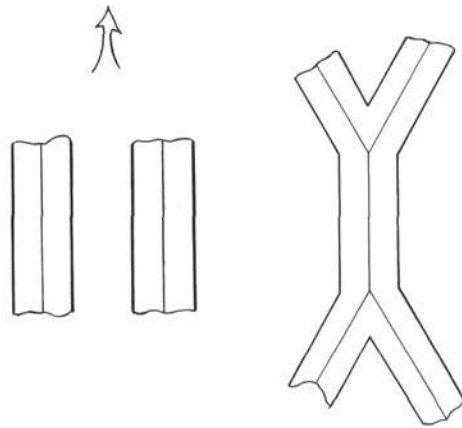
Fig. 1. A trilaminary structure arising from two approaching bilayers.

bon tails, penetrating into the membrane, thus changing the voluminal relationship between polar heads and the hydrocarbon part, and increasing the proportion of the latter. As a result, membrane fluidity increases. One of the consequences of the high fluidity is that the angle between hydrocarbon chains and the normal to membrane surface grows larger, thus affecting the bilayer stability (Breisblatt and Ohki 1975). On the contrary Papahadjopoulos (1978) has presumed it is not the liquid state that provides the condition for fusion. In his opinion, the decisive circumstance is the simultaneous presence in the membrane of liquid and gel-like (crystalline) phases, the boundaries of which prove to be unstable.

Eventually, the third condition relates to the geometrical characteristics of membrane. Portis et al. (1979) have discussed an assumption that a large curvature of membranes must promote their fusion. However, a comparative study of small and large liposomes (Wilshut et al. 1980) has revealed that a large curvature is by no means absolutely necessary. Nevertheless, the rate of fusion and the number of liposomes involved in this process were found to be higher for small as compared to the large liposomes.

We have reviewed the main concepts and trends of fusion membrane studies. There exist suggestions concerning more detailed aspects of this phenomenon, e.g. the hypothesis on the influence of the degree of hydration of lipid polar heads (Sunder and Papahadjopoulos 1981). However, from our point of view, no modern hypothesis provides an experimental support which would be strong enough.

Classification of Fusion Mechanisms

The absence of a single viewpoint concerning the conditions necessary for membrane fusion suggests that this phenomenon has not yet been adequately
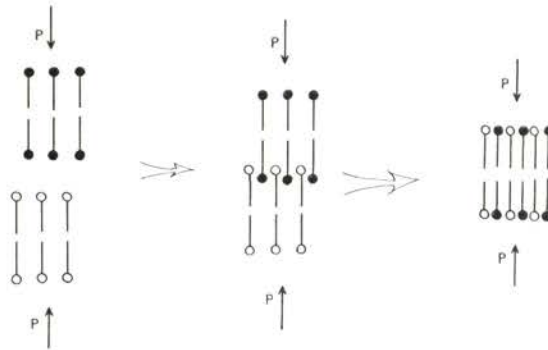
Fig. 2. A hypothetic squeezing of membranes into one another; under normal conditions, this process is impossible.

investigated and that there may exist different mechanisms underlying this process. Several hypotheses are available on this topics in the literature (Poste and Nicolson 1978). Some of them are mutually excluding, others partially overlap. They deal with possible reorganization of membranes during fusion and with various kinds of intermediate structures. One of these structures, known as trilaminary (Gingell and Ginsberg 1978), deserves special attention. This theory is based on the formation of a single bilayer from two mutually approaching bilayers (Fig. 1). The appearance of a trilaminary structure was observed during the fusion of two BLM (Neher 1974; Melikyan et al. 1982) and during the fusion of chromaffin granules (Edwards et al. 1974) as an intermediate stage, but it can also be the final result of the fusion (Grishin et al. 1979).In addition this structure is obviously a result of the interaction of bacteriorhodopsin-containing liposomes with planar membranes during the implant this proton pump into BLM (Hermann and Rayfield 1978).

How can a trilaminary structure arise? Let us imagine the simplest variant: the mutual penetration of two bilayers as the teeth of two combs (Fig. 2). Let us estimate the pressure necessary for such a process to occur. For the sake of simplicity we shall consider a bilayer consisting of neutral lipid molecules with polar heads having a dipole moment μ. As seen in Fig. 2, during the process of reciprocal penetration the head groups of lipids go over a medium with a high dielectric permeability, ε_w, (equal to 80) into another with a low dielectric permeability ε_M,(close to 2). It is easy to evaluate the energy of polar heads in the medium:

$$W = \frac{\mu^2}{\delta^3 \varepsilon_0 \varepsilon} \qquad (1)$$

where ε_0 is the dielectric permeability of the vacuum; ε is the relative dielectric permeability of the medium; and δ is the size of the polar head. As follows from the expression (1), the energy of the polar head is the higher, the lower the
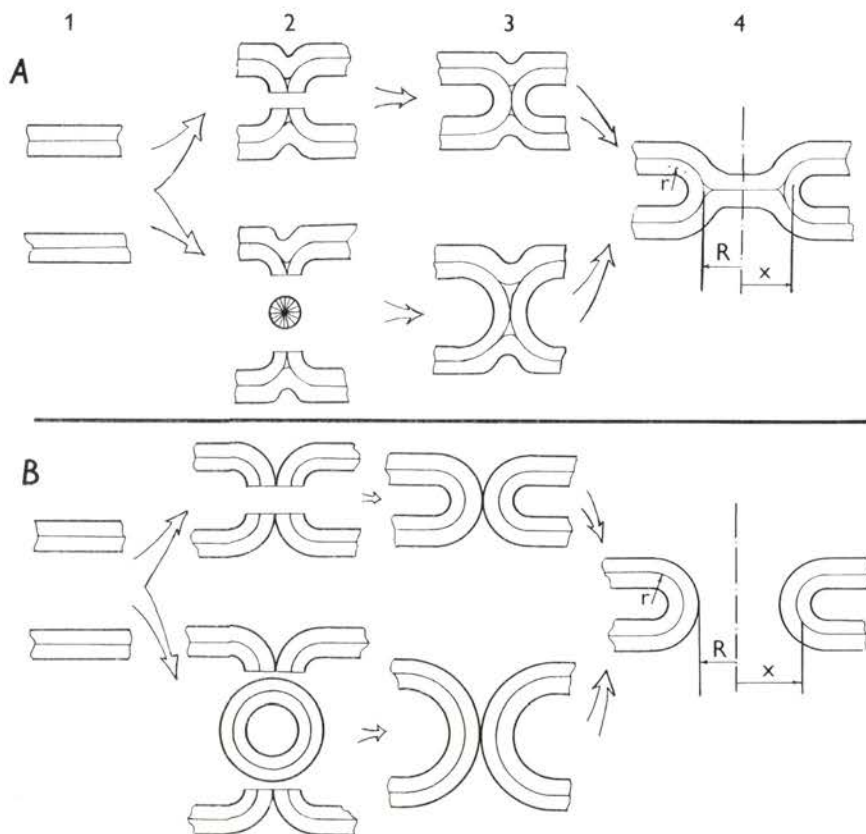
Fig. 3. The stalk mechanism of membrane fusion; the figure shows cross-sections of the figures of revolution arising during the process of fusion. A — The monolayer variant: 1) approaching original membranes; 2) simultaneous formation of bulging defects capable to close one upon another directly or to interact through a micelle; 3) the appearance of a stalk with a zero radius; 4) expansion of the stalk. Thick lines indicate hydrophilic surfaces, thin lines represent hydrophobic surfaces, interrupted lines indicate the intermediate surface; R is the radius of the stalk, $2r$ is its height, $2h$ is the thickness of the stalk wall, x — is the distance from the point on the intermediate surface to the axis of revolution. B — The bilayer variant. The stages of fusion are the same as in the case of the monolayer stalk. The closure of bulging defects may proceed through liposomes. The intermediate surface of the stalk coincides with the surface separating the monolayers.

dielectric permeability of the surrounding medium. Consequently, for reciprocal penetration of bilayers with a molecule density n and a thickess of $2h$ (Fig. 2), the work performed by the external pressure P has to be

$$Ph = \left(\frac{1}{\varepsilon_M} - \frac{1}{\varepsilon_W}\right) \frac{\mu^2 n}{\varepsilon_0 \delta^3} \tag{2}$$
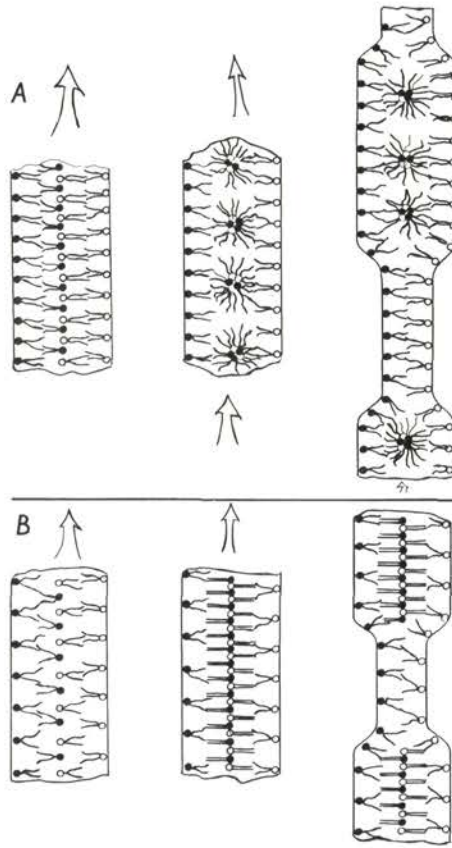
Fig. 4. The adhesion mechanism of fusion: *A*: adhesion-micellar, *B*: adhesion-condensational.

Assuming $\delta \approx 0.5$ nm, $n \approx 0.002$ nm^{-2}, and the dipole moment μ set up by elementary charges e_0 and $-e_0$ lying at a distance of the order of 0.3 nm, we shall obtain $P \approx 10^9$ N/m^2. To obtain such a pressure, a water column 100 km high would be needed! In the case of charged lipids, an even higher energy would be needed, since the energy of a charge in the medium is higher than that of a dipole. Consequently, the above mechanism in reciprocal penetration of bilayers without disturbing their structure appears impossible, and the role of hydrostatic pressure as reported by Neher (1974) seems to consist in establishing a tight contact between two membranes over a relatively wide area; such a contact results in monolayer fusion.

It is natural to suppose that every fusion process starts with a more or less deep disturbance of the bilayer. Due to this, we believe that it is feasible to differentiate

between fusion mechanisms according to their primary stage or primary act. At this stage a crucial change in the membrane structure, the enigma of fusion, occurs. The subsequent stages are more or less a logical continuation of the primary act.

From the viewpont of the primary act there apparently exist, only two possibilities: either a lipid bridge becomes formed at a certain distance from the approaching membranes, or the membranes approach until a tight junction and a consequent reorganization of the bilayer occur. Let us analyse both possibilities.

The first type of fusion mechanism associated with the formation of a lipid bridge between two membranes is illustrated in Fig. 3. The lipid bridge consisting of a curved monolayer (Fig. 3A) or bilayer (Fig. 3B) region which connects two membranes is called a stalk (Gingell and Ginsberg 1978). Therefore, this type of fusion mechanism is called ,,stalk" mechanism. A stalk can arise from a single monolayer or from an entire bilayer. The evolution of a monolayer stalk (expansion of the curved region) leads to the formation of a trilaminary structure, while a bilayer stalk immediately results in complete fusion (Fig. 3B). If a stalk is to be formed between membranes, there local structural bulging defects have to appear, "growing" towards one another; they resemble to a certain extent a stalactite and stalagmite. These prominences can close upon one another directly or, as presumed in the model of Lucy (1970) as modified by Gingell and Ginsberg (1978), through other lipid formations, e.g. micelles. In the first case, the stalk is formed of the substance of the membranes, in the other one of some additional material.

Another type of primary act involves reorganization as a result of a tight contact of the membranes; we shall call it adhesional. It brings about following chain of events: Initially a tetralayer structure is formed, in which the two inner monolayers may, apparently, partially penetrate one another (Fig. 4). As a result, the structure becomes with a high probability bilayer due to the removal of the two inner monolayers. As a result, a trilaminary structure arises (Fig. 4). Investigation of the adhesion mechanisms shall be the subject of separate communications. In the present paper we shall deal in detail with the stalk mechanism of membrane fusion, and bring the experimental evidence of stalk existence.

Stalk Mechanism Theoretical

Formulation of problem. Membranes resist bending; the expression for the surface density of the energy of a bent membrane (Helfrich 1974) is

$$w_e = \frac{D}{2} (K_m + K_p - 2K_S)^2, \tag{3}$$

where K_m and K_p are the values of principal curvatures of the surface at a given point; K_S is a spontaneous curvature of the membrane, D is the coefficient of bending rigidity, the value of which in artificial bilayers was estimated to be

5×10^{-20} J (Helfrich 1974; Evans 1980). The total bending energy is obtained by integrating over the entire membrane surface.

It can reasonably be expected that a strongly curved stalk membrane is endowed with a significant bending energy. However, the primary membrane from which the stalk was formed, may possess bending energy, if its geometric curvature differs from the spontaneous one. The stalk has an opportunity to arise, if the bending energy of its membrane is inferior to that of the primary membrane. We shall call the difference between these energies the stalk energy, W_S. Our purpose is to find and to analyse its dependence on stalk dimensions. This will permit us to form an idea about the possibility for the stalk to expand, about intermediate structures arising during the fusion, and conditions necessary for complete fusion.

Stalk energy. Let us consider a stalk formed as a result of a direct closure upon one another of bulging defects in two membranes having an initial curvature, K_0, and a spontaneous curvature, K_S. Let us draw the so-called intermediate surface between the inner and the outer stalk surfaces (Fig. 3A3, 3B3) and assume that it was formed by the revolution of a semicircle of a diameter $2r$ which corresponds to the stalk height. Let us denote h the half-thickness of the stalk wall and R the minimal radius of the channel formed by the inner surface of the stalk (inner radius).

The meridional curvature of the stalk membrane is $K_m = -1/r$, as in the case of figures of revolution the other, "parallel", curvature is interrelated with the meridional one by the equation (Pogorelov 1965)

$$K_p + x\,\frac{dK_p}{dx} = K_m, \tag{4}$$

where x is the distance from a given point on the intermediate surface to the axis of revolution (Fig. 3 A4, B4). The boundary condition for equation (4) implies, on considerations of symmetry, that at the points on the intermediate surface with the coordinate $x = R + h$ the "parallel" curvature must be $K_p = (R + h)^{-1}$. By solving the equation (4) we obtain
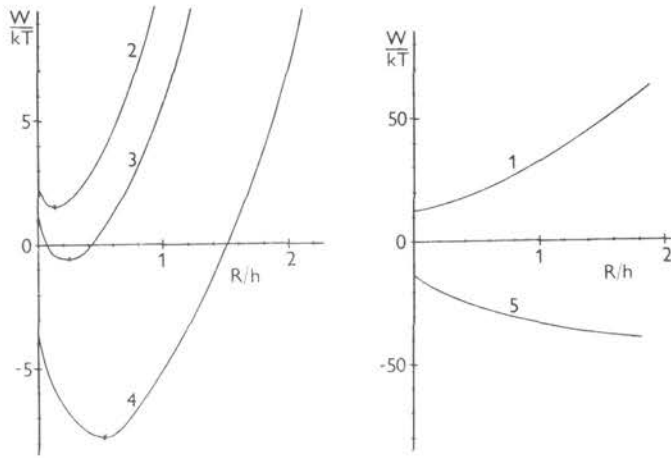
$$K_p = \frac{(r + R + h)}{r}\frac{1}{x} - \frac{1}{r}. \tag{5}$$

Using the expression (5), we obtain

$$W_s = 2\pi D \left\{ \int dS \left(\frac{r + R + h}{rx} - \frac{2}{r} - 2K_s\right)^2 - \int dS \,(2K_0 - 2K_s)^2 \right\} \tag{6}$$

The first integral in the right-hand part of the equation (6) represents the bending energy of the stalk membrane over the integral surface of which integration is carried out. The second integral is equal to the bending energy of the initial

Fig. 5. The energy of the stalk in kT units versus its dimensionless radius $\varrho \equiv R/h$. The membrane rigidity is $D = 5 \times 10^{-20}$ J, temperature $T = 300°$K, the stalk semiheight $r = h$. The curves correspond to different values of the spontaneous curvature:
1) $K_s = -(7h)^{-1}$; 2) $K_s = -(5.7h)^{-1}$; 3) $K_s = -(5.5h)^{-1}$; 4) $K_s = -(5h)^{-1}$; 5) $K_s = -(4h)^{-1}$.

membrane. It is taken over the intermediate surface of that area of the initial membrane from the stalk was formed. As a result, we obtain

$$W_s = 2\pi D \left\{ \left[\left(\frac{2}{r} + 2K_s \right)^2 - (4K_0 - 2K_s)^2 \right] \times \right.$$

$$\times \left[\frac{\pi}{2} r \left(r + h + R \right) - r^2 \right] - \pi r \left(\frac{2}{r} + 2K_s \right) \frac{(r+h+R)}{r} + \tag{7}$$

$$\left. + \frac{2}{r} \frac{(r+h+R)^2}{\sqrt{(R+h)(2r+h+R)}} \, \text{arctg} \, \sqrt{\frac{2r+h+R}{R+h}} \right\}$$

The evolution of the stalk. The energy of the stalk depends on its radius, R. An a priori determination of the character of such a dependence is difficult because with increasing R, the surface area of the stalk grows, and the surface energy density declines due to the decreasing curvature K_p, while K_m remains constant. The rivalry of these two tendencies may be responsible for the fact that the energy may behave differently with the rising R. The possible variants are given in Fig. 5.

The energy may monotonously increase with the growing radius (curve 1); then, the formation and widening of the stalk is impossible. Under other conditions the function $W_s(R)$ may reach a minimum (curves 2—4). In such a situation the stalk tends to assume a radius at which its energy is minimal. Finally, there may be cases

in which the energy declines with the growing radius (curve 5); this corresponds to the tendency of the stalk to expand unlimitedly.

The parameters determining the energy versus radius dependence of a stalk are r and K_s. We shall assume that the semiheight r is constant and equals h. This corresponds to the case when membranes approach to a distance smaller than the monolayer thickness and the stalk starts expanding during a time period which is small compared to the characteristic time of the divergence of membranes.

Presuming for the sake of simplicity that the curvature of initial membrane is negligible, since $|K_0| \ll |K_s|$, we shall rewrite the expression (7) in the form

$$W_s = 2\pi D \left\{ 2 K_s h \left[\pi(\varrho + 2) - 4 \right] + 2 \frac{(\varrho + 2)^2}{\sqrt{(\varrho + 2)^2 - 1}} \, \mathrm{arctg} \sqrt{\frac{\varrho + 3}{\varrho + 1}} - 4 \right\} \qquad (8)$$

where ϱ dimensionless radius $\varrho \equiv R/h$ is introduced.

An analysis of the expression (8) (illustrated in Fig. 5) shows that the stalk energy $W_s(\varrho)$ reaches a minimum, if the spontaneous curvature satisfies the inequality (curves 2, 3, 4)

$$-(4h)^{-1} < K_s < -(6.6h)^{-1}, \qquad (9)$$

i.e. if the absolute value of the radius of the spontaneous curvature, $R = 1/K_s$, varies within $4h < |R_s| < 6.6h$. In this case, the stalk formed will be expanding until its energy becomes minimal. However, the stalk formed as a result of such expansion may be endowed with different properties. If $5.4h < |R_s| < 6.6h$, the energy W_s for $\varrho = 0$ is positive (curves 2, 3). This means that for a stalk to be formed the membranes have to overcome an energy barrier equal to $W_s(\varrho = 0)$. To break off a stalk, barrier equal to the difference between the energy at $\varrho = 0$ and the energy in the minimum must be overcome. If the minimal energy is positive, the energy barrier of separation is smaller than that of formation (curve 2), the stalk being unstable in this case; consequents, when the minimum of the stalk energy is negative (curve 3), the stalk is stable. If $4h < |R_s| < 5.4h$ the membranes need not overcome a barrier associated with bending energy, for a stalk to be formed since $W_s(\varrho = 0) < 0$; moreover, the value of the energy in the minimum being negative, the stalk is stable (curve 4).

It is worth noting that for the greater part of values of K_s corresponding to the presence of the energy minimum, the radius R^* at which this minimum is reached has the magnitude of the order of several units of h (curves 2—4), i.e. the stalk radius is small compared to such quantities as the liposome radius, which amounts to tens of h.

If the absolute value of the radius of the spontaneous curvature is small, i.e.

$$|R_s| < 4h, \qquad (10)$$
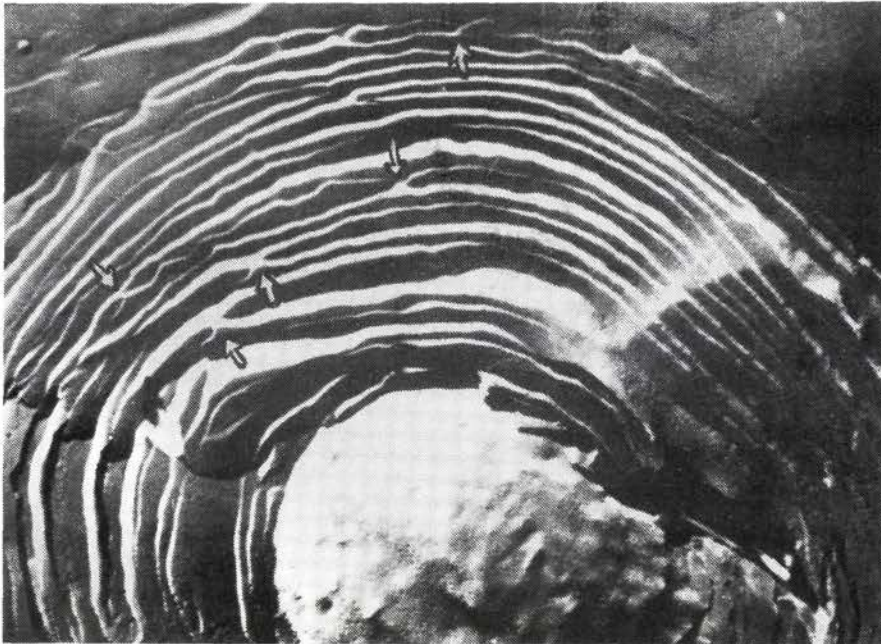
Fig. 6. Freeze-fracture electron micrograph of a 1:1 molar mixture of egg phosphatidylcholine and cardiolipin multilamellar liposome. The sample was frozen after 2 hours of incubation with 10 mmol/l $CaCl_2$. Numerous intermembrane contacts can be seen (arrows).

the stalk formed will expand without any limit (curve 5). The result of such an expansion may be monolayer fusion, in the case of a monolayer stalk, or a complete fusion in the case of a bilayer stalk.

The entire variability interval of the radius of the spontaneous curvature corresponding to transition from the situation when no stalk is formed to that of its unlimited expansion is fairly narrow and amounts to $2.6h$.

Materials and Methods

Appropriate mixtures of Cardiolipin (Sigma Chemical Company) and egg yolk lecithin (Lipid Products, Nutfield, England) molar ratios of at 1:1 and 1:3 were removed from ethanol solutions by rotary evaporation. Excess (70 % by weight) water was added to the dry lipids and after extensive shaking definite amounts of $CaCl_2$, $MgCl_2$, $MnCl_2$ or $CdCl_2$ (3 mmol/l to 10 mmol/l) were added and vortexed from 0.5 hour to 6 hours at +18°C. Samples for freeze-fracture electron microscopy were taken (in various time intervals following the addition of Morinated metal salts) and frozen into liquid propane at −190°C. Other details were described elsewhere (Borovjagin et al. 1982). The replicas were cleaned in
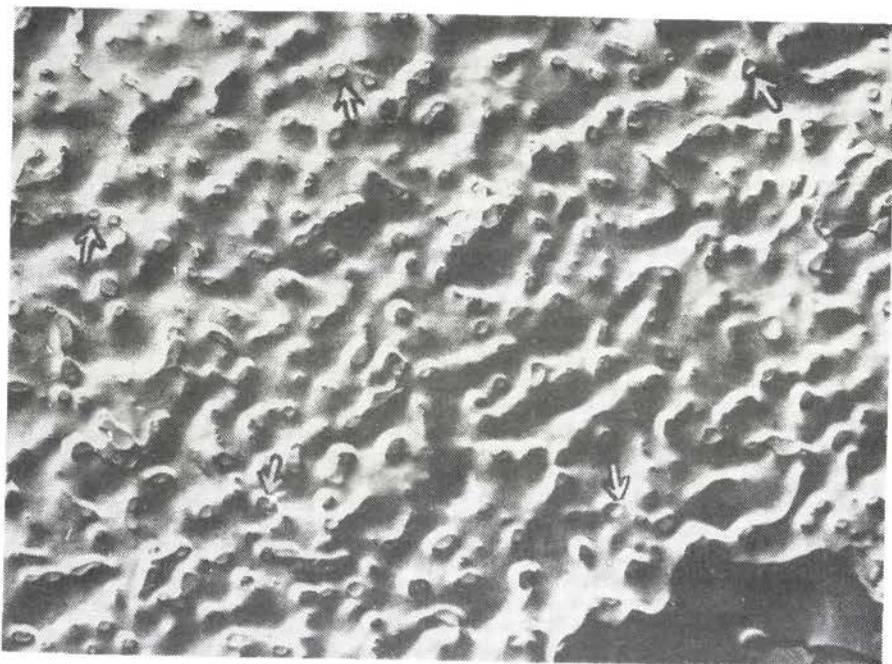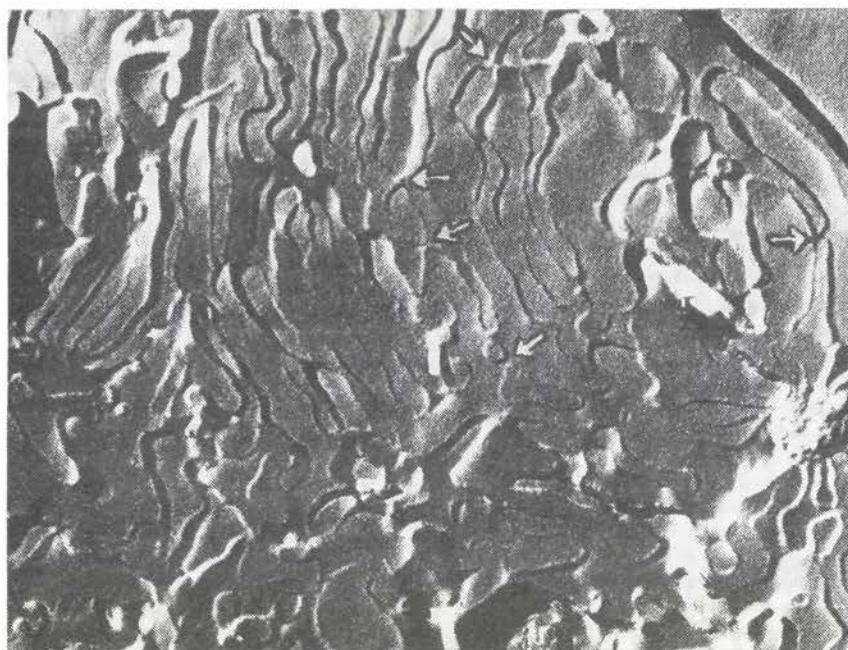
Fig. 7. The same as Fig. 6, after 2 hours of incubation with 4 mmol/l MgCl$_2$. Fracture face of lipid membrane has a numerous crater-like (stalks) structures (arrows).

nitric acid, washed in water, picked up on uncoated 300 mesh grids, and examined with an IEM-100B electron microscope.

Results

Samples of multiwalled liposome suspensions incubated over 1—2 hours following the addition of 3×10^{-3} mol . 1^{-1} or 5×10^{-3} mol . 1^{-1} metal chloride showed many examples of intermembrane contacts and points of first steps of fusion (Fig. 6, arrows). These contacts correspond to numerous well known lipidic intramembrane particles (Cullis et al. 1980) and pits revealed on membrane fracture faces. After longer incubation periods with the metal chloride extensive fusion and a number of stalk structures (the crater-like structures, Fig. 7, large arrow) are formed. As a result of the stalk evolution a fragmentation of multiwalled liposomes and formation of small unilamellar vesicles and tubular structures progressively occurred (Figs. 8 and 9).

Fig. 8. The same as Fig. 6, after 6 hours of incubation with CaCl₂. Stalk structures and numerous points of membrane fragmentation (arrows) are seen.

Discussion

The experiments mentioned above have shown that intermembrane contacts-stalks can arise in a multibilayer system comprised of egg phosphatidylcholine and cardiolipin mixture. Now let us consider the correlation between these observations and the theory.

A cardiolipin molecule consists of two phosphatidylglycerol molecules having a common glycerol residue. If the negatively charged heads of cardiolipin are neutralized with monovalent cations, e.g. Na^+, the cardiolipin molecules assume a shape very close to that of a cylinder, and this lipid produces stable bilayer structures. If cardiolipin is neutralized with divalent cations, e.g. Ca^{2+}, the cations tighten up the phosphate groups of the same molecule, and the molecule assumes a conical shape. In other words, the difference in the cross-section area of polar and hydrophobic regions increases. The asymmetry of volumes of the polar and apolar areas of the molecule increases with the raising temperature.

A monolayer consisting of asymmetrical molecules, e.g. of a cardiolipin-Ca^{2+} complex, must display a spontaneous curvature K_s. As follows from geometrical
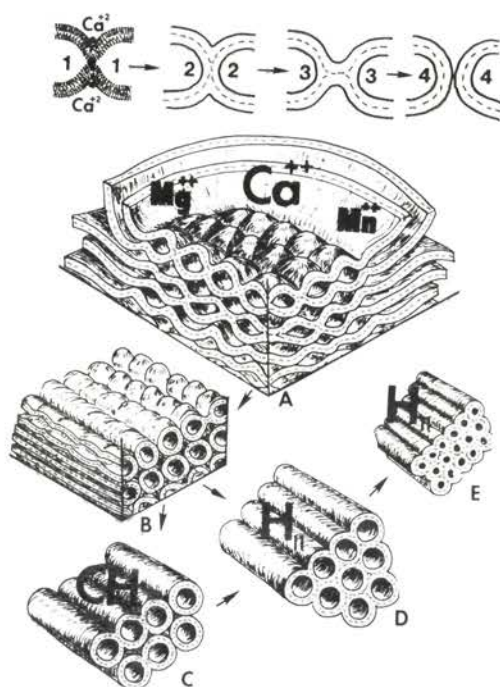
Fig. 9. Three-dimensional drawings (A) of the intermediate stage in the lamellar to complex hexagonal (CH) — hexagonal (H) phase transition. Multilamellar liposomes enriched by cardiolipin or pure cardiolipin liposomes after the addition of metal chloride. 1—4 — the intermediate stage of stalk formation.

considerations, the spontaneous curvature K_s is negative for this complex (Israelachvili et al. 1980), as the monolayer has a tendency to assume a concave shape. Due to negative K_s, cardiolipin can form inverted micelles.

The spontaneous curvature of mixed monolayers is equal to the mean spontaneous curvature of all lipids in the mixture (Markin 1981). In the case of an equimolar lecithin-cardiolipin mixture, the spontaneous curvature of monolayer is equal to half of that of cardiolipin, because lecithin has a symmetrical shape and its spontaneous curvature is zero (Israelachvili et al. 1980). The stalk formation in this system is less profitable. The membrane in these experiments might be expected to have a nonhomogenous structure: cardiolipin might form clusters with a large (in absolute value) spontaneous curvature. However, recent spectroscopic investigations (Vasilenko et al. 1982) have demonstrated that both lipids are present in the curved regions. Nevertheless there is a close correlation between the amount of cardiolipin in the mixture and the number of intramembrane particles curved structures raising between the adjacent bilayers (Borovjagin et al. 1982). This

proves that highly asymmetric cardiolipin molecules play a decisive role in stalk formation and the possibility that enriched cardiolipin microdomains occur in the region of intermembrane contacts cannot be ruled out. The formation of stalks in this region becomes advantageous. If these regions have the proper spontaneous curvature the stalk can have a finite-radius, as demonstrated in the theoretical part of the paper. Fig. 7 proves that this is the case.

Now we consider the specific role of calcium ions in the process of fusion. We have shown that these cations are necessary for the stalk formation in the membrane system consisting of a lecithin-cardiolipin mixture. We believe that this particular case illustrates the general rule which is pertinent to the majority of other system used for the investigation of membrane fusion (Portis et al. 1979; Papahadjopoulos 1978; Papahadjopoulos et al. 1974; Yegorova et al. 1981). For example, calcium ions can drastically shift the phase transition temperature in lipid bilayers (Markin and Kozlov 1983a, b) resulting in so called adhesion-condensation mechanism of fusion.

There exist three major effects related to the presence of Ca^{2+} in the system.

First, when Ca^{2+}-cardiolipin complex is formed a neutralization of the negative charge of the molecules occurs. It eliminates the lateral electrical repulsion between cardiolipin molecules and can promote the appearence of enriched cardiolipin microdomaines. As shown here, this makes the stalk formation more advantageous.

Second, the stalk formation is preceded with the appearance of some defects in the membrane structure — bulging, which is the nucleus of the future stalk (Fig. 3 $A2$). Neutralization of the membrane charge with Ca^{2+} promotes membrane approaching; as a result, the opposing bulgings can close into one another and a stalk structure arises.

Third, the stalk nucleus has a hydrophobic boundary (Fig. 3 $A2$, $B2$). Thus, in the process of stalk formation the system has to overcome an energy barrier associated with both the elastic energy of membrane bending (Fig. 5, curves 2, 3) and the interaction of defects with the surrounding medium W_γ. The latter barrier can be expressed as

$$W_\gamma = 4\pi\gamma h, \tag{11}$$

where γ is the energy per unity length of the membrane edge, which equals 10^{-11} N (Helfrich 1974). In this case, the hydrophobic energy barrier W_γ equals 40 kT. However, the membrane contact can be dehydrated in the presence of Ca^{2+} (Portis et al. 1979). In this case, the linear tension of the edge of the stalk nucleus must significantly decrease, leading to a decrease in the barrier W_γ. This means that the stalk formation becomes more probable.

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