MEMBRANE FUSION AND LIPID POLYMORPHISM

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INTRODUCTION

Membrane fusion is an extremely important phenomenon in biology. During this process two membranes, which can be two different membranes or two sites of one membrane, come in close contact, join and subsequently fuse, resulting in an intermixing of membrane lipids and proteins of the two membranes. Moreover, aqueous compartments, which were separated before the fusion, will intermix (see Fig. 1). If fusion is stopped at the stage of joining and the two membranes stay connected one may call it arrested fusion.

Among the important biological phenomena where membrane fusion is involved are: (i) fusion of the sperm and the egg membrane which leads to fertilization; (ii) secretion of neurotransmitters, insulin and other hormones plus digestive enzymes of the respective storage vesicles inside the gland cells called exocytosis, and (iii) the uptake of particles, bacteria (phagocytosis) and the uptake of viruses and the removal of receptors from the surface (receptor-mediated endocytosis).

In fact, membrane fusion is the potency of any membrane and this potency may be revealed more in one membrane than in the other. In most intracellular membrane types like endoplasmic reticulum, lysozymes, Golgi system, fusion events take place continuously.

Many studies have been undertaken to understand the fusion process itself and internal parameters which are involved in and/or activity modulate the fusion process. In recent years this basic interest in the membrane fusion process has been further stimulated.

One realized that the application of artificially-induced fusion is a powerful tool for hybridization of cells in order to produce monoclonal antibodies, for introducing membrane components in a cell membrane, and also in relation with the potential to introduce drugs in a cell (targeting).

At present, many effectors are known to trigger membrane fusion (Schramm et al., 1982), like Ca^{2+} in exocytosis, antibodies and hormones in receptor-mediated endocytosis, pH during the fusion of endocytotic vesicles and the lysosomes. Moreover, the involvement of many other substances like ATP, cAMP, GTP, drugs and Ca^{2+} binding proteins, including calmodulin and synexin, has been reported. Also membrane proteins appear to be involved in the fusion (Schramm et al. 1982), including the state of the cytoskeleton, the extent of glycosylation and the distribution of membrane spanning proteins. With respect to the latter aspect, it was assumed that the membrane proteins are cleared from the fusion site before actual fusion can start. This was based on freeze-fracture experiments which exhibited smooth bilayer patches without intramembraneous particles (IMP). However, later on it has been demonstrated that this phenomenon can be attributed to the use of cryoprotectants and/or chemical fixation. No particle clearance has been found using fast-freezing methods (Chandler and Heuser, 1979). So there is no requirement for a visibled lateral reorganization of intrinsic proteins before membrane fusion. In fact, only a small area of lipid may be enough for fusion, which is consistent with the local point fusion hypothesis.

As discussed above, many factors are thought to play a role in membrane fusion, but lipids actually fuse. The lipids of the bilayer have to become in close opposition, which requires a reduction in electrostatic repulsion and in the hydration forces of the lipids.

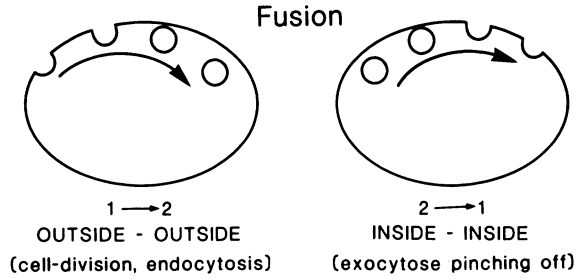


Fig. 1.

Subsequently, the lipids of both bilayers have to join, which implies that they temporarily will leave the bilayer configuration at the fusion point. Finally, both bilayers fuse with each other, which includes bilayer restabilization.

The question is: "Is there one general mechanism involved in the fusion with respect to the lipids?" From model studies a variety of possible factors have been proposed, including the presence of lysolecithin (Lucy, 1979), the presence of solid and lipid domains which can be triggered isothermally in mixtures of phosphatidylserine and neutral phospholipid (Papahadjopoulos et al., 1978). Although these principles may play a role in membrane fusion they only fit for some distinct fusion processes. In this respect it may be mentioned that one can divide biological fusion in two types. Firstly, there is fusion in which the outer (exoplasmic) monolayer of the membrane is involved, like in endocytosis and cell fusion. Secondly, the opposite situation is encountered in exocytosis, fusion between intracellular organelles and cell division in which the inner (cytoplasmic) monolayer is primarily involved (Fig. 1).

So, the involvement of for instance phosphatidylserine can only be relevant for exocytosis of eukaryotic cells, since this phospholipid is present in sufficient quantity only in the cytoplasmic monolayer of the plasma membrane and exocytotic vesicles, phosphatidylserine as a result of phospholipid asymmetry in these membranes (Verkleij et al., 1973; Rothman and Lenard, 1977). Furthermore, the fact that phospholipid compositions of biomembranes in nature may vary widely - compare the lipid composition of the membranes of gram-negative bacteria, the plasma membrane of eukaryotic cells and the chloroplast membranes - it is quite clear that membrane fusion does not require the presence of a special lipid.

HEXAGONAL II LIPIDS

Recently it has been proposed that lipids which prefer the hexagonal II phase upon isolation from the membrane are involved in membrane fusion (Cullis and Hope, 1978; Verkleij et al., 1979; Cullis and De Kruijff, 1979). Such a type of lipid is present in almost every membrane. The organization of lipids in the hexagonal II phase can be detected with X-ray diffraction (Luzzati and Husson, 1962; Luzzati et al., 1968, 1974), freeze fracturing (Deamer et al., 1970; Verkleij and De Gier, 1981) and ^{31}P Nuclear magnetic resonance (^{31}P NMR; Cullis and De Kruijff, 1978). Figure 2 shows the characteristic ^{31}P NMR spectra and freeze-fracture morphology of the bilayer and hexagonal II phase.

Examples of hexagonal II phase-forming lipids are unsaturated phosphatidylethanolamines (Reis-Husson, 1967; Rand et al., 1971; Cullis et al., 1978a), monogalactosyldiglycerides (Shipley, 1973) and monoglucosyldiglycerides (Wieslander et al., 1978; De Kruijff

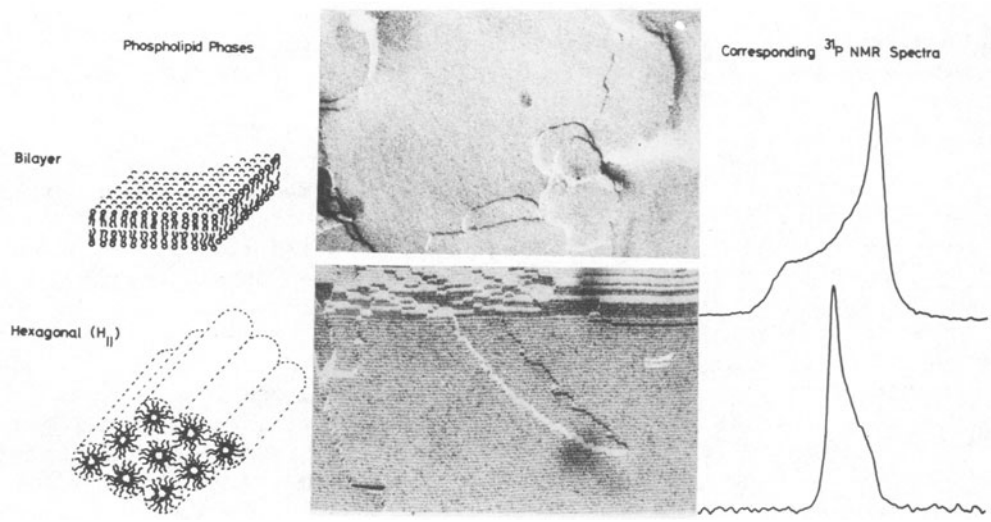


Fig. 2. Molecular arrangements of phospholipids in the bilayer and hexagonal II phases, with their characteristic freeze-fracture morphology and ^{31}P NMR. Micrographs x 50,000.

et al., 1979). The lipid cylinders in the hexagonal II structures have diameters of about 50-70 Å (Luzzati et al., 1968; Rand and Sengupta, 1972). Other hexagonal II phase-forming lipids are the negatively charged phospholipids cardiolipin (Deamer et al., 1970; Rand and Sengupta, 1972; Cullis et al., 1978b) and phosphatidic acid at neutral pH (Papahadjopoulos et al., 1976; Verkleij et al., 1981), which can be converted isothermally from the bilayer structure to the hexagonal II phase by the addition of Ca^{2+} and Mg^{2+} ions, respectively. In the case of cardiolipin and phosphatidic acid it has been shown that the local anaesthetics dibucaine and chlorpromazine can produce a similar phase change (Cullis et al., 1978a; Verkleij et al., 1981). Recently it has been found that hydrophobic peptides like gramicidin can induce the hexagonal II phase in phosphatidylcholine bilayers (Van Echteld et al., 1981).

The bilayer-hexagonal II transition in systems of purified unsaturated phosphatidylethanolamines is remarkably abrupt and occurs within a temperature range of only a few degrees. Differential scanning calorimetric studies show that the enthalpy change involved in this structural rearrangement is very small compared to the amount of heat taken up needed to melt the solid bilayer phase. Since the polymorphic transition occurs above the gel-liquid-crystalline transition of the bilayers, it is likely that the hexagonal II phase is in the liquid-crystalline state.

MIXTURES OF BILAYER AND HEXAGONAL II FORMING LIPIDS

The presence of hexagonal II forming lipids in mixtures with bilayer forming lipids will tend to destabilize the bilayer structure and possibly allows the occurrence of non-bilayer phases in membranes. With ^{31}P NMR an unexpected behavior in these lipid mixtures was found revealing the presence of hexagonal II phase, the lamellar phase and of a narrow spectral component, indicating isotropic motional averaging. With freeze fracturing a variety of structural features have been encountered which in principle can be found in a variety of lipids of which one of the lipids prefers the hexagonal II phase.

First of all, one can find a conglomerate of particles like in phosphatidylcholine/cardiolipin in excess of Ca^{2+} (Verkleij et al., 1979; Van Venetië and Verkleij, 1981), cardiolipin and chlorpromazine (Verkleij et al., 1981), monogalactosyldiglyceride/digalactosyldiglyceride (Sen et al., 1981), phosphatidylcholine/phosphatidylethanolamine (Hui et al., 1982) and monoglucosyldiglyceride/diglucosyldiglyceride (Fig. 3). These particles vary from 100 to 150 Å in diameter in dependency of the system. The particles are packed in a hexagonal or rectangular lattice, which is consistent with inverted micelles in a close packing. Alternatively, this structure may reflect a cubic phase in which two separated aqueous compartments are present, a model which has been put forward by Fontell (1981).

A second feature found in these lipid mixtures is the presence of cylinders with different diameters. Figure 4 shows two examples of this feature. Table 1 shows the diameter of the hexagonal II cy-

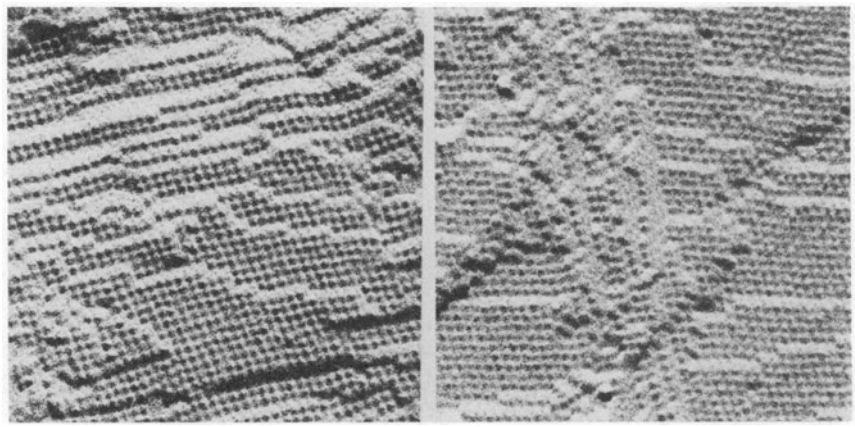


Fig. 3. Monoglucosyldiglyceride/diglucosyldiglyceride in a molar ratio of 3:1 in excess of water. x 100,000 (Wieslander and Verkleij, to be published), frozen from 50°C with jet-freezing.

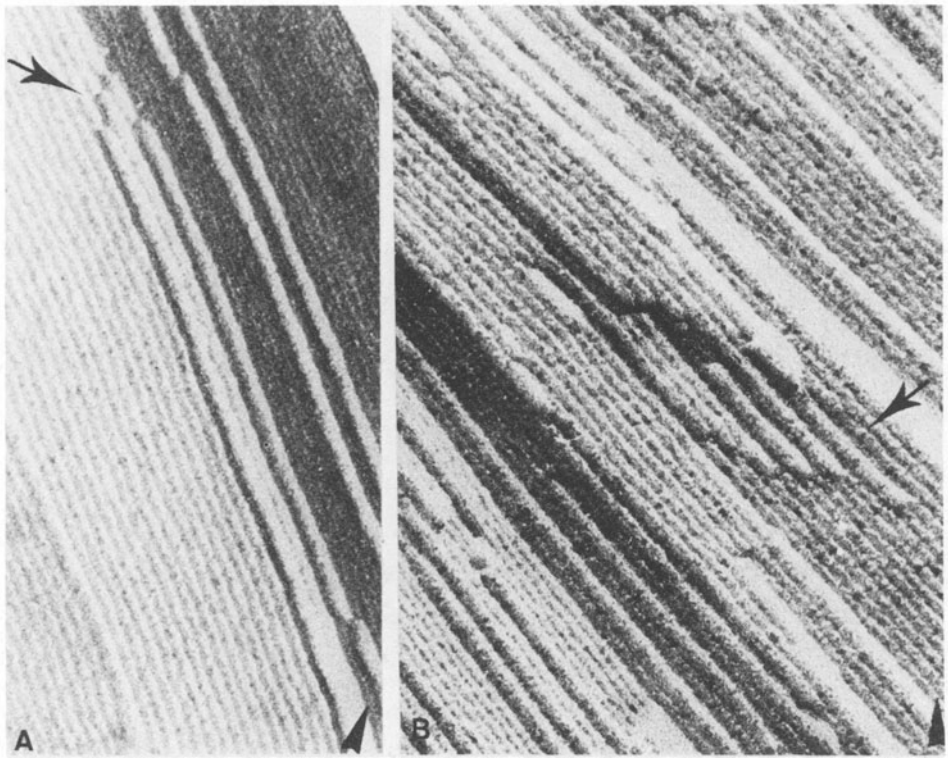


Fig. 4. Two types of hexagonal II tubes with distinct diameters exist in phosphatidylcholine/cardiolipin mixtures, in the presence of Ca^{2+} (A) and Mg^{2+} (B). The arrows indicate thick type tubes. x 200,000.

linders in pure hexagonal II phases and those in mixed lipid systems (Van Venetië and Verkleij, 1981).

A third feature is the presence of smooth fracture faces being continuous with respect to their fracture plane with the hexagonal II phase tubes, i.e., the tubes divergate into smooth fracture faces as for instance in Fig. 5 (Van Venetië and Verkleij, 1981). A model for this transition can be seen in Fig. 6.

Finally, one finds "lipidic particles" and their complementary pits on smooth fracture faces. The particles may be arranged in three dimensional agglomerates as mentioned above or lined up, frequently changing into hexagonal II phase tubes (Verkleij et al., 1980; Van Venetië and Verkleij, 1981) or they can be present solely on fracture faces of smooth fracture faces (De Kruijff et al., 1979; Fig. 7).

Other types of particles (cusp-like) have been found as well in addition to the more well-defined particles (Hui and Stewart, 1981).

Table 1. Periodicity measurements of the hexagonal II phase tubes.

	Diameter (nm)			
	H_{II}	H_{II}*	H_{II}**	Lipidic particle
DOPE	7.4			
DOPE/DOPC/cholesterol	8.6	-	-	9.5
Ca/DPG	5.2			
Ca/DPG/PC	-	7.3	14.0	8.5
Mg/DPC	6.5			
Mg/DPG/PC	6.4	11.5	-	13.0
Mn/DPG	7.5			
Mn/DPG/PC	7.3	9.7	-	13.0

H_{II}* indicates the thick type tubes. H_{II}** represents the extra thick tube type, as found in the Ca/DPG/PC system only. The diameter value of the lipidic particles is represented as the mean of the particle and pit diameter.

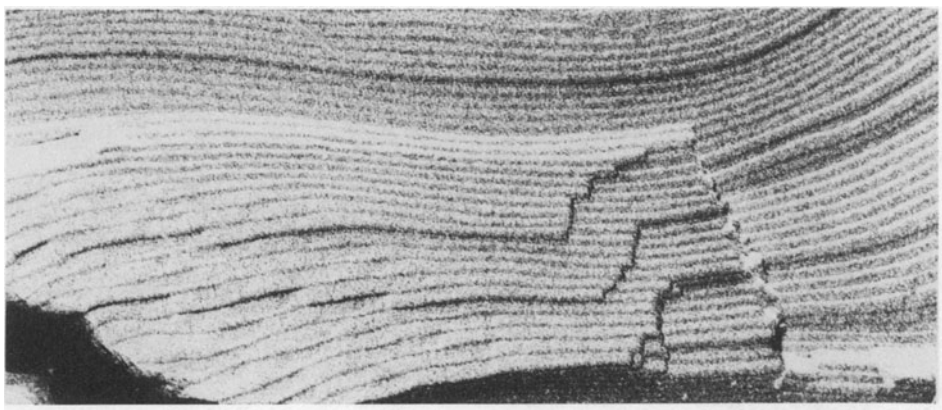


Fig. 5. Hexagonal II tubes, gradually divergating into stacked layers in dioleoylphosphatidylethanolamine/dioleoylphosphatidylcholine/cholesterol mixture (molar ratio: 3:1:2). x 100,000.

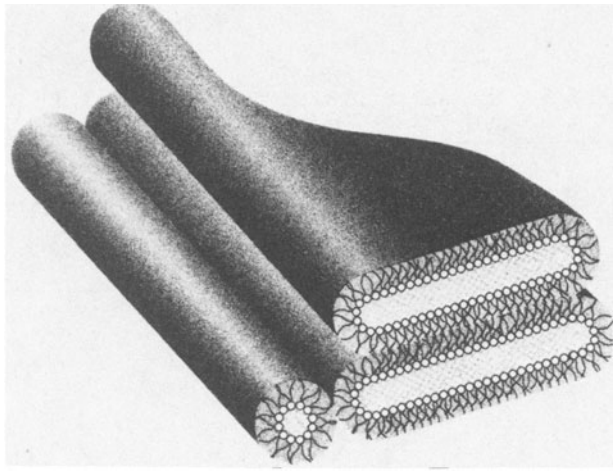
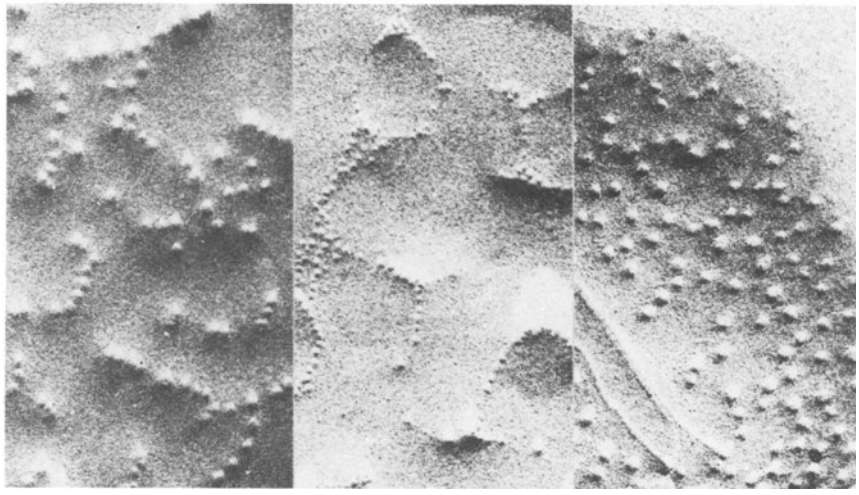


Fig. 6. The hexagonal II to lamellar phase transition.



CARD/PC MGDG/PC PE/PC/CHOL

Fig. 7. Examples of phospholipid systems showing intramembraneous particles; cardiolipin and egg phosphatidylcholine (CARD/PC; molar ratio 1:1) in the presence of Ca^{2+}; monoglucosyldiglyceride and egg phosphatidylcholine (MGDG/PC; molar ratio 1:1) at 10°C, after being heated to 60°C; dioleoylphosphatidylethanolamine/dioleoylphosphatidylcholine/cholesterol (PE/PC/CHOL; molar ratio 3:1:2) at 10°C, after being heated to 60°C. x 200,000.

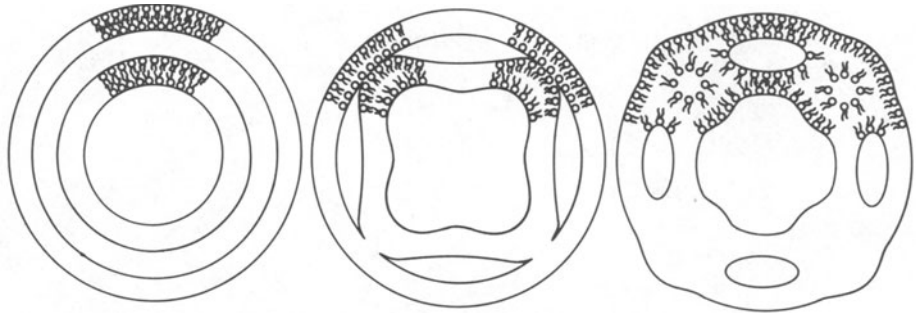


Fig. 8. Model for fusion between bilayers via inverted micelles.

At present the discussion with respect to the nature of the particles is not completed but it is generally accepted that these lipidic particles represent structures at the fusing site or nexus of two or more membranes. The cusp-shaped particles could be prefusing contact structures whereas the well-defined lipidic particles are inverted micelles at the fusing site as depicted in Fig. 8.

MEMBRANE FUSION

As visualized in Fig. 8, non-bilayer structures like inverted micelles and small tubes of lipids, organized in a similar way as the hexagonal II phase, could be intermediary stages in membrane fusion. Such an intermediary structure has been proposed earlier (Lau and Chan, 1975; Pinto da Silva and Noqueira, 1977). This hypothesis has been precised by Cullis and Hope (1978) who suggested that the fusogenic capacity, which proceeds via a non-bilayer "inverted micelle", is derived from lipids which prefer the hexagonal II phase.

The involvement of hexagonal II type lipids in fusion has been confirmed by experiments with model membrane systems. It has been shown that vesicles composed of an equimolar mixture of bovine heart cardiolipin and egg-lecithin fuse upon the addition of Ca^{2+} (Fig. 9; Verkleij et al., 1979).

Similar phenomena have been observed with vesicles of dioleoylphosphatidylethanolamine/dioleoylphosphatidylcholine/cholesterol at a molar ratio of 3:1:2 (Verkleij et al., 1980). These vesicles, obtained by sonication, fuse upon increasing the temperature. This behavior has been found for many mixtures of lipids of which one of the phospholipids prefers the hexagonal II phase (Nayar et al., 1982).

It has been found that in all these systems the fusion is associated with the formation of lipidic particles, frequently found at the fusion interface (Fig. 10). This phenomenon has led to the

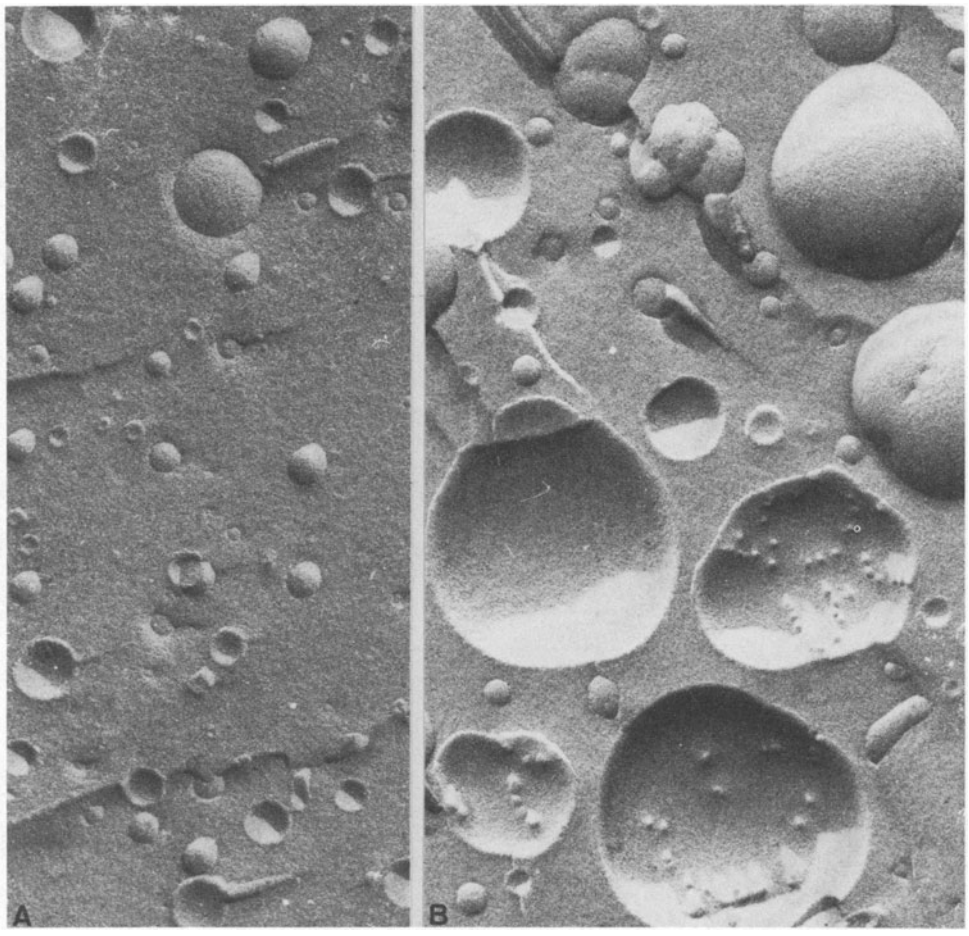


Fig. 9. Fusion of lipid vesicles composed of an equimolar mixture of cardiolipin and lecithin before (A) and after (B) addition of Ca^{2+}.

hypothesis that these lipidic particles represent inverted micelles, which are intermediary structures in fusion. Such micrographs were obtained after incubation times longer than 10 min. Moreover, glycerol as cryoprotectant has been used.

Recent kinetic experiments (Wilschut et al., 1982) have demonstrated that fusion of vesicles prepared from an equimolar mixture of cardiolipin and lecithin is extremely fast (on the time scale of sec) and that this fusion is non-leaky. Therefore we (Verkleij and Wilschut, unpublished results) have repeated our initial fusion experiments using the fast-freezing method of spray freezing. Vesicles

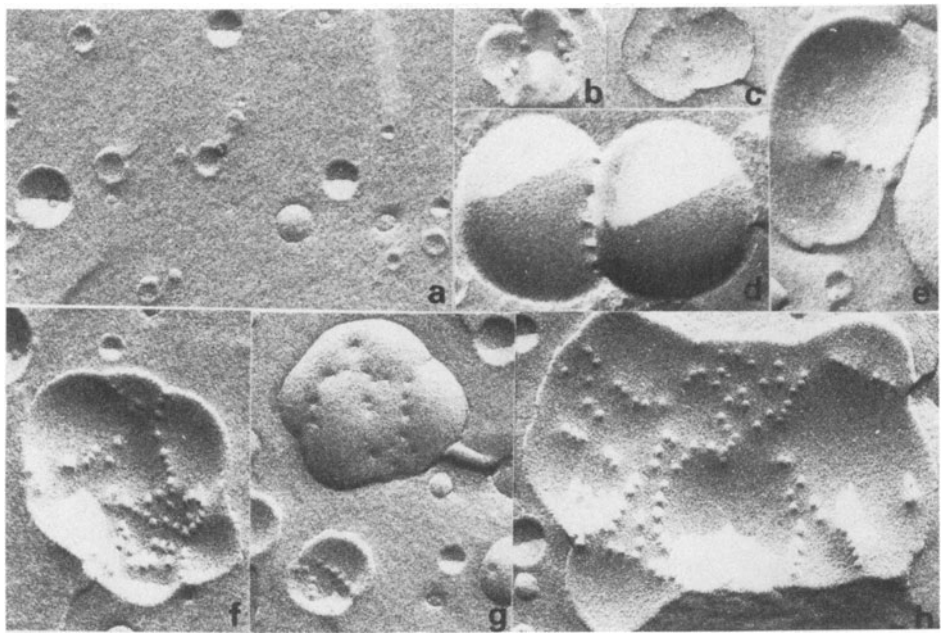


Fig. 10. Fusion of lipid vesicles composed of an equimolar mixture of cardiolipin and egg-lecithin. (a) Without Ca^{2+}, (b-h) representative for Ca^{2+} concentrations between 2-10 mM $CaCl_2$. x 100,000.

composed of cardiolipin and lecithin were quenched within seconds upon addition of Ca^{2+}. Figure 11 shows that larger vesicles are formed within this time scale. However, lipidic particles at the points of fusion between vesicles have not been identified. Similar results have been obtained in other mixtures like phosphatidylethanolamine/phosphatidylcholine/cholesterol/phosphatidylserine upon addition of Ca^{2+}. These mixtures show lipidic particles upon longer incubation times. So at present it is not clear whether lipidic particles are really intermediary in membrane fusion or only equilibrium structures after fusion. On the other hand, it is not excluded that the life time of initial intermediate fusion structures is too short (less than a thousand of a second) to be visualized with freeze fracture electron microscopy.

In summary, it is clear that vesicles composed of a lipid mixture, of which one of the lipids prefers the hexagonal II phase, fuse. This fusion is extremely fast and, as has been shown for one mixture, non-leaky. On the other hand, the nature of the intermediate structure during fusion is not revealed.

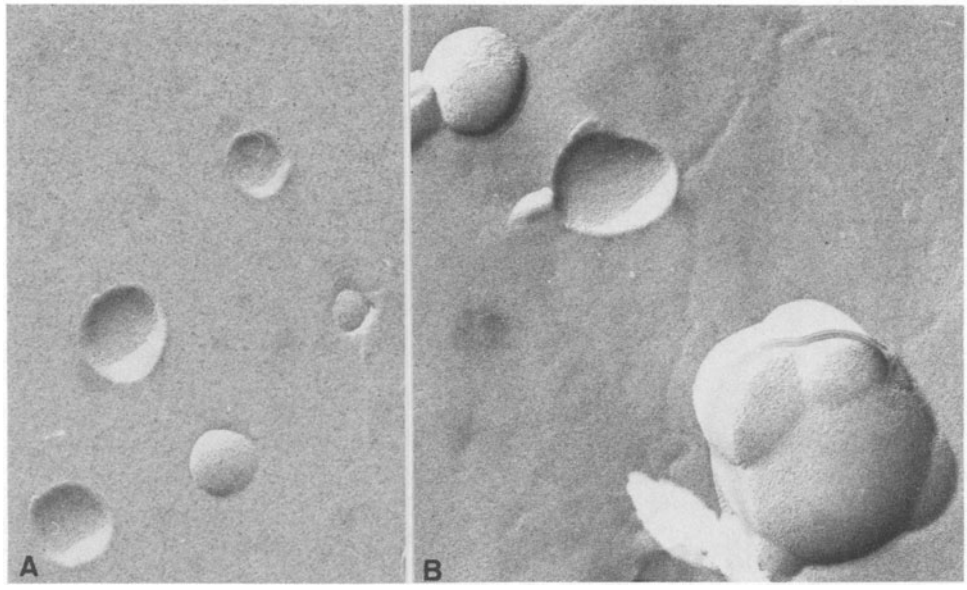


Fig. 11. Fusion of lipid vesicles composed of an equimolar mixture of cardiolipin and egg-lecithin made by reverse phase evaporation (Wilschut et al., 1982), quenched by spray freezing. (A) Without Ca^{2+}, (B) 2 sec after addition of Ca^{2+} in concentrated form (20 mM) resulting in a final Ca^{2+} concentration of 5 mM.

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