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# Fusion in Phospholipid Spherical Membranes
## I. Effect of Temperature and Lysolecithin

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*Summary.* A study concerning membrane contact and fusion phenomena was made for phospholipid spherical bilayer systems with respect to temperature. Specific temperatures were obtained for the spherical bilayer membranes of phosphatidyl choline (PC) and phosphatidyl serine (PS) which indicated a greater degree of membrane fusion and were designated $T_f$ (the fusion temperature – PC: 43 °C, PS: 38 °C). These temperatures were reduced by about 10 °C for the membranes incorporated with 20% lysophosphatidyl choline. The results of the contact and fusion observed in the spherical membranes are compared and discussed with the conductance characteristics of the PC and PS planar bilayer membranes as well as dissolution study on the phospholipid monolayers formed at the air/water interface with respect to temperature. Also, a possible molecular mechanism of membrane fusion is discussed in terms of the fluidity and instability of the membrane.

The use of model membranes has become an important tool for explaining functional and structural changes in biological membranes. Recently, a great deal of attention has been focused on the physical states of lipids in relation to membrane structure and the effect this may have on many cellular functions (Oldfield & Chapman, 1972). The degree of fluidity of the hydrocarbon chains of the lipid bilayer allows the chains to undergo changes from an ordered to a disordered configuration, which could be significant for membrane function, not only in the area of membrane transport but also in the phenomena of cell-cell contact and fusion (Steim *et al.*, 1969; Esfahani *et al.*, 1971; Traüble & Haynes, 1971; Papahadjopoulos *et al.*, 1973*b*; Chapman & Urbina, 1974; Traüble & Eibl, 1974). In this respect some authors have tried to correlate the phase transition of lipid bilayers with membrane fusion (Papahadjopoulos, Poste & Schaeffer, 1973*a*; Papahadjopoulos, Poste, Schaeffer & Vail, 1974; Prestegard & Fellmeth, 1974). Although the phase transition may be related to membrane fusion, since most naturally occurring phospholipids are already in the liquid crystalline state in the membrane at physiological temperatures, it seems relevant that our attention should be centered about the degree of fluidity of the membrane in order to characterize

the phenomena of cell contact and fusion for biological systems. Fluidity was shown to be an important criteria for fusion as demonstrated in the chemically and thermally induced fusion studies of Ahkong *et al.* (1973). In a recent review on membrane fusion, Poste and Allison (1973) proposed a mechanism for fusion which relies on the fluidity of the membrane as one of the prerequisites for membrane fusion.

There is evidence that many cellular processes depend upon what has been characterized as increased fluidity in the membrane structure. It has been suggested that changes in the ionic environment can cause conformational changes in the bilayer structure (Ohki & Aono, 1970; Traüble & Eibl, 1974). Incorporation of some substances could affect the membrane fluidity and increased temperature could also induce conformational changes in the bilayer structure as demonstrated by Oldfield and Chapman (1972) and by Chapman and Urbina (1974). Chapman and co-workers have clearly established that at the critical temperature $T_c$, a lipid phase transition occurs which results in a "fluidization" of the bilayer, or an increase in the mobility of the hydrocarbon chains of the lipid molecules (Chapman *et al.*, 1966, 1967).

A great variety of techniques have been used to study the degree of fluidity of lipid bilayers, ranging from differential scanning calorimetry (DSC) to the use of NMR, ESR, X-ray diffraction and fluorescence spectroscopy (Chapman & Salsbury, 1966; Hubbell & McConnell, 1971; Oldfield & Chapman, 1972; Papahadjopoulos *et al.*, 1973*b*). Although these techniques are mainly useful for establishing the point of phase transition, they give us little insight as to the effect of increased fluidity on the bilayer structure of the membrane.

Recently, Papahadjopoulos and co-workers have demonstrated that the fusion of phospholipid vesicles was not only directly related to membrane fluidity, but a significant percentage of membrane fusions occurred at or above the transition temperature of the phospholipid studies (Papahadjopoulos, Poste & Schaeffer, 1973*a*; Papahadjopoulos, Poste, Schaeffer & Vail, 1974). The fusion of dimyristoyl-phosphatidylcholine (DMPC) vesicles has been studied by Prestegard and Fellmeth (1974) by the technique of proton magnetic resonance (PMR). Their results seem to be in agreement with the other studies, showing that the rate of fusion dramatically increases at or above the hydrocarbon phase transition of the phospholipid vesicle.

Since it is difficult to study the molecular mechanism of membrane fusion in biological systems, many researchers have relied on model membrane systems, especially the vesicle system as developed by Bangham

*et al.* (1965). While the vesicle system offers many advantages over other model membrane systems, the use of spherical bilayer membranes (Pagano & Thompson, 1967) may offer promise in studying the mechanism of the cell contact and fusion phenomena. Since the spherical bilayers are quite large and are visible with a light microscope, their electrical parameters can be studied by the injection of microelectrodes into the membrane. Bilayer formation can be clearly observed, membrane size can be regulated and the dynamic behavior of the membrane in the cell fusion process can be photographed.

Liberman and Nenashev (1972*a*, *b*) have successfully investigated membrane contact and adhesion using hemispherical bilayer membranes. In their experiments, membranes were not detached into solution and they measured changes in resistance between membranes as they came into contact. Neher (1974) has also recently studied hemispherical bilayer membrane fusion using an apparatus similar to Liberman's, but in these experiments fusion is not a measure of two membranes forming one, but rather the formation of a new structure over only part of the membrane. In our studies, membrane fusion is the result of two membranes coming into contact and forming one.

The study of contact and fusion in spherical phospholipid bilayers (PC and PS) with temperature, allows us to examine the increasing effects of membrane fluidity which appears to be responsible for fusion. Monolayer dissolution studies of PC and PS provide a good indicator for fluidity in the lipid molecules and give us correlation for our bilayer studies. The study of bilayer conductance which obtains membrane breaking points with varying temperature and pH is helpful for examining the structural distrubances that may give insight into our experimental results on the spherical bilayers. In conclusion, fusion will be discussed in reference to both membrane structure and membrane fluidity.

## Materials and Methods

Phospholipids for the spherical membrane studies were purchased from Applied Sciences and Supelco Laboratories. Applied Science samples of 10 mg were stored in chloroform solution, and Supelco samples of 25 mg were divided into three samples of 8.3 mg each which were also stored in chloroform in the refrigerator at −15 °C. The purity of these samples is said to be more than 97%. All lipid samples were chromatographically pure and showed only a single spot under TLC analysis. In preparing the membrane-forming solution (after the evaporation of chloroform with nitrogen gas) each 10 mg of phosphatidylcholine (egg) was dissolved in 0.4 ml chloroform, 0.3 ml methanol and 0.3 ml *n*-decane. For 10-mg samples of phosphatidylserine (bovine), the membrane-forming solution contained 0.5 ml chloroform, 0.2 ml methanol and 0.4 ml *n*-decane. The concentrations of the individual solvents were of extreme importance and had to be kept at the proper ratios

in order for the membranes to form properly. It was also observed that chloroform is the key solvent for having a proper membrane-forming solution, and it had to be constantly checked to compensate for its evaporation in the air. When lipid samples were not in use they were stored under nitrogen gas in the freezer at −15 °C. For the experiments with lysolecithin (Applied Science), 20% lysolecithin (egg) by weight was incorporated into the samples of PC and PS. Solvent concentrations were kept the same, in order to maintain proper membrane-forming solutions.

Spherical bilayer membranes were formed in a layered concentration gradient containing a base solution (bottom phase) of 4.5 M NaCl with an upper solution of 0.01 M NaCl. The inside solution for the membrane was 0.2 M NaCl. This procedure was similar to that used by Pagano and Thompson (1967). The inside solution was stored in a microsyringe and was delivered through a polyethylene tube. Lipid was drawn into the tube and was then pushed out with the inside solution forming a bubble; the diameter of the bubble was in a range of a few to several millimeters. Through manipulation, the bubble was detached into the gradient, and the bubble membranes remained in the upper gradient phase. Membranes were observed through a low power microscope and the bubble membranes were illuminated with a microscope illuminator (American Optics). Bilayer formation was observed as the spherical bilayer membranes started to thin out (Fig. 1*a* and *b*). The bilayer formation took place on the bottom of the membrane as the black lipid film began to form. Capacitance measurements were used to confirm that the black membrane in Fig. 1*b* was a bilayer. Total bilayer formation took approximately 15 min and stopped at the membrane cap (composed mostly of excess lipid and decane). Membranes were found to be extremely stable once bilayer formation had occurred. Temperature was varied during the

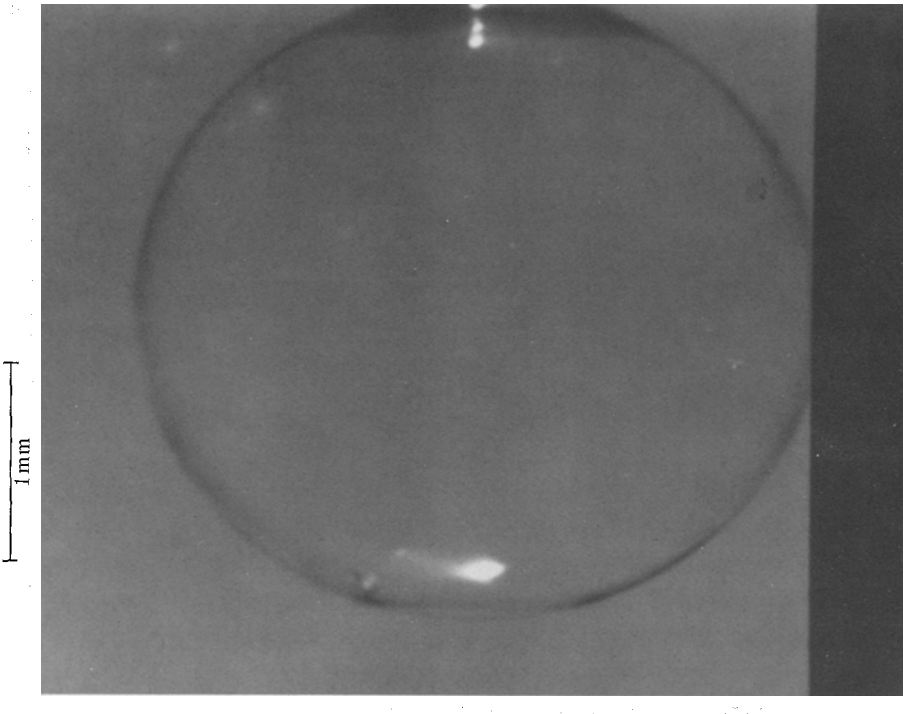


Fig. 1. (*a*) Spherical membrane prior to bilayer formation. (*b*) Spherical bilayer membrane, (an arrow indicates the bilayer formation region)

experiment by using a water jacket and a thermo regulator (Neslab Instruments). pH measurements were taken at the beginning and end of each study to insure that the proper pH range was not exceeded.

Membranes were formed in close proximity (within several millimeters) to facilitate membrane contact. For each study, approximately 10 membranes were made and all temperature-dependent studies were started once bilayer formation was complete. Temperature was increased at 5-min intervals after the temperature of the solution had reached a stationary value equivalent to the experimental temperature. Studies were started at 25 °C and were completed at 60 °C. Also to insure proper interpretation to these results where the temperature was increased successively, another series of experiments were also done by forming membranes at fixed temperatures in the range of 25–50 °C and observing the results at the individual fixed temperatures. All photography was done with a 35 mm Leica camera that adapted into the binocular scope. Kodak SO4 film was used, which was a photomicrograph film with very high contrast. Some color photography was also done, and for this work Kodak PCF 135 was used, which was also a photomicrograph film with high contrast.

In the monolayer studies phospholipids were the same as in the spherical membrane studies. Those phospholipid samples showing a single spot by thin-layer chromatography were used in experiments. The phosphatidylcholine as purchased was dissolved in chloroform. After driving out the chloroform with nitrogen gas, the PC was dissolved in benzene and stored in refrigeration. Monolayers of these phospholipids were prepared by spreading an aliquot of the phospholipid-benzene solution on the aqueous surface by using a microsyringe. After the evaporation of benzene the surface tension was measured. The benzene (Baker Instra-Analyzed) used for spreading solvent was purified over silica gel, and was found to leave no measurable degree of surface pressure increase after evaporation even when compressed to 1/10 of the original area. Water used for substrate was distilled three times

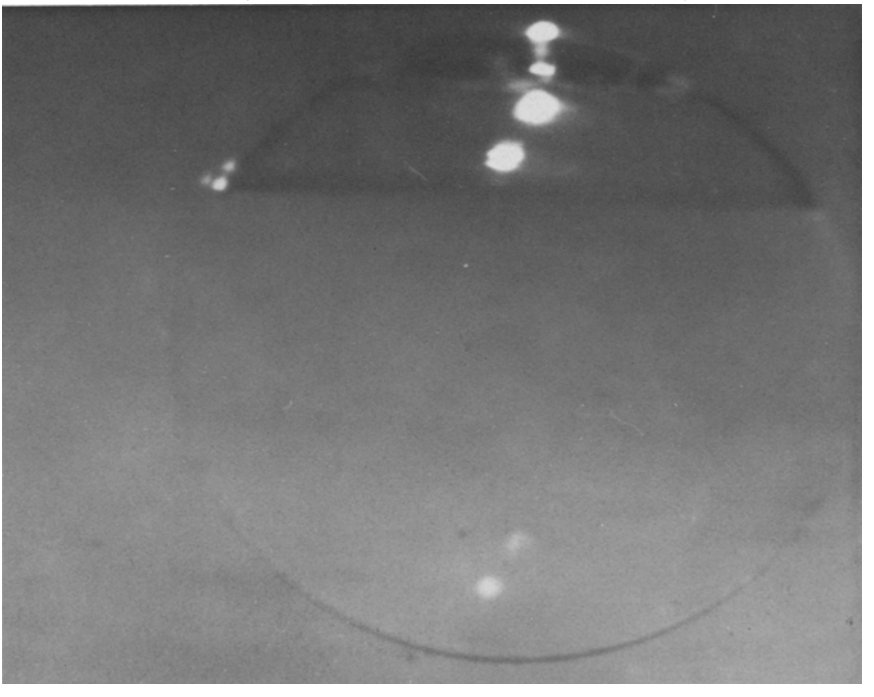

Fig. 1b

including the process of alkaline permanganate distilling. Surface tension was measured by a Cenco du Nouy surface balance using a $17 \times 17 \times 0.2$ mm microscopic cover slip as a Wilhelmy plate. Great care had to be taken for wettability of the glass plate to ensure a contact angle of 0°, and to make sure the receding condition was always satisfied. For a further explanation of the monolayer technique refer to Seimiya and Ohki (1972).

For the planar bilayer studies the phospholipids used were as previously stated. The phospholipid samples were dissolved in chloroform which was driven out by blowing nitrogen gas. The membrane-forming solutions of PC and PS were prepared by dissolving 10 mg of the lipid into 0.5 ml of *n*-decane (Fluka).

Bilayer membranes were formed over the hole of a Delrin vessel with a diameter of 1.59 mm. For the procedure of preparing a Delrin vessel refer to Ohki (1969). The membrane films were prepared by smearing the membrane-forming solution across the hole of a Delrin vessel with a teflon applicator. The membranes were formed in 0.1 M NaCl at a temperature of $25 \pm 1$ °C. The films formed across the hole were observed through a low power microscope (Unitron MSF) in light reflected by the film from a microscope illuminator (American Optics). In order to obtain a membrane resistance, a constant d–c voltage of 20 mV was applied across the bilayer. Membrane current was measured by a sensitive ammeter (DC Micro Volt-Ammeter, Hewlett Packard) and recorded by a strip chart recorder (Bausch-Lomb VOM7). Temperature was varied by the use of a water jacket and a thermo regulator (Neslab Instruments). Temperature of the solution surrounding the membrane was used for all measurements. pH was controlled by a Tris-HCl buffer solution and was measured by a standard pH meter (Radiometer).

## Results

Spherical bilayer membranes of phosphatidylcholine and phosphatidylserine were formed in a concentration gradient in close proximity to each other. We examined membrane contacts and fusions with increasing temperature for each phospholipid. For the purpose of this experiment membrane contact is defined as the formation of a stable doublet as shown in Fig. 2. Our observations indicate that the contact region between two spherical bilayers can be defined by the approach of two planar bilayers (as is indicated in Fig. 2). At this time, we are not sure whether this contact occurs at the primary minimum or secondary minimum (as described by the theoretical work of Weiss & Harlos, 1972). Fusion is defined as the two cells (doublet) forming one. Figs. 3 and 4 indicate the results obtained for phosphatidylcholine and phosphatidylserine membranes. The contact curves for PC and PS membranes appear to be very similar; but it should be noted that the cell contact curve is not an accurate indicator of the effect of temperature for membrane contact as other factors may also be involved (e.g. convection current, gradient diffusion). Our measurement of membrane fusion was based on only those membranes that had come into contact. In the case of PC membranes approximately 80% of the membranes came into contact (the remaining 20% either broke or did not enter contact) and 70% of these ultimately fused. Of

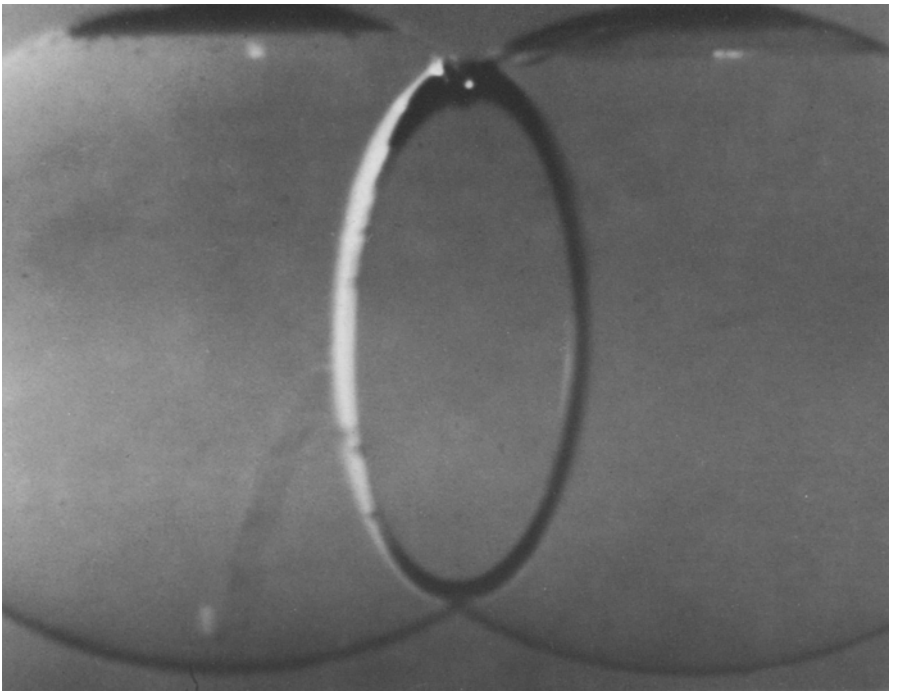

Fig. 2. Two spherical membranes in contact

the remaining 30% that did not fuse, approximately 8% remained as stable doublets, while the remaining ones (22%) broke. The curve for PC membrane fusion indicates a steep rise in the percentage of fusions at 43 °C. Our observations indicate that this temperature seems to be a critical point (which is not due to the occurrence of a phase transition) for PC membranes and may represent some structural change in the bilayer membrane due to increased fluidity. The PC curve was compiled from 20 studies and contained the results of over 200 membranes. As stated in Materials and Methods, another series of experiments were done to confirm the results obtained. Using our second technique, in which membrane fusion was looked at, at specific fixed temperatures, the results were equivalent as the greatest increase in membrane fusion occurred between 43–45 °C. The PC membranes exhibited a breaking point in the range of 52–56 °C. This agrees well with our conductance studies of planar bilayers done with this phospholipid. The pH of the solution changed from 6.0 to 6.6 during the study which should have no effect on the above results. Diffusion of the concentration gradient was checked with a dye and was found to be stable with temperatures up to 65 °C and for time intervals of 2 hr or more.

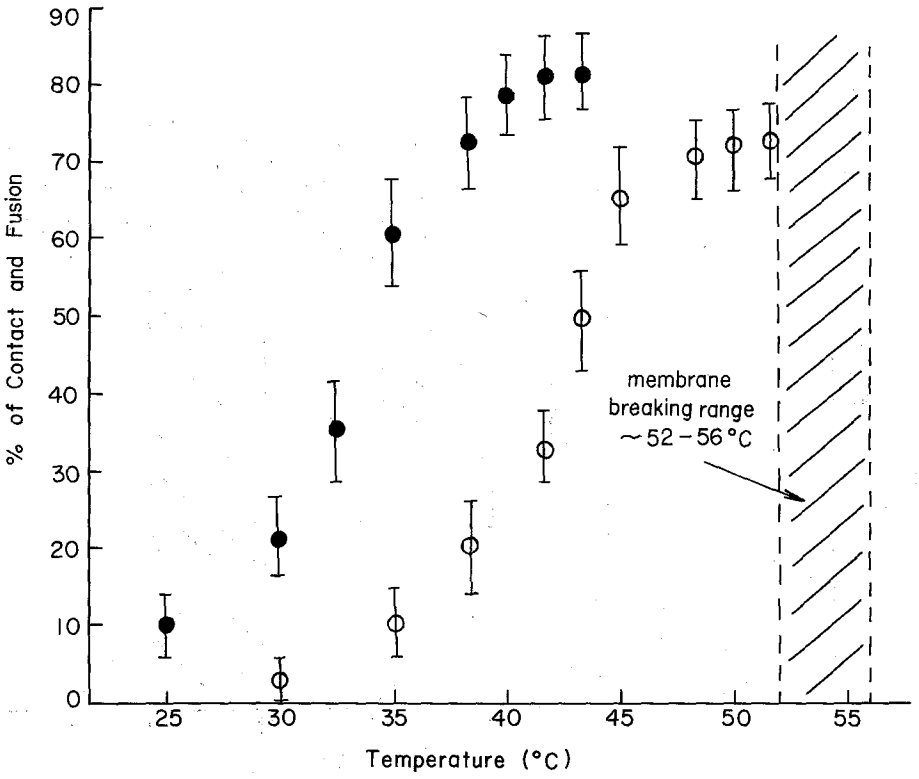


Fig. 3. Contact and fusion in PC membranes. ●,% of PC membranes which come into contact as a doublet; ○,% of PC membranes in contact which fuse. Error bar was calculated using the standard error (SE) formula:

$$SE = \left\{ \sum_{i}^{N} (x_i - \bar{x})^2 / N - 1 \right\}^{1/2}$$

For PS spherical bilayers (Fig. 4) 75% (the remaining 25% either broke or did not come into contact) of the membranes came into contact and approximately 65% of these fused. Of the remaining 35% that did not fuse only 5% remained as stable doublets, as close to 30% of the membranes in contact broke. It should be mentioned that PC spherical bilayers form more stable membranes than the PS bilayers and could account for the above results. A sharp rise in the curve for PS membrane fusion occurs at 38 °C. As pointed out in the case of PC spherical bilayers, we believe that this point of increased fusion is significant and may correspond to a certain degree of structural change in the PS bilayer membrane. For the PS membranes, 20 individual studies were done, representing the results of close to 200 membranes. The breaking point for PS membranes occurred in the range of 45–50 °C, somewhat lower than the PC

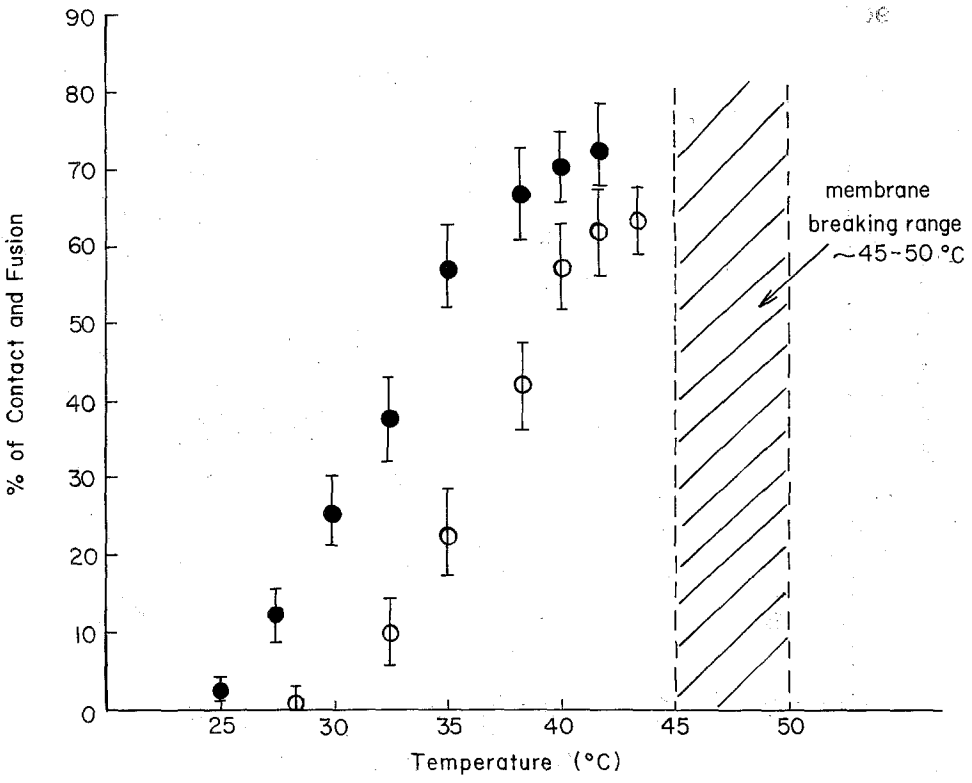


Fig. 4. Contact and fusion in PS membranes. ●,% of PS membranes which come into contact; ○,% of PS membranes in contact which fuse. Error bar was calculated using the SE formula

membranes. We also looked at membrane fusion by using our second technique in which membrane fusion was examined at specific fixed temperatures. The results closely resembled each other as the greatest percentage of membrane fusion occurred between 36–39 °C.

Spherical bilayer studies were also done by incorporating lysolecithin into the membranes of phosphatidylcholine and phosphatidylserine (Figs. 5 and 6). The same criteria were used for these studies as in the other spherical bilayer work. Figs. 5 and 6 indicate the results obtained for PC and PS membranes with 20% lysolecithin by weight, and as can be seen, only the fusion curve is represented which is an indicator of the percentage of membranes in contact that fuse. For PC membranes (Fig. 5) with 20% lysolecithin the results seem to resemble the PC membrane curve without lysolecithin. The only difference is that the curve for membrane fusion rises steeply at 32 °C, approximately 10 °C less than without lysolecithin. The breaking range for the PC membranes was also lowered probably because the lysolecithin caused a great deal of membrane

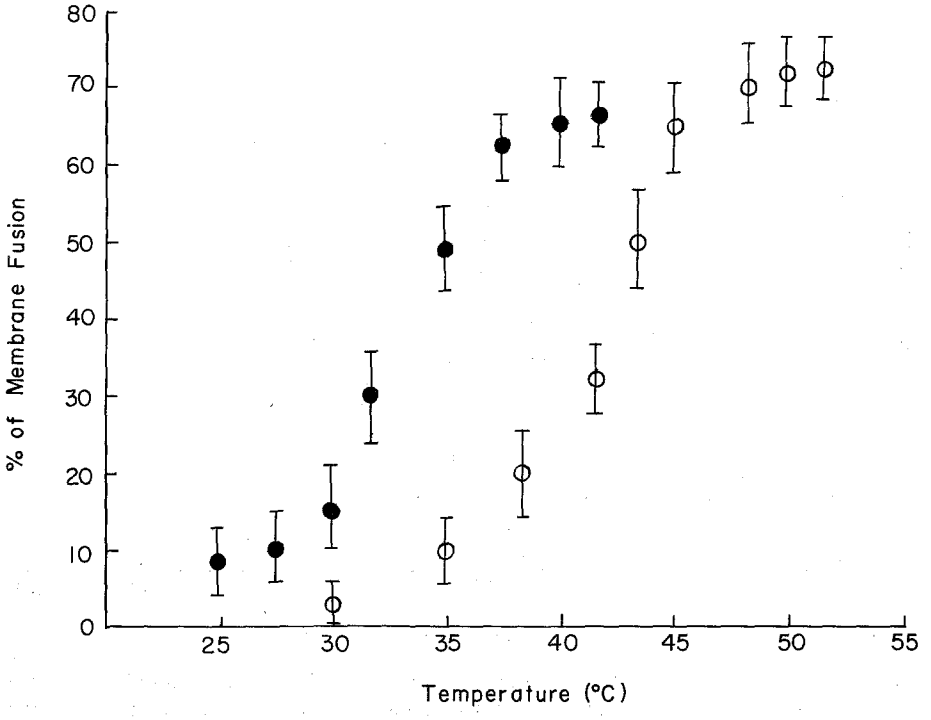


Fig. 5. The effect of lysolecithin on PC membrane fusion. ●,% of PC +20% lyso PC membranes in contact which fuse; ○,% of PC membranes in contact which fuse. Error bar was calculated using the SE formula

instability (40–45 °C). For spherical bilayers of PS (Fig. 6) with 20% lysolecithin the fusion curve rises sharply at approximately 26 °C, which is almost 10 °C less than the results observed without lysolecithin (similar to the results obtained with PC). Higher concentrations of lysolecithin were tried but membrane formation was extremely difficult, and membranes were very unstable.

Our monolayer studies investigated the dissolution of phosphatidylcholine and phosphatidylserine monolayers with increased temperature. A similar study was conducted by Seimiya and Ohki (1972) but only PS was studied at pH 6.0. By calculating $\pi$-$A$ ($\pi$ is a measure of surface pressure) and $\pi$-$t$ curves for PC and PS monolayers, the rate of dissolution can be calculated. An estimate of the error in $\pi$ was calculated and found to be $\pm 0.5$ dynes/cm. Also surface tension of water was calculated at different temperature and was found to compare with the known values by $\pm 0.3$ dynes/cm. Fig. 7 is a measure of $\dot{\pi}(=d\pi/dt)$ with temperature which is an indicator of the rate of surface pressure decrease with temperature for the monolayers of PC and PS. $\dot{\pi}$ is calculated from the $\pi$-$t$ (in

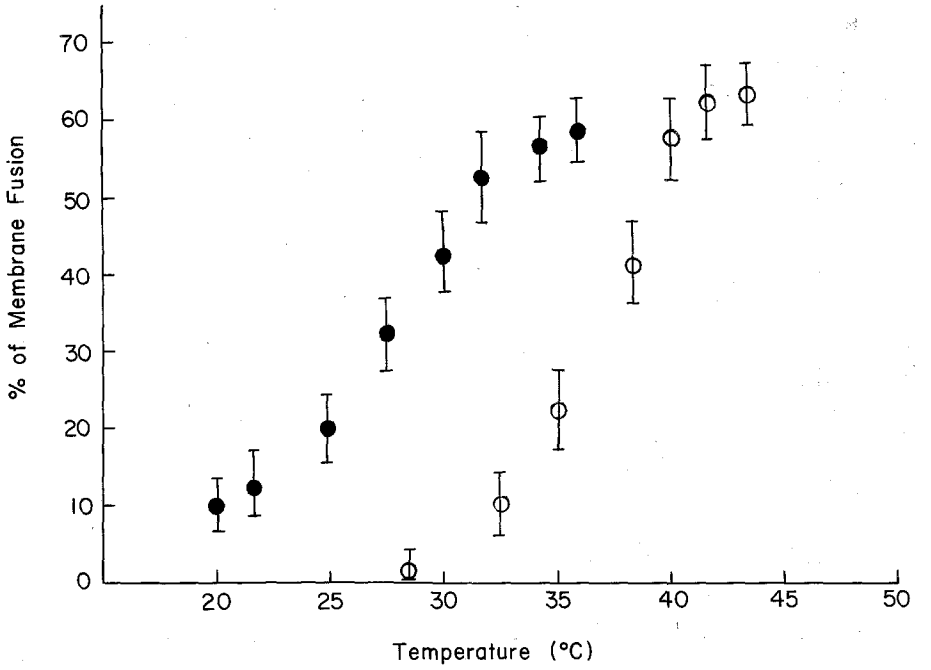


Fig. 6. The effect of lysolecithin on PS membrane fusion. ●,% of PS+lyso PC membranes in contact; ○,% of PS membranes in contact which fuse. Error bar was calculated using the SE formula

which the initial surface pressure corresponded to an area of 65 Å$^2$ per molecule for both phospholipids used) curve and corresponds to the slope taken at 7.5 min after the spreading of the monolayer. Since this is a first-order kinetic process (indeed we observed that the plot of ln $k$ vs. $1/T$ is a straight line as would be expected in a first-order kinetic process), the slope can be taken at any point in the curve except for the first few minutes. The reason for this is that within the first few minutes, the dissolution process does not follow a first-order kinetic model possibly due to the effect of evaporation of solvent. As Fig. 7 indicates, the dissolution of PS monolayers rises sharply at approximately 38 °C, and the dissolution of PS appears to be very rapid. While the dissolution of PS monolayers is a very rapid process, PC monolayer dissolution is slower and in fact it is of the order of 10 times less than PS. While there is no sharp rise in the dissolution of PC monolayers, its rate does seem to be accelerated at approximately 47 °C. It seems probable that the temperature dependence of the dissolution process may have some relevance to the fusion process.

Planar bilayers of phosphatidylcholine and phosphatidylserine were made over the hole of a Delrin vessel in 0.1 M NaCl. At three different

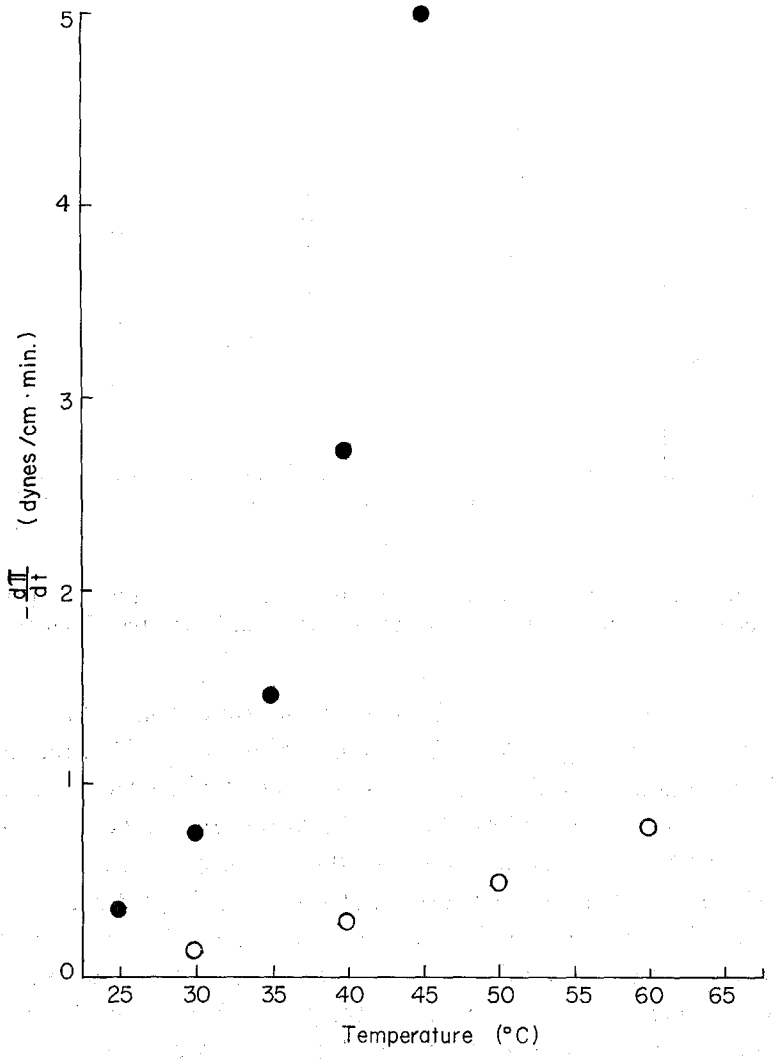


Fig. 7. Rate of surface pressure decrease with temperature for PC and PS monolayers ($\dot{\pi}$ *vs.* T).
● PS monolayer; ○ PC monolayer

pH's (3.5, 5.5 and 7.2) temperature-dependent studies were done on the bilayers to test for the breaking point of the membrane. Twenty millivolts of d-c potential were applied across the membrane and variations in conductance with temperature were measured on a strip chart recorder. Fig. 8 illustrates the results of these experiments. PS membranes exhibit a breaking point at approximately 42 °C at pH 7.2, while PC membranes tend to show a somewhat higher breaking point of 48 °C at pH 7.2. Our conductance measurements of the (PC and PS) bilayers also seemed to indicate

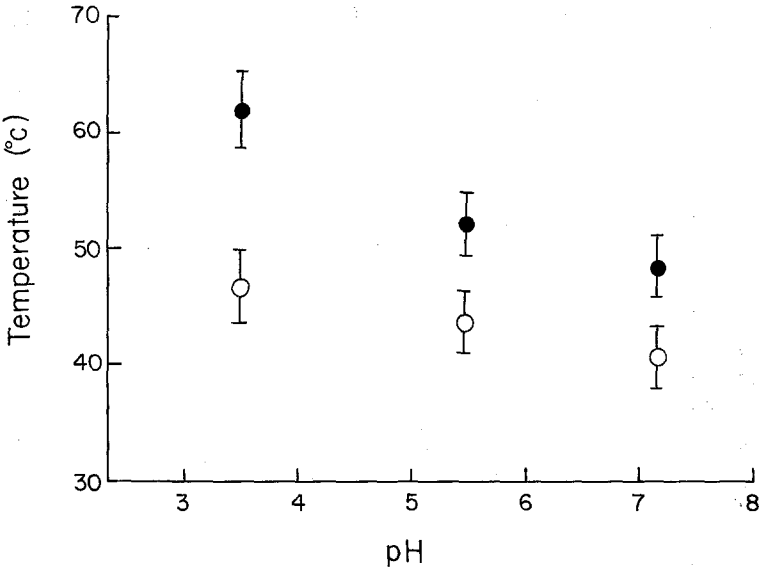


Fig. 8. Membrane breaking point in planar bilayers; Effect of temperature and pH. ● PC membranes; ○ PS membranes. Error bar was calculated using the SE formula

some sudden increasing variations in membrane conductance which occurred on the average 5 °C below the breaking point of the membrane. These conductance variations seem to be associated with structural changes in the membrane. It should be pointed out that these conductance measurements are at best approximations, but they do show some similarity to other bilayer studies done with different techniques. In particular, Ohnishi and Ito (1974), using spin label techniques, showed a certain critical temperature for the phospholipid bilayers of the naturally occurring lipids. Their data seem to correspond well to the planar bilayer results obtained here. The relevance of this data, to the cell fusion process, will be discussed in the Discussion.

## Discussion

Spherical bilayers of phosphatidylcholine and phosphatidylserine undergo most of their fusion at certain temperatures ($T_f$-fusion temperature), while at the same time monolayer and bilayer studies of these phospholipids indicate some physical change to be taking place in the membrane with increasing temperature especially at the corresponding fusion temperatures. It is clear that at 25 °C both these phospholipid (PC and PS) membranes are in the state well above their liquid crystalline phase transi-

tion point ($T_c$). Any increase in the temperature should serve to increase the molecular motion of the hydrocarbon chains of the bilayer.

It is also clear from the data presented that the fusion of spherical phospholipid bilayers does not only depend on temperature (Figs. 3 and 4) but other factors are also involved in membrane fusion as is correlated with the results of membrane fusion with lysolecithin (Figs. 5 and 6). The temperature for fusion, indeed, was lowered (by 10 °C) by the presence of lysolecithin in the membrane. Lucy (1970, 1974) has shown that lysolecithin will have a destabilizing effect on the membrane, and lysolecithin may even induce micelle formation in the membrane structure (Poole, Howell & Lucy, 1970). Lucy has suggested that micelle formation will lead to fusion through the interdigitation of micelles between two membranes. Our results may support some of his ideas. The results obtained with planar bilayer studies and monolayer dissolution studies may offer further evidence for a structural modification of the bilayer membrane. Our conductance measurements showed sudden variations in membrane conductance at about 42 °C for PC and 37 °C for PS, respectively (which were approximately 5 °C below the breaking point temperatures of these membranes), which may indicate some modification of membrane structure. As pointed out earlier, spin label studies of phosphatidylserine and phosphatidylcholine bilayers show a similar critical temperature (Ohnishi & Ito, 1974). By plotting the order parameter against temperature, Ohnishi and Ito detected a critical temperature that may be related to a large change in membrane structure (40–50 °C for PC and about 40 °C for PS). The temperatures detected by the above authors correlate fairly well with the fusion temperatures observed for our spherical bilayers PC–43 °C, PS–38 °C) as well as the sudden increased variations observed in the planar bilayer studies (PC–42 °C, PS–37 °C, values at pH 7.2).

Dissolution of PS monolayers rises sharply around 38 °C and may have an effect on the membrane. Whether dissolution can actually be related to the fusion process brings up an interesting point. Dissolution certainly can lead to instability or micellization of the membrane (Shapiro, 1974). The fact that PS dissolution is extremely rapid above a certain temperature seems to suggest that the membrane components consisting of PS molecules become unstable above that certain temperature. On the other hand, a neutral lipid such as PC appears to be rather inert in the membrane with temperature and our dissolution study provides some evidence for this. Yet we have observed a significant percentage of PC membrane fusions in our artificial system, in the temperature range of 40–50 °C. This would appear to be inconsistent with the above argu-

ment. However, this can be explained by the following: Dissolution will probably be related to the fusion process through the instability or micellization of membranes but other factors may be more responsible for the fusion process. Factors such as molecular motion in the hydrocarbon chains, and the expansion of phospholipid molecules should provide most of the motivating forces for cell fusion.

It is also possible that membrane fusion may occur through a localized instability in the membrane. Recent studies on membrane systems seem to indicate that the membrane may be able to form high density regions or patches of certain phospholipids (acidic phospholipids) within its heterogeneous lipid composition (Ohki&Papahadjoboulos, 1970; Ito & Ohnishi, 1974; Ohnishi & Ito, 1974). Cell fusion might also be initiated by the action that occurs in such regions.

Many detailed spin labeled studies using paramagnetic resonance have given a great deal of knowledge on the physical state of lecithin bilayers (McFarland & McConnell, 1971). McFarland and McConnell, have demonstrated that the fatty acid chains near the polar head group are very rigid and are bent at a 30° angle relative to the normal plane of the bilayer. Recent studies indicate that with increased fluidity in the membrane with temperature there is an increase in the angle of tilting (from the normal to the membrane surface) of the hydrocarbon chains (Traüble & Haynes, 1971; McConnell *et al.*, 1972). This would agree with the bilayer conductance measurements and spin label studies which indicate a structural change in the bilayer occurring above the phase transition. With increased mobility in the chains and the increase in area per molecule that accompanies increases in fluidity, it would be possible for the hydrocarbon chains to be exposed to the bilayer surface. In fact, if two membranes are in close apposition, hydrophobic interactions could result from the hydrocarbon chains being able to interact with each other. This type of membrane configuration would resemble a micelle configuration (Lucy, 1970), and could be termed a semi-micelle.

This semi-micelle membrane configuration fits in well with the data obtained for fusion in spherical bilayers with and without lysolecithin. If two membranes in a semi-micelle configuration have their surfaces in contact, this structure could be very unstable and might lead to fusion.

While the present study presents some possible ideas as to what is happening in this system, more current work must be done to elucidate such a mechanism for fusion.

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