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Alterations in Phospholipid Polymorphism by Polyethylene Glycol

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Summary. The fusogen polyethylene glycol is shown to alter the polymorphism of dimyristoyl phosphatidylcholine, soybean phosphatidylethanolamine, bovine phosphatidylserine, egg phosphatidylcholine/cholesterol mixture, dilinoleoylphosphatidylethanolamine/palmitoyl-oleoylphosphatidylcholine mixture, and egg lysolecithin. Suspension of these lipids in 50% polyethylene glycol (mol wt = 6000) reduces both the lamellar and the hexagonal II repeat spacings as measured by X-ray diffraction. An increase in the gel to liquid crystalline and bilayer to hexagonal transition temperatures are observed by freeze-fracture, X-ray diffraction, differential scanning calorimetry and ^{31}P NMR. Freeze-fracture electron micrographs revealed different bilayer defects depending on the physical states of the lipid. Lipidic particles in mixtures containing unsaturated phosphatidylethanolamine is eliminated. Some of the influences of polyethylene glycol on lipids may be explained by its dehydrating effect. However, other nonfusogenic dehydrating agents failed to produce similar results. These findings are consistent with the proposal that close bilayer contact and the formation of bilayer defects are associated with the fusogenic properties of polyethylene glycol.

Key Words polyethylene glycol · lipid bilayer · phase transition · freeze fracture · differential scanning calorimetry · X-ray diffraction · ^{31}P NMR

Introduction

Membrane fusion can be artificially induced by polyethylene glycol (PEG). During the process of PEG-induced fusion between cells, regions devoid of intramembrane particles (IMP) in cell plasma membranes have been observed by freeze-fracture electron microscopy (Maul et al., 1976; Knutton, 1979; Robinson et al., 1979). These IMP-free regions, possibly regions of pure lipid, are thought to be sites of cell fusion. Once bilayers are in direct contact, an interruption in bilayer continuity is needed to allow for the mixing of membrane components preceding fusion (Prestegard & Kantor, 1978). Thus, a form of bilayer discontinuity or transient destabilization of the bilayer is necessary for fusion to occur (Ahkong et al., 1975; Papahadjopoulos et al., 1977; Hui et al., 1981*a*; Hui et al., 1983*b*). Pure lipid systems are ideal for observing any associated nonbilayer configurations in connection with the fusogenic effect of PEG.

Various aspects of PEG effects on lipids have been reported. These include the changes of transition temperatures and entropies in saturated phospholipids (Tilcock & Fisher, 1979), decrease of the motion of the choline methyl group (Ohno, Maeda & Tsuchida, 1981), inductions of vesicle aggregation, lipid exchange (Tilcock & Fisher, 1982) and fusion (Boni et al., 1981*a*), and the reduction of membrane potential (Maggio, Ahkong & Lucy, 1976; Arnold, Pratsch & Gawrisch, 1983). PEG's from various sources and of different purity have been tested for fusion efficiency and bilayer destabilization (Honda et al., 1981; Smith et al., 1982; Tilcock & Fisher, 1982). We reported that the addition of a commercial grade PEG to egg phosphatidylcholine multilamellar vesicles induces the formation of nonbilayer configurations (Boni et al., 1981*b*). In this work, we used purified PEG and examined the effect of PEG on different lipids. Multilamellar vesicles of dimyristoyl phosphatidylcholine (PC) are used in order to investigate the effect of PEG on the gel-liquid crystalline phase transition. Bovine phosphatidylserine (PS) vesicles are studied to see the PEG effects on a negatively charged lipid, where an electrostatic repulsive force exists between bilayers. Dispersions of soy phosphatidylethanolamine (PE) are used to observe how PEG affects a lipid in the hexagonal II phase. Any stabilizing effects attributable to cholesterol are investigated in a 2 : 1 egg PC/cholesterol mixture. The effect of PEG on lipidic particles, often considered an intermediate during fusion processes (Verkleij et al., 1979; Hui et al., 1981*a*), is examined in an 85 : 15 DLPE/POPC dispersion. Lysolecithin is used to exhibit the dehydrating capabilities of PEG on the bi-

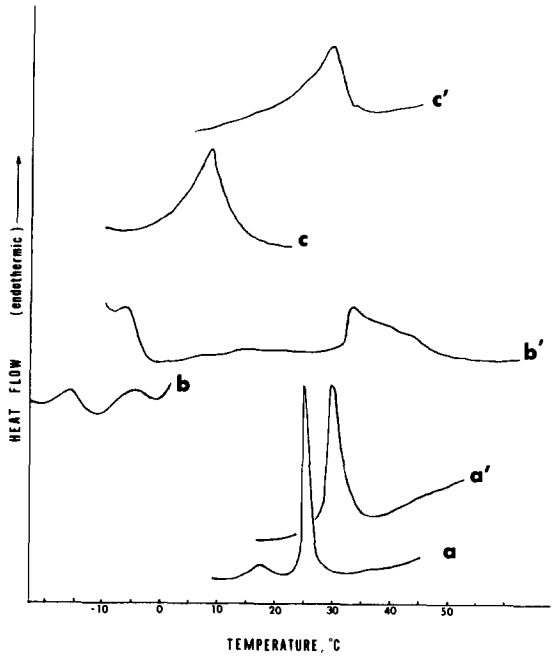

Fig. 1. Differential scanning calorimetry tracings of dispersions of: (*a*) dimyristoylphosphatidylcholine, (*b*) soy phosphatidylethanolamine and (*c*) bovine phosphatidylserine. Primed letters correspond to the above dispersions in 50% 6K PEG

layer to hexagonal I transition. The relation between numerous alterations resulted from PEG treatment and the fusogenic capabilities of PEG is discussed.

Abbreviations

DLPE	dilinoleoylphosphatidylethanolamine
DMPC	dipalmitoylphospatidylcholine
DSC	differential scanning calorimetry
H_{II}	hexagonal phase
LIP	lipidic particles
MLV	multilamellar vesicles
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
PC	phosphatidylcholine
PE	phosphatidylethanolamine
PS	phosphatidylserine
PEG	polyethylene glycol
POPC	1-palmitoyl-2-oleoylphosphatidylcholine
SUV	small unilamellar vesicles
Tc	transition temperature

Materials and Methods

Sample Preparation

Hen egg phosphatidylcholine (egg PC) and bovine brain phosphatidylserine (bovine PS) were purified by the method of Papahadjopoulos and Miller (1967). Cholesterol was purchased from Sigma Chemical Co. (St. Louis, Mo.). All other phospholipids used were purchased from Avanti Polar-Lipids (Birmingham, Ala.). All lipids were stored in chloroform and were shown to be pure by thin-layer chromatography. Polyethylene glycol of molecular weight 6,000 (PEG 6K) and other polymers were purchased from Fisher Scientific (Fair Lawn, N.J.). Purification of PEG was carried out according to Honda et al. (1981).

Multilamellar vesicles (MLV) were prepared by evaporation and resuspension in 7 mM Tris, 0.2 mM EDTA at pH 7.4 as previously described (Boni et al., 1981*a*). All samples were pelleted from 20 μM lipid dispersion and resuspended in 1.5 ml 50% (wt/vol) PEG 6K by vigorous vortexting.

Experimental Procedures

The procedure for ^{31}P NMR was described in Boni et al. (1981*b*). The protocols for freeze-fracture electron microscopy, X-ray diffraction and differential scanning calorimetry (DSC) were given by Hui et al. (1983*a*) and Stewart et al. (1979). Unless specified that Balzers cups were used in freeze-fracture experiments, rapid freezing by the copper sandwich method was employed throughout the text.

Results

The common effects of PEG on the various phospholipids studied are (1) the creation of numerous structural defects in bilayers, (2) an upward shifting of transition temperatures (Fig. 1) which is consistent with results of Tilcock and Fisher (1979) for saturated PC and PE, and (3) a reduction in all repeat spacings measured by X-ray diffraction (Table). These results will be discussed in the corresponding sections.

Dimyristoylphosphatidylcholine (DMPC)

In comparing DSC endotherms of DMPC MLV in aqueous buffer and in 50% PEG (Fig. 1*a* and *a'*, respectively), an upward shift of 6°C along with a broadening of the main transition and loss of the pretransition peak is observed. These results are consistent with those of Tilcock and Fisher (1979) for DPPC MLV in PEG, and are indicative of lipids in a dehydrated state (Ladbrooke & Chapman, 1969). X-ray diffraction data agree with this PEG-induced shift in phase transition, as seen by the wide angle reflection at 4.2 Å persisting up to 28°C, along with the corresponding changes in the lamellar repeat spacings with temperature (Janiak, Small & Shipley, 1976). The decrease in the lamellar repeat spacings in PEG as compared to the fully hydrated MLV are consistent with the bilayers being

less hydrated, where the 55 Å repeat at 18°C corresponds to a 15% hydration (Janiak et al., 1976).

Freeze-fracture electron micrographs contain a number of interesting features. Figure 2*a*, where the sample was freeze quenched from below the shifted Tc, exhibits smooth fracture faces containing numerous random folds across lamellar fracture planes. Screw dislocations are also apparent (arrow). Upon quenching the same sample from 40°C, large bumps were noted, much like those found for egg PC in PEG above the Tc (Boni et al., 1981*b*). A wrinkled surface is also seen that resembles a jumbled structure (Stewart et al., 1979). In areas containing more regular ripples, a ripple repeat of approximately 150 Å is consistent with a 20% hydration state (Janiak et al., 1976). Although the pretransition peak is not observed by DSC, which is expected due to lower hydration (Janiak et al., 1976), ripple structure nevertheless exist at temperatures within the broadened main phase transition, which might overlap the pretransition. The folds observed below the Tc for DMPC MLV in PEG were also seen in Fig. 2*c*, a fusion product of DMPC SUV in 25% PEG at 10°C. These folds appear to converge or radiate from one area where they resemble a P_β type structure. The P_β feature was often seen at this percentage of PEG when the sample was heated to 20°C. This suggests that the ripples of the P_β phase are a result of these random folds increasing in density until an ordered ripple is formed. The relation is similar to that between the banded and the terrace structures (Stewart et al., 1979).

^{31}P NMR spectra for DMPC MLV below and above the PEG-shifted phase transition showed a broadening of the anisotropic lineshape attributable to a reduction in motion of the headgroup (Seelig, 1978). A narrow resonance noted previously (Boni et al., 1981*b*) is due to a water-soluble impurity in the commercial grade PEG. As monitored by ^{31}P NMR, the phosphorus-containing impurity in the commercial grade PEG is removable by dialysis. Both the purified and the commercial grade PEG induce a slight broadening of the resonance from samples above the Tc, but a more pronounced change in lineshape is apparent below the Tc, where a broadening of the high field component is seen (*results not shown*). This could be due to a change in the polar headgroup with respect to its motion (rate and type) or conformation (Seelig, 1978; Thayer & Kohler, 1981). An increase in the dipolar interactions can result in the flattened spectrum (Yeagle & Romans, 1981). The application of a stronger proton decoupling power in an inverted gated pulse routine to the dispersion in 50% PEG at 20°C revealed the characteristic anisotropic lineshape of a bilayer dispersion.

Table. X-ray diffraction spacings

Lipid	Temperature (°C)	Spacing[a] (Å)	
		W/O PEG	In 50% PEG
DMPC	10	60.5(4.2)	55.5(4.2)
	18	65.5(4.2)	55.5(4.2)
	28	64.5	56.5(4.2)
	40	64	51
Bovine PS	0	73d(4.2)	53.5, 64(4.3)
	10	73d	53.5, 64(4.3)
	20	73d	53.5, 65(4.3)
	39	—	53.5
Soybean PE	0	53	49.5
	24	$65.5H_{II}$	47
	40	—	$43H_{II}$
2:1 egg PC/cholesterol	22	65	59

[a] The numbers indicate the lamellar repeat spacings including the water space, unless followed by H_{II} where it indicates the spacing of the first-order reflection of the hexagonal II phase. The numbers in parentheses are the sharp high angle diffraction spacings of the acyl chains. 'd' indicates a diffuse reflection. Dash indicates data not taken.

Bovine Phosphatidylserine (PS)

An appreciable upshift in the DSC endotherm of over 20°C is observed for bovine PS in 50% PEG (Fig. 1*c* and *c'*). This increase is greater than that found by Jacobson and Papahadjopoulos (1975) for neutralized PS MLV at pH 2.5, and is most likely due to both dehydration and charge neutralization (PEG causes a decrease in pH; Kao & Michayluk, 1974).

Dispersions of PS in aqueous buffer form loosely packed MLV, as indicated by the diffuse lamellar repeat spacings (*see* Table). Sharp diffraction patterns indicative of tightly packed lamellar were observed for PS in 50% PEG. Two different lamellar repeat spacings were noted, the 64-Å spacing became less intense than the 53.5-Å spacing with increasing temperature. This may be a consequence of two coexisting domains. The 53.5-Å spacing represents the stacking of multilamellae of PS maximally dehydrated (Papahadjopoulos, Portis & Pangborn, 1978). The sharp wide angle reflection at 4.3 Å indicates the lipid is in the gel state but not in a cochleate form (Hui et al., 1983*a*), in agreement with the DSC results.

Freeze-fracture electron microscopy of PS in 50% PEG at 4°C, which is below the Tc, yields stacked lamellar with a smooth surface texture and numerous cross fractures through the lamellae (Fig. 3*a*). Samples frozen at the onset of the phase transition retain a smooth surface texture but also contain

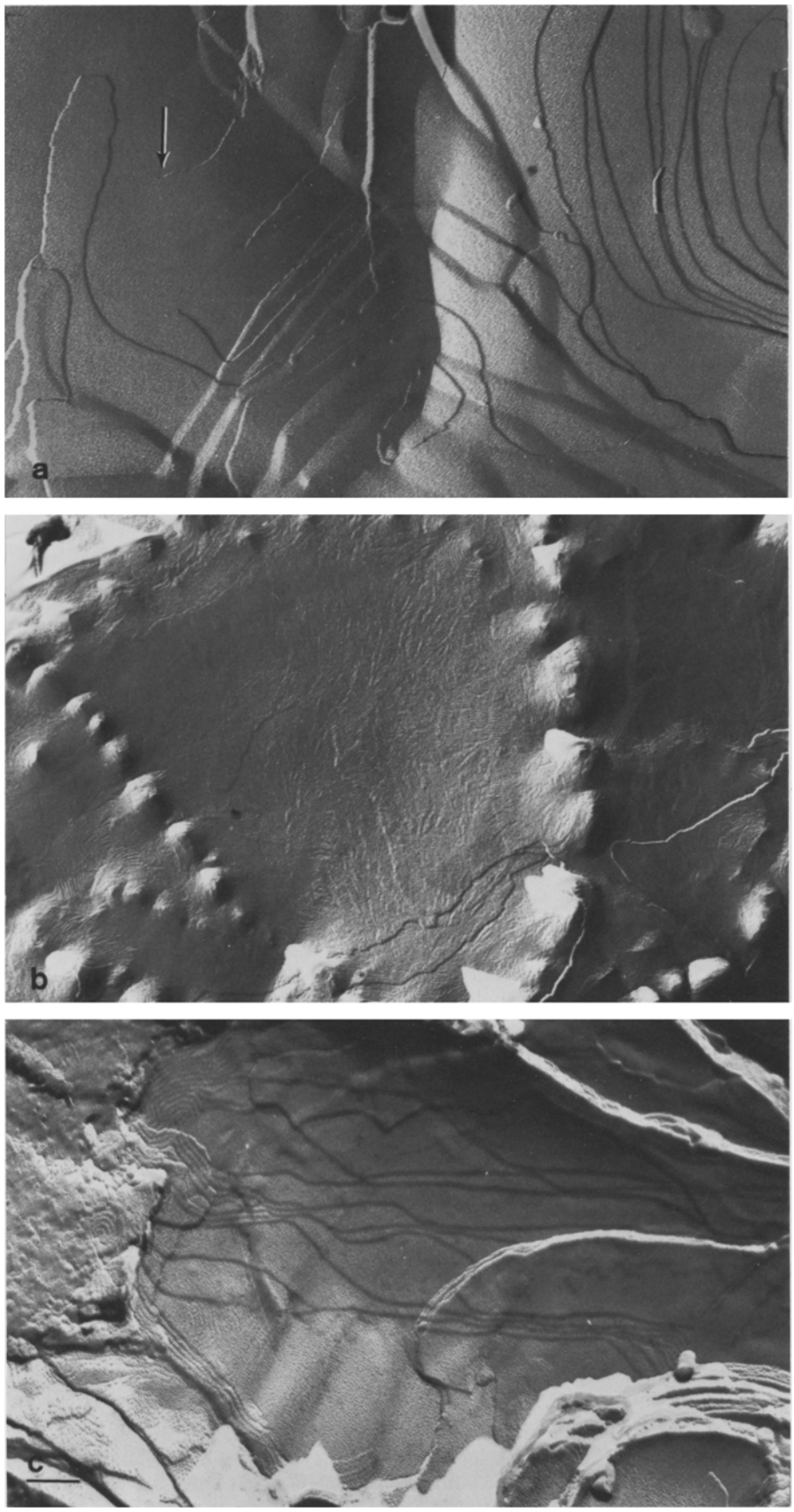

Fig. 2. Freeze-fracture electron micrographs of DMPC MLV in 50% PEG quenched from *a*) 20°C and *b*) 40°C using Balzers cups. *c*) is a fusion product of DMPC SUV in 25% PEG, incubated and frozen from 10°C. Bar = 0.1 μm. Arrow indicate screw dislocations

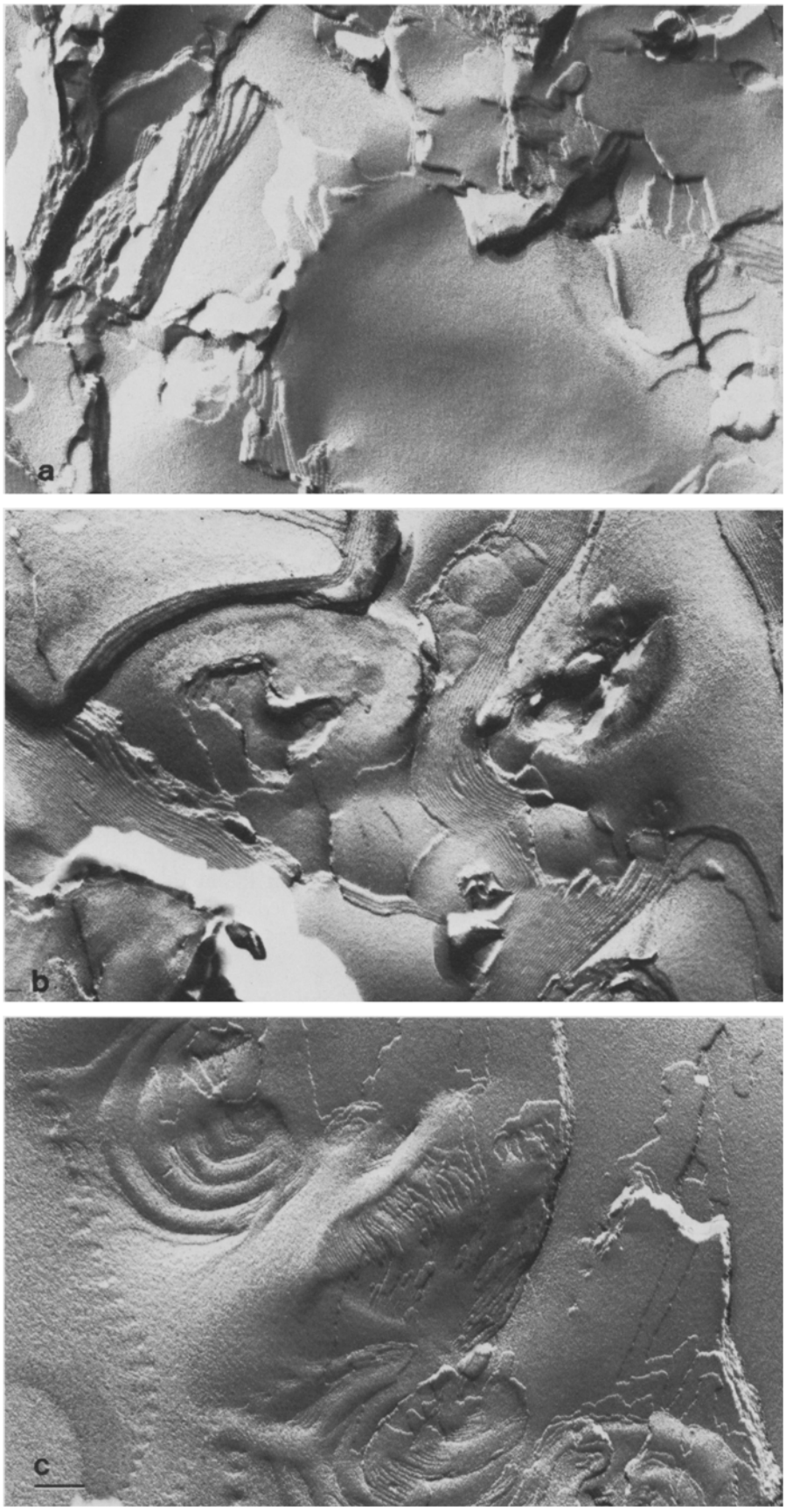

Fig. 3. Freeze-fracture electron micrographs of bovine PS dispersions in 50% PEG quenched from *a*) 4°C, *b*) 20°C and *c*) 39°C, using Balzers cups. Bar = 0.1 μm

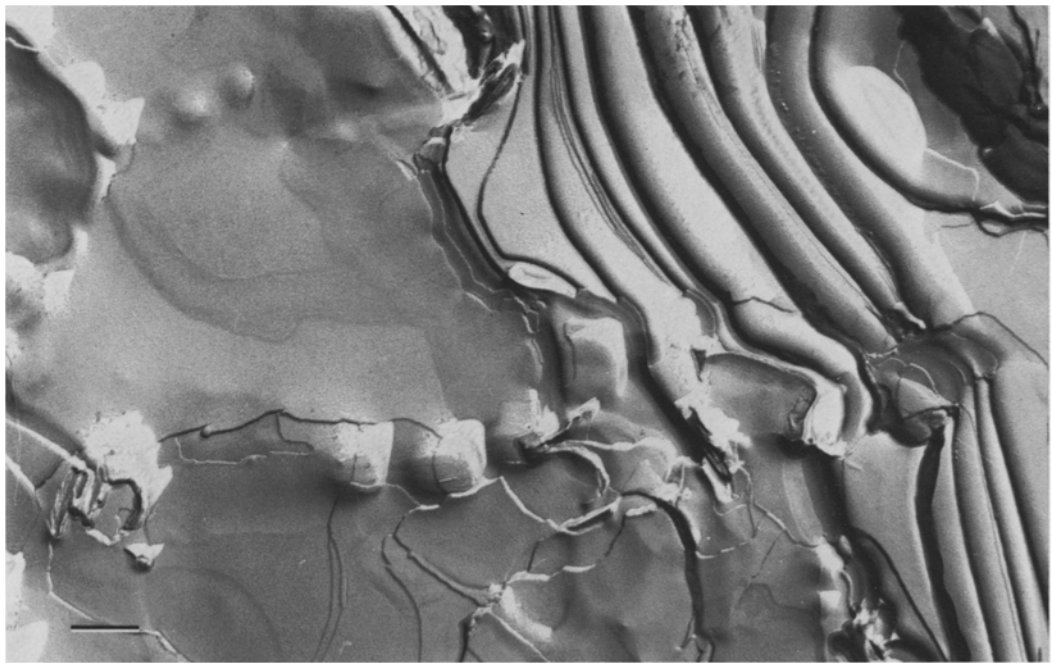

Fig. 4. Freeze-fracture electron micrographs of a 2 : 1 molar ratio egg PC/cholesterol dispersion in 50% 6K PEG quenched from 20°C. Bar = 0.1 μm

some regions with numerous striations and bubbles (Fig. 3*b*). The striations could either be ripples of a bilayer or cross fractures of lamellae. The possibility of these striations being hexagonal tubes cannot be verified by the other techniques because of their rarity in existence. If they are indeed a hexagonal phase lipid, it would agree with ^{31}P NMR results for egg PS at pH 3.5 (Hope & Cullis, 1980). Replicas of samples above the Tc revealed a stitch-type texture, along with screw dislocations (Fig. 3*c*). A number of smaller vesicles were also observed.

2 : 1 Egg PC/Cholesterol

A 2 : 1 mixture of egg PC and cholesterol was studied to see if cholesterol has any obvious stabilizing properties compared to egg PC MLV in 50% PEG. X-ray diffraction reveals a decrease in the lamellar repeat from 65 to 59 Å caused by PEG (Table), but not as much as that found for egg PC in 50% PEG (53 Å) (Boni et al., 1981*b*). This could be due to the increased hydration of phosphatidylcholines caused by cholesterol incorporation (Huang, 1977), thus allowing greater competition from the lipid over the PEG for water.

Freeze-fracture electron micrographs (Fig. 4) showed many peaks and hollows in the bilayer, most likely confocal domains (Kleman et al., 1977). Large tubular type structures, possibly folds in the bilayer, were also apparent and seemed to converge at various points. The typical jumbled texture noted for egg PC in 50% PEG (Boni et al., 1981*b*) was not seen in this mixture. This could be that cholesterol is completely miscible in PC at this mole percent (Hui & He, 1983) and leaves no pure domains of PC.

Soybean Phosphatidylethanolamine (PE)

Soybean PE was shown to have a broad transition from liquid-crystalline to H_{II} phase centering at −5°C, and have a 77-Å spacing between cylinders at 24°C by X-ray diffraction and freeze-fracture studies (Hui et al., 1981*b*). Upon incubation in 50% PEG, the gel to liquid-crystalline transition is shifted upward along with a dramatic shift in the bilayer to H_{II} transition (Fig. 1*b* and *b'*). This transition was monitored by ^{31}P NMR (Fig. 5), X-ray diffraction (Table) and freeze-fracture electron microscopy (Fig. 6). This shift in bilayer to H_{II} transition is similar to the result of dehydrating natural PE (Shipley, 1973), and is most likely a consequence of the acyl chains becoming more rigid and thus less capable of undergoing the increase in gauche conformers necessary for a nonbilayer phase. The decrease in both lamellar repeat and dis-

tance between hexagonal phase cylinders for PE in 50% PEG agrees with a 10 to 25% hydration (Shipley, 1973).

Freeze-fracture electron microscopy of PE in 50% PEG indicates numerous screw dislocations ending in large bubbles, possibly water pockets (Fig. 6*a*). No lipidic particles were observed. ^{31}P NMR spectra show no isotropic resonance during the transition from bilayer to H_{II} (Fig. 5). Instead, the formation of tubes directly from screw dislocations or line defects is revealed (Fig. 6*b*), a mechanism proposed by us (Hui et al., 1983*b*) and different from that proposed by Van Venetie and Verkleij (1981). The tubes being formed at 40°C also appear larger than those seen at 60°C (Fig. 6*c*). The decrease in tube dimension with increasing temperature may be a result of further exclusion of water, an important step in the bilayer to H_{II} transition (Hui et al., 1983*b*).

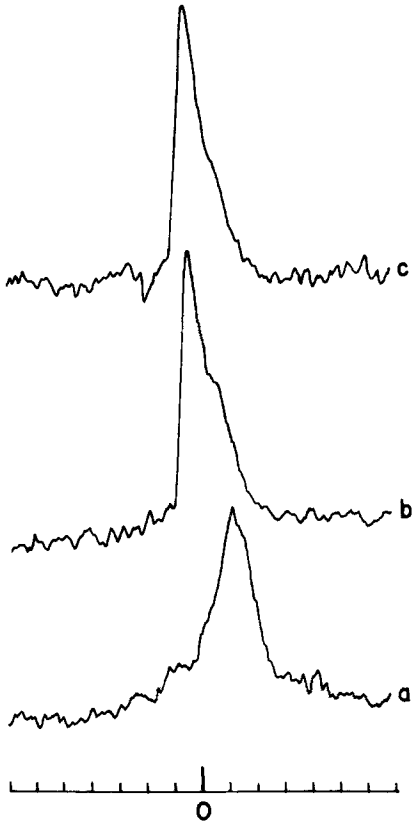

Fig. 5. 81 MHz ^{31}P NMR spectra of soy PE dispersions in 50% PEG taken at *a*) 20°C, *b*) 40°C, and *c*) 50°C

85 : 15 Dilinoleoylphosphatidylethanolamime/1-Palmitoyl-2-Oleoylphosphatidylcholine (DLPE/POPC)

Under certain conditions, mixtures containing high concentrations of unsaturated PE show numerous lipidic particles (LIP) which have been implied to affect membrane fusion and functional aspects (Hui et al., 1981*b*). The region of particular interest in these systems is where both bilayer and nonbilayer phases co-exist (Hui et al., 1981*b*; Boni & Hui, 1983). Thus the examination of an 85 : 15 DLPE/POPC mixture incubated in 50% PEG would be of interest.

In the absence of PEG, 85 : 15 DLPE/POPC in aqueous dispersion is in a bilayer conformation at 20°C. Freeze-fracture electron micrographs show that the multibilayers are interrupted by numerous LIPs (Boni & Hui, 1983). Upon heating to 40°C, it transforms to a mixture yielding ^{31}P NMR spectra indicative of a mixture of phospholipids in the H_{II} phase and those undergoing isotropic motion (Fig. 7*a–d*). Freeze-fracture electron micrographs of this mixture at 40°C reveal regions containing H_{II} tubes, rows of lipidic particles and amorphous lipids (Fig. 8*a*). Upon heating the sample to 50°C and cooling it down to 20°C, the isotropic resonance persists, and freeze-fracture micrographs show both lipidic particles and amorphous lipids (Boni & Hui, 1983), indicating a lack of reversibility (or strong thermal hysterisis) between these phases.

When the above heat-cycled sample was mixed with 50% PEG at 20°C, the system is reverted to a bilayer conformation. Freeze-fracture results exhibited regions of smooth bilayer which were seen along with hollows and peaks resembling confocal domains shown in Fig. 2*b*. At some portions of the sample, tubes forming along the plane of the bilayer were seen (Fig. 8*b*). Upon heating to 40°C, more tubes were observed, suggestive of a transition from the bilayer to the hexagonal II phase. Screw dislocations are also apparent (Fig. 8*c*), yet no amorphous lipid nor lipidic particles were found. This mixture of hexagonal II and lamellar phase lipids found by freeze fracture agrees with the ^{31}P NMR results. At 20°C and in the presence of 50% PEG, this mixture yields a bilayer-type resonance. Upon heating to 40°C, a hexagonal II-type resonance begins to appear. The bilayer-hexagonal II transition is completed at 50°C (Fig. 7*e, f* and *g*). No isotropic resonance was seen. Thus the addition of 50% PEG to this mixture completely removes the lipidic particles and the isotropic ^{31}P NMR peak during the bilayer to hexagonal II transition. Cooling the system back down to 20°C regained the bilayer structure, and upon reheating reformed the H_{II} phase, thus preserving the reversibility (eliminating the thermal hysterisis) of the bilayer to H_{II} phase transition.

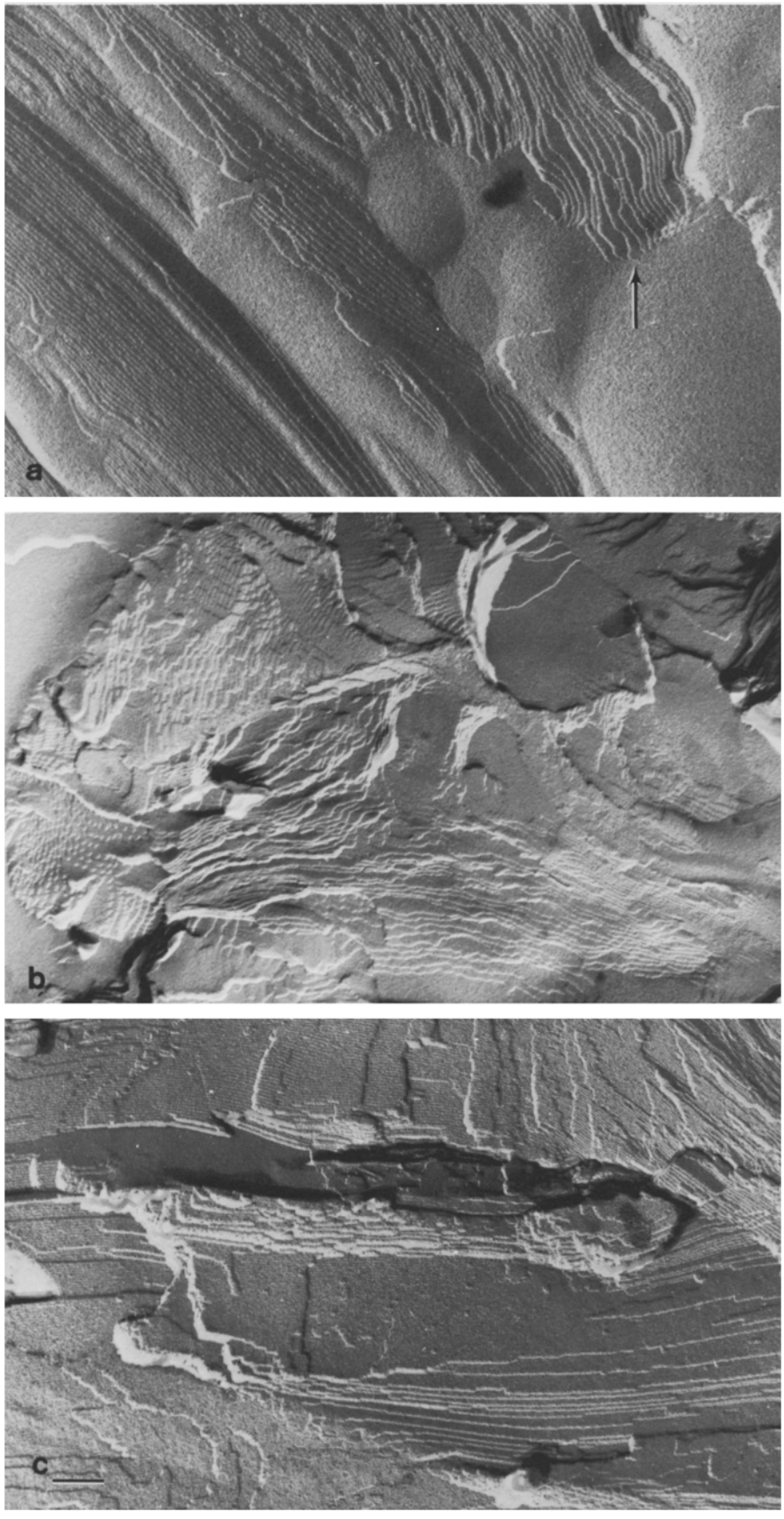

Fig. 6. Freeze-fracture electron micrographs of soy PE dispersions in 50% 6K PEG quenched from *a*) 24°C, *b*) 40°C and *c*) 60°C. Bar = 0.1 μm

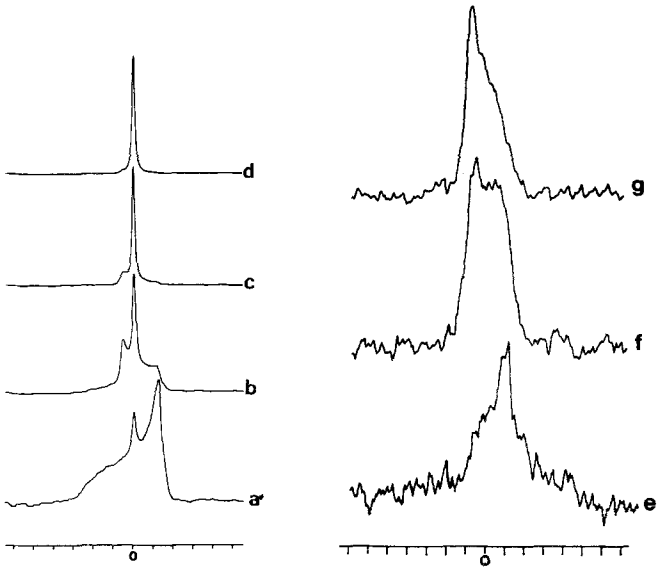

Fig. 7. 81 MHz ^{31}P NMR spectra of an 85 : 15 molar ratio DLPE/POPC dispersion at *a*) 20°C, *b*) 40°C, *c*) 50°C and *d*) cooled down to 20°C. The spectra of the same lipid mixture in 50% PEG are shown in *e*) 20°C, *f*) 40°C and *g*) 50°C. Spectra (*a–d*) were recorded with 14,000 scans each, and the lipid concentration of the sample was 20 mM. Spectra (*e–g*) were recorded with 5,000 scans each, and the lipid concentration was 10 mM. Each notch represents 10 ppm

Egg Lysolecithin

DSC of 20 mM egg lysolecithin in 44% PEG revealed a broad phase transition between 22 and 27°C (*not shown*). The X-ray diffraction pattern at 20°C gave a lamellae repeat of 50 Å and a wide angle spacing of 4.2 Å, indicating lipid in the gel phase and agreeing with results of Rand et al. (1975) for the minimum repeat found for a lyso-PC/water system at a 2/1 ratio or greater. At 40°C only one small angle diffraction spacing of 51 Å was observed.

Freeze-fracture electron microscopy was used to further elucidate the transition. In 44% PEG, multilamellar fracture surfaces and cross fractures are clearly discernible (Fig. 9*a*). At 40°C (Fig. 9*c*) a striated and textured surface suggestive of hexagonal tubes with an inter-tube distance of 66 Å can be seen. If the 51-Å spacing found by X-ray diffraction is the (100) reflection of a hexagonal diffraction pattern, it would infer an inter-tube distance of 59 Å. These results are similar to the X-ray and freeze-fracture results of Deamer et al. (1970) for egg lyso-PC/water in a 3/2 ratio at 37°C. Combining the above information and the lyso-PC phase diagram of Reiss-Husson (1967) it is reasonable to attribute the observed phase transition to be a gel phase bilayer to a hexagonal I transition. A direct gel bilayer to hexagonal transition without going through a liquid-crystalline bilayer phase was also observed by Marsh and Seddon (1982) for various lipid dispersions. Further information on the nature of this transition is seen in the freeze-fracture results taken at 26°C, during the transition. Lamellae become striated and directly form tubes (Fig. 9*b*). Numerous screw dislocations between lamellae are also present throughout.

An unexpected result was obtained by ^{31}P NMR. The lyso-PC/44% PEG dispersion at 20°C yielded a narrow anisotropic spectrum with an apparent half-width of 7.6 ppm (Fig. 10). This is much narrower than typical anisotropic patterns for hexagonal II tubes. Furthermore, the system has been shown to be in the lamellar phase at this temperature, and is therefore expected to give an anisotropic spectrum of the opposite sense (Seelig, 1978). A possible explanation would be a change in conformation of the phosphorus group relative to that typically found in phospholipid dispersions (Thayer & Kohler, 1981). This would imply a tilt in the headgroup of lyso-PC different from that of PC, and would agree with results found by Yeagle (1979) in ^{31}P NMR NOE experiments. Upon raising the temperature through the phase transition, no obvious change in the spectra was observed. Perhaps the change in the spectrum upon phase transition is buried under other contributions to the spectral width.

Discussion

A dehydration effect of 50% PEG on a number of lipid dispersions has been illustrated through DSC,

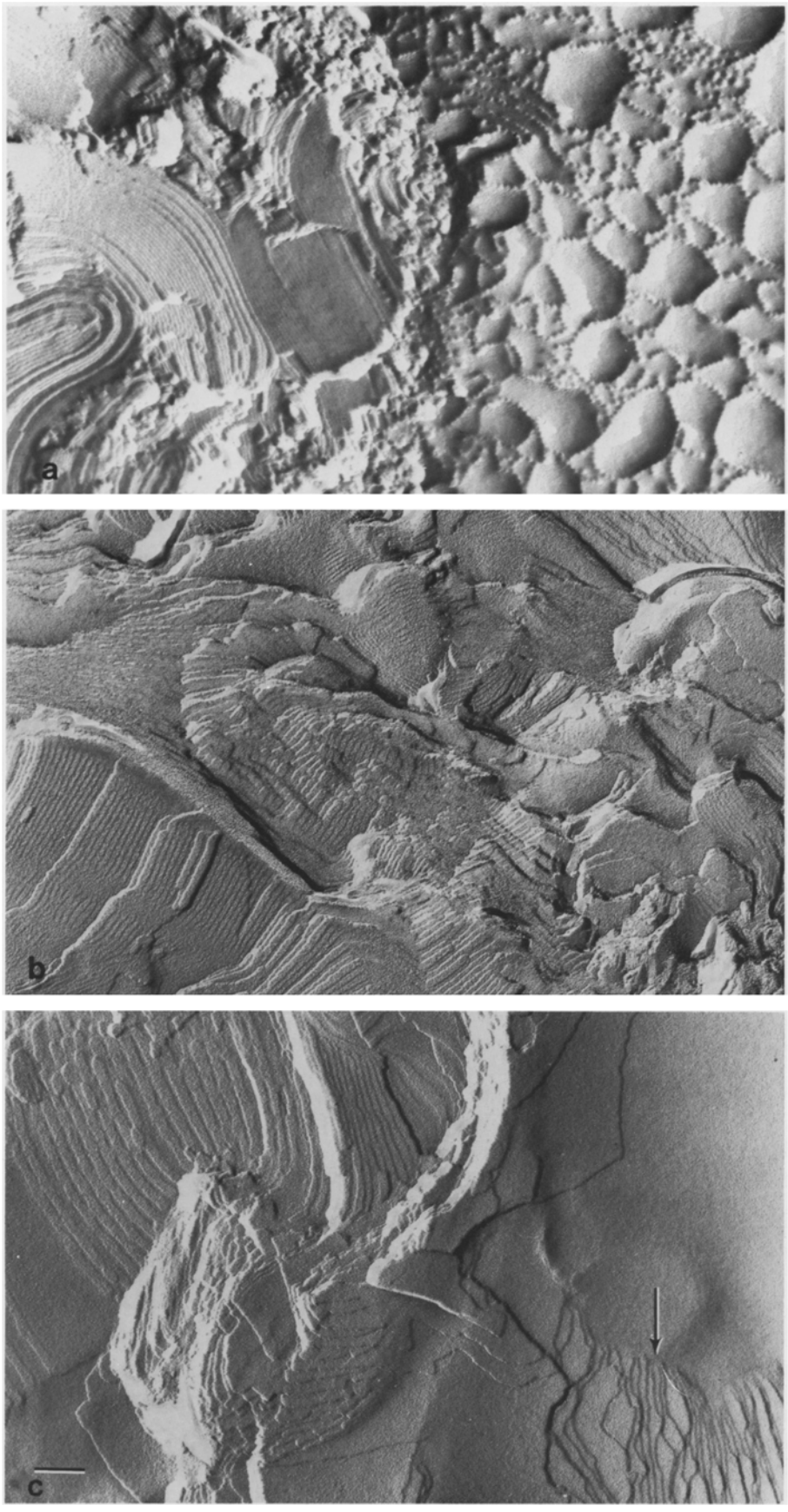

Fig. 8. Freeze-fracture electron micrographs of an 85 : 15 molar ratio DLPE/POPC dispersion *a*) quenched from 40°C, *b*,*c*) cooled to 20°C, suspended in 50% 6K PEG and quenched from 40°C. Bar = 0.1 μm

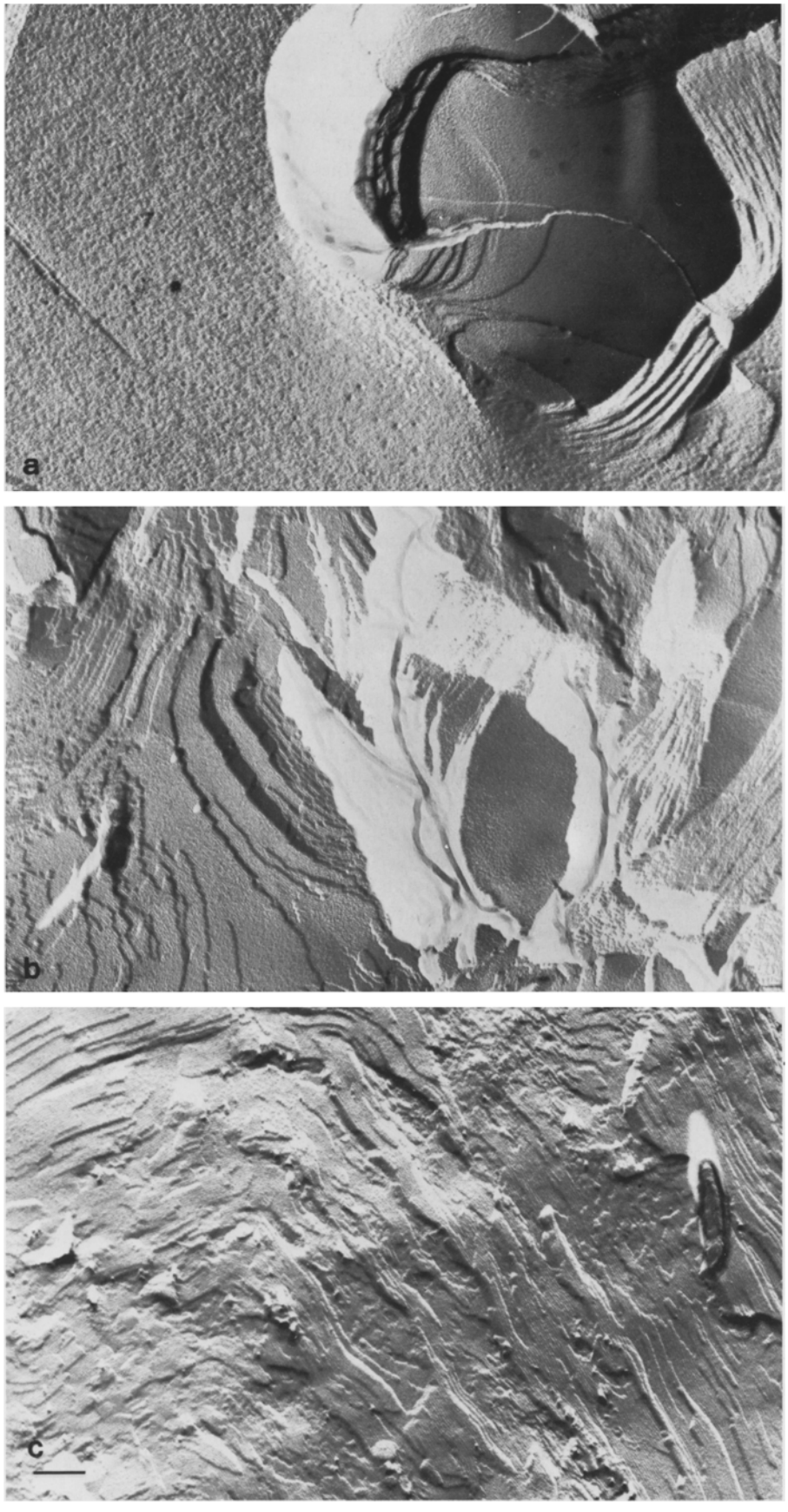

Fig. 9. Freeze-fracture electron micrographs of egg lyso-PC in 44% PEG quenched from *a*) 20°C, *b*) 26°C and *c*) 40°C. Bar = 0.1 μm

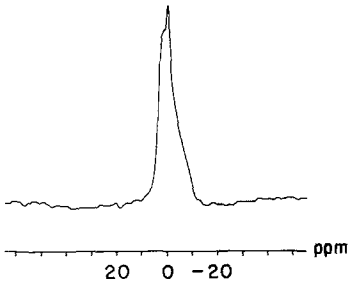

Fig. 10. 81 MHz ^{31}P NMR spectrum of 20 mM egg lyso-PC in 44% PEG at 20°C

X-ray diffraction, NMR and freeze-fracture electron microscopy. All dispersions were shown by DSC to have their main transitions shifted upward and broadened. Although the pretransition for DMPC was abolished in 50% PEG, a rippled structure nevertheless persisted. X-ray diffraction indicated a decrease in lamellae repeat spacings, which is most likely a result of a decrease in the water space between the bilayers. Freeze-fracture electron microscopy revealed numerous morphological features, some of which are known to occur in lipids 10 to 20% hydrated (Kleman et al., 1977). This dehydration effect agrees with previous studies which show that no unbound water exists in solutions of PEG-6,000 above 45% (wt/vol) (Blow et al., 1978). Only 11 of the 23 water molecules per phospholipid molecules are said to remain at the presence of 47% PEG (Arnold et al., 1983).

Morphological alterations observed in this study, however, appear to be unique to PEG. Other dehydrating agents such as dextran, poly(vinyl alcohol), poly(acrylic acid) did not induce any similar morphological alterations in egg PC MLV (*results not shown*). We have observed that PEG treatment alone led to the formation of nonbilayer structures such as screw dislocations and confocal domains, and to modify pre-existing defects such as lipidic particles in the bilayer to hexagonal transition. PEG was able to overcome the charge repulsion between PS lamellae. These properties of PEG are common to both the purified and the commercial grade PEG we used, therefore they are not related to the impurities isolated by the method of Honda et al. (1981). The effect of impurity in PEGs on their fusogenic property have been shown (Smith et al., 1982: *our unpublished results*) not to be a general concern. The influence of PEG appeared to be dependent on the particular phospholipid employed and its physical state.

Many of the lipid dispersions in 50% PEG exhibited evenly spaced peaks and hollows in rows (Figs. 2*b* and 3*c*). These have been attributed to a relaxation strain between large confocal domains (Kleman et al., 1977; De Gennes, 1974), which are liquid crystal defects adjusting to uneven surface conditions (such as local dehydration). The stacked layers can bend and slip over each other in a splay fashion keeping the interlayer thickness constant but resulting in regions of high curvature. It is interesting that when multilamellar systems are in the gel state, as shown for DMPC and bovine PS, the flexibility to form these focal conics is lost. This is possibly due to the lamellae being too rigid to bend, and that there are other defects such as screw dislocations at the microscopic level that can accommodate external strains.

Indications of screw dislocations, another bilayer defect, were also present. These dislocations connect hydrophobic regions of neighboring layers, allowing for rapid diffusion of molecules from one layer to another. PEG is known to promote rapid transfer of fluorescent lipid probes between two apposed membranes prior to fusion (Wojcieszyn et al., 1982). These screw dislocations were seen to occur more frequently in systems with the tendency to form highly curved structures, such as in both PE-containing dispersions and in lysolecithin, and may be instrumental in the bilayer to H phase transition. Such bilayer contacts may also be related to the greater fusion capacity of lipid mixtures high in PE content (Cullis & de Kruijff, 1979; Hui et al., 1981*a*).

In conclusion, the presence of a fusogenic concentration of PEG 6K dehydrated to a large extent the membrane systems and forced them into making close contact. Moreover, PEG uniquely induces various bilayer defects in different lipids, which may be instrumental in the fusion mechanism. This unique property of PEG may be associated with its depolarizing effect (Arnold et al., 1983) which alters the partition of hydrophobic molecules between the interior and the exterior of the bilayer. Experiments to clarify this point are in progress.

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