Modulation of the Phase Transition Behavior of Phosphatidylethanolamine by Cholesterol and Oxysterols†

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ABSTRACT: Cholesterol lowers the bilayer to hexagonal phase transition temperature of phosphatidylethanolamines up to a mole fraction of about 0.1. At cholesterol mole fractions above about 0.3, the effect of this sterol is to stabilize the bilayer phase. The relatively weak effects of cholesterol in altering the bilayer to hexagonal phase transition temperature can be explained on the basis of lateral phase separation. This is indicated by the horizontal liquidus line for the gel to liquid-crystalline transition in the phase diagram for mixtures of cholesterol with dielaidoylphosphatidylethanolamine (DEPE) as well as the fact that cholesterol does not greatly decrease the cooperativity of the bilayer to hexagonal phase transition. The enthalpy of this latter transition increased with increasing mole fractions of cholesterol. Two oxidation products of cholesterol are 5-cholesten-3β,7α-diol and cholestan-3β,5α,6β-triol. Compared with cholesterol, 5-cholesten-3β,7α-diol had a greater effect in decreasing the bilayer to hexagonal phase transition temperature and broadening this transition. It is suggested that its effectiveness is due to its greater solubility in the DEPE. In contrast, cholestan-3β,5α,6β-triol raises the bilayer to hexagonal phase transition temperature of DEPE. This is due to its larger and more hydrophilic head group. In addition, its length, being shorter than that of DEPE, would not allow it to pack efficiently in a hexagonal phase arrangement. We suggest that this same effect is responsible for cholesterol raising the bilayer to hexagonal phase transition temperature at higher mole fractions.

Cholesterol is a major lipid component of most mammalian membranes. The effects of cholesterol in altering membrane properties have received considerable attention [for a recent review, see Yeagle (1985)]. Cholesterol has marked effects on the order, mobility, and phase transition properties of membrane lipids. Such effects are best studied in model membranes composed of a small number of molecularly homogeneous lipid components. There have been many such studies evaluating the effects of cholesterol on liposomes composed of synthetic phosphatidylcholines but relatively few studies of the effects of cholesterol on the properties of other phospholipids. In particular, phosphatidylethanolamine is of interest because of its property of undergoing transitions to the hexagonal phase. Substances which promote hexagonal phase formation in membranes may also induce membrane fusion (Cullis et al., 1985, 1986). In addition to cholesterol, we have also studied the effects of two oxidized forms of cholesterol on the polymorphic phase properties of phosphatidylethanolamines. Oxidized forms of cholesterol occur in vivo as metabolic intermediates as well as arising through oxidative damage to membranes (Smith, 1981). Cholesterol oxidation products have been suggested to play an important role in certain pathological conditions, particularly in arterial injury (Yachnin et al., 1979; Peng et al., 1985), and studies of their effects on biological (Rooney et al., 1985) and model phospholipid membranes (Theunissen et al., 1986) have attracted recent renewed interest.

Experimental Procedures

Materials. Dielaidoylphosphatidylethanolamine (DEPE)[1] and 1-palmitoyl-2-oleoylphosphatidylethanolamine (POPE) were obtained from Avanti Polar Lipids. The phosphatidylethanolamines exhibited a sharp gel to liquid-crystalline phase transition, indicating a high degree of purity. The gel to liquid-crystalline phase transition of both DEPE and POPE shows a small degree of asymmetry, with a shoulder on the low-temperature side of the transition peak, a characteristic of gel to liquid-crystalline phase transitions of phosphatidylethanolamines (Chowdhry et al., 1984).

Cholesterol (5-cholesten-3β-ol) was purchased from Nu Chek Prep, Elysian, MN, and was repurified by recrystallization from water–methanol. The dried crystals were stored under argon at −20 °C. Cholestan-3β,5α,6β-triol was purchased from Makor Chemicals, Jerusalem, Israel, and 5-cholesten-3β,7α-diol from Steraloids, Wilton, NH. All three sterols were shown to be pure by TLC using *n*-hexane, diethyl ether, and glacial acetic acid (70:30:1) as the developing solvent.

Sample Preparation. The phospholipid and additive were dissolved together in a solution of chloroform and methanol (2:1 v/v). The solvent was evaporated with a stream of dry nitrogen so as to deposit the lipid as a film on the walls of a glass test tube. Last traces of solvent were removed into a liquid nitrogen trap by placing the samples in a vacuum oven at 40 °C. The apparatus was maintained under high vacuum for at least 90 min. The lipid film was then suspended in a pH 7.40 buffer of 20 mM Pipes, 1 mM EDTA, 150 mM NaCl, and 0.02 mg/mL NaN_3 by warming the tube to about 45 °C and vortexing vigorously for about 30 s. The final concentration of phospholipid was 5 mg/mL with varying amounts of sterols admixed. The buffer and lipid suspensions were degassed under vacuum before being loaded into the calorimeter.

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[1] Abbreviations: DEPE, dielaidoylphosphatidylethanolamine; POPE, 1-palmitoyl-2-oleoylphosphatidylethanolamine; DSC, differential scanning calorimetry; EDTA, ethylenediaminetetraacetic acid; Pipes, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); TLC, thin-layer chromatography.

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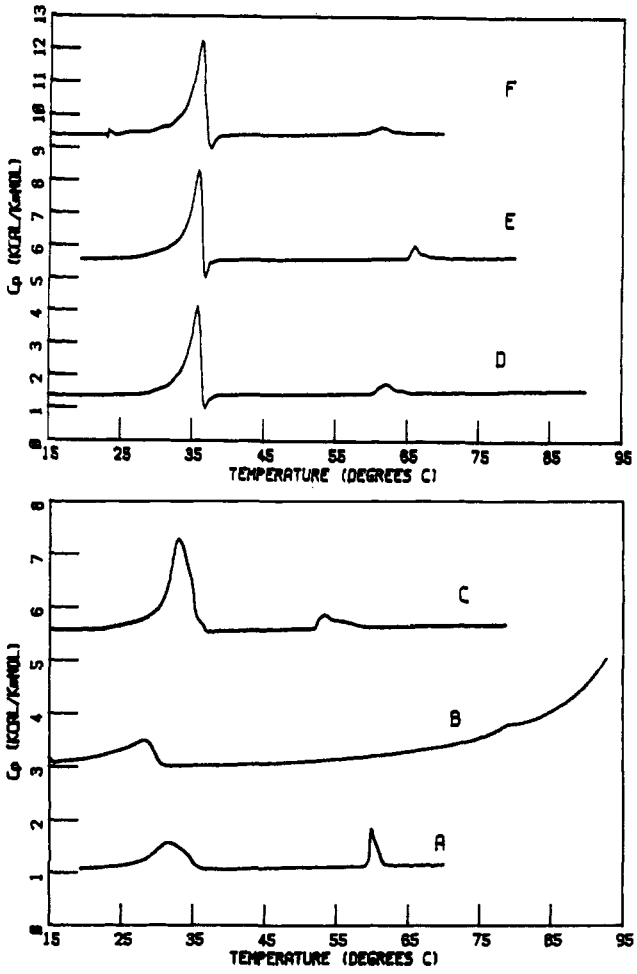

FIGURE 1: Representative DSC scans of DEPE with cholesterol or oxysterols. Scan rate, 0.7 K/min. Buffer, 20 mM Pipes, 1 mM EDTA, and 0.15 M NaCl containing 0.02 mg/mL NaN_3, pH 7.40. DEPE concentration, 7 mM, with the following mole fractions of added sterol: (A) 0.25 cholesterol; (B) 0.26 cholestan-3β,5α,6β-triol; (C) 0.25 5-cholesten-3β,7α-diol; (D) 0.06 cholesterol; (E) 0.06 cholestan-3β,5α,6β-triol; (F) 0.06 5-cholesten-3β,7α-diol. The small deviation seen in scan F just below 25 °C is not a reproducible feature of DSC scans of this sample.

Differential Scanning Calorimetry (DSC). Lipid suspension or buffer was loaded into the sample or reference cell, respectively, of an MC-2 high-sensitivity scanning calorimeter (Microcal Co., Amherst, MA). A scan rate of 39 K/h was generally employed. Second heating scans on the same sample were essentially superimposable on the first scan except for pure lipid samples which were heated above 80 °C. These latter samples exhibited an increase of approximately 0.1 °C in the bilayer to hexagonal phase transition temperature. A sufficient amount of lipid was used in order to accurately measure the low enthalpy bilayer to hexagonal phase transition. This large amount of lipid caused an overshoot of the DSC scan at the high-temperature side of the gel to liquid-crystalline transition. This apparent exotherm is not observed with more dilute suspensions or with broader transitions at high sterol concentrations. It is an instrumental artifact.

RESULTS

Representative DSC curves of mixtures of DEPE with cholesterol, cholestan-3β,5α,6β-triol, or 5-cholesten-3β,7α-diol are presented in Figure 1. At a low mole fraction of 0.06 of additive (Figure 1, curves D–F), the typical asymmetric gel to liquid-crystalline transition phosphatidylethanolamines are observed at about 35 °C. The much less endothermic bilayer

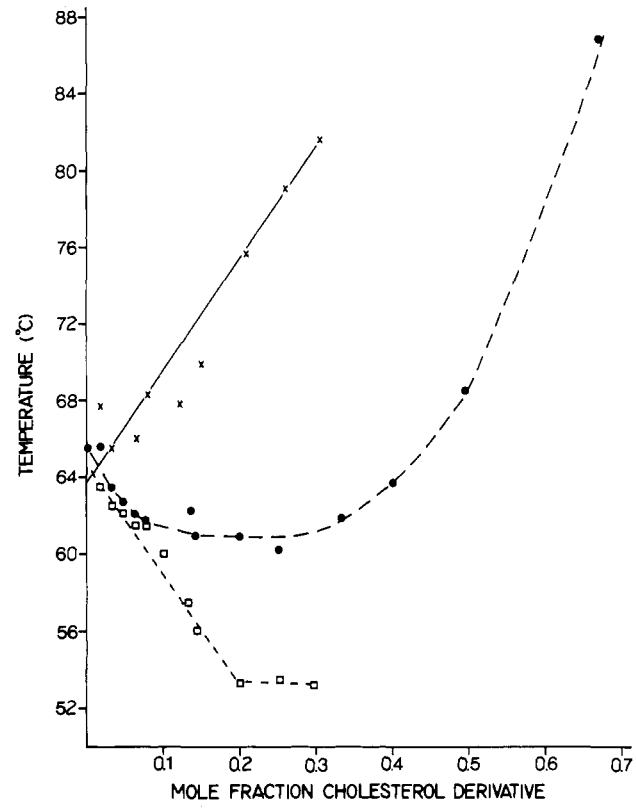

FIGURE 2: Dependence of the bilayer to hexagonal phase transition temperature of DEPE on added cholesterol (●), cholestan-3β,5α,7β-triol (×), or 5-cholesten-3β,7α-diol (□).

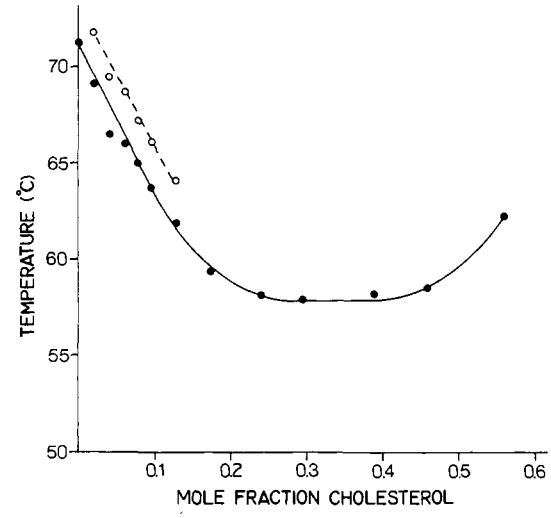

FIGURE 3: Dependence of the bilayer to hexagonal phase transition temperature of POPE on added cholesterol. Below a mole fraction of 0.15 cholesterol, the observed transition was fitted to two van't Hoff components.

to hexagonal phase transition can also be discerned at about 65 °C. At a higher mole fraction of 0.25 of additive (Figure 1, curves A–C), the gel to liquid-crystalline transition is further broadened and shifted to lower temperatures. The bilayer to hexagonal phase transition becomes more prominent, particularly for cholesterol (Figure 1, curve A), where it retains a high degree of cooperativity.

The bilayer to hexagonal phase transition of DEPE is shifted to lower temperatures with 5-cholesten-3β,7α-diol and to higher temperatures with cholestan-3β,5α,6β-triol (Figure 2). The temperature of this transition was determined by fitting the observed scan to a single van't Hoff component using software provided by Microcal Co. In the case of cholesterol,

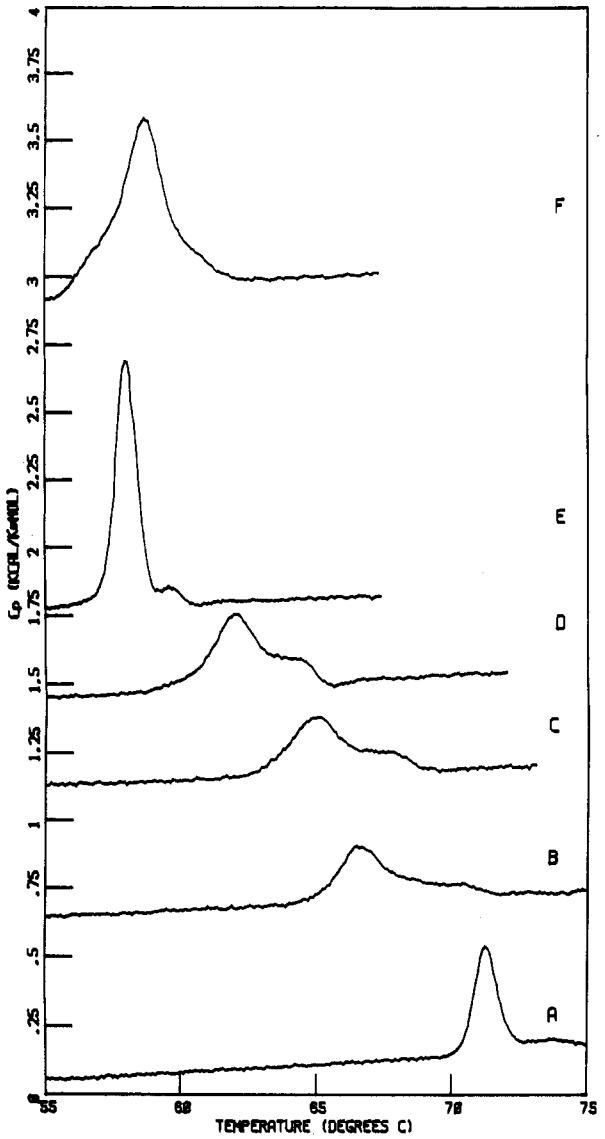

FIGURE 4: Representative DSC scans of POPE with cholesterol. Curves expanded to show the bilayer to hexagonal phase transition. Curve A, pure POPE and with the addition of the following mole fractions of cholesterol: (B) 0.04; (C) 0.08; (D) 0.13; (E) 0.30; (F) 0.46. Other conditions as for Figure 1.

the temperature at which DEPE is converted to the hexagonal phase is lowered up to about 0.1 mole fraction of cholesterol and is raised above 0.3 mole fraction of cholesterol. We did not determine whether 5-cholesten-3β,7α-diol also exhibits this biphasic behavior because of the high cost of this sterol and because the result would not have direct biological relevance. The effect of cholesterol on the bilayer to hexagonal phase transition temperature of POPE (Figure 3) is similar to its effects on DEPE. However, in the case of POPE, the bilayer to hexagonal phase transition exhibits a more complex shape at cholesterol mole fractions below about 0.15 (Figure 4). Cholesterol also increases the enthalpy of the bilayer to hexagonal phase transition of both DEPE (Figure 5) and also POPE (Figure 6). This effect is also observed for 5-cholesten-3β,7α-diol but not for cholestan-3β,5α,6β-triol (Figure 5). Because of the error involved in these measurements, we cannot be certain that the relationship between enthalpy and mole fraction of cholesterol derivative is linear, although it is drawn this way in Figures 5 and 6 to indicate the direction and magnitude of the change.

For the gel to liquid-crystalline phase transition, the temperature at which the maximal excess heat capacity occurs

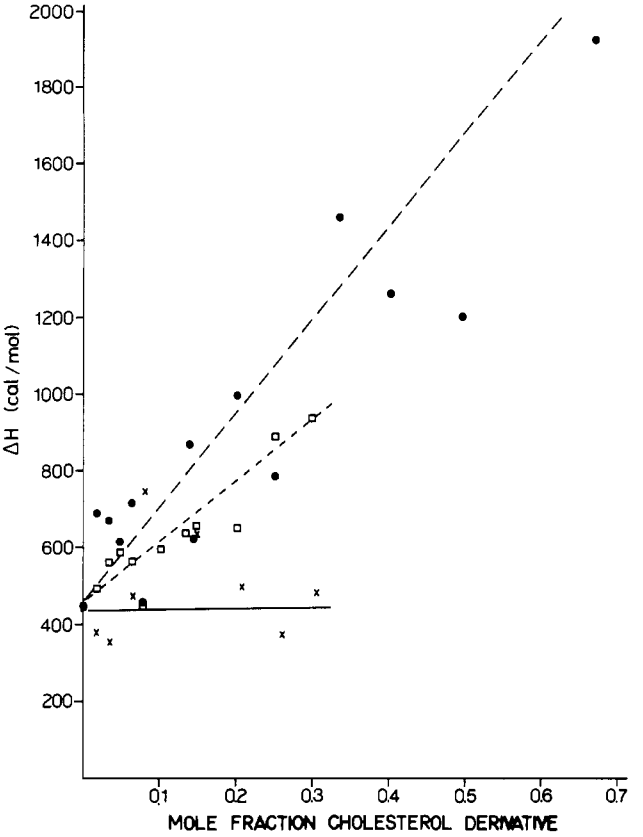

FIGURE 5: Effect of sterols on the enthalpy of the bilayer to hexagonal phase transition of DEPE. Cholesterol (●); 5-cholesten-3β,7α-diol (□); cholestan-3β,5α,6β-triol (×).

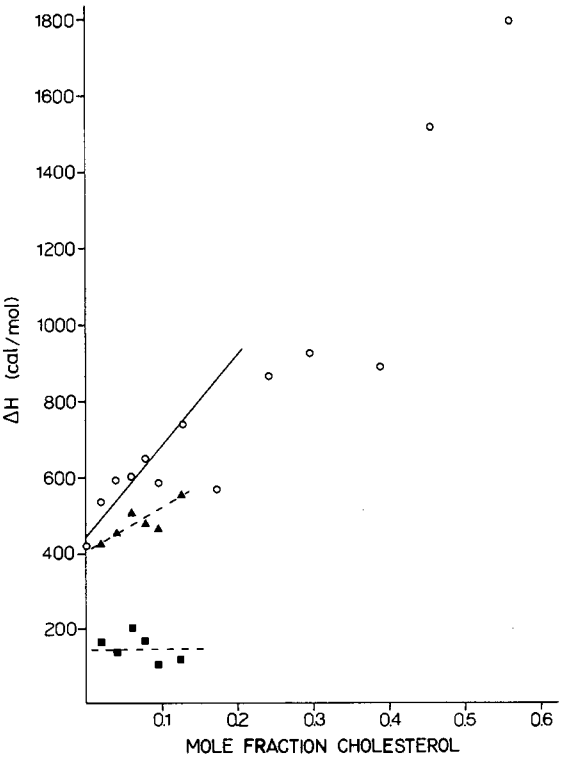

FIGURE 6: Effect of cholesterol on the enthalpy of the bilayer to hexagonal phase transition of POPE. Total enthalpy (○); lower melting component (▲); higher melting component (■).

(i.e., the peak position of the DSC curve) decreases with increasing mole fraction of sterol (Figures 7 and 8). The initial slopes of these plots are similar for all of the systems studied. There appears to be some derivation from linearity at high

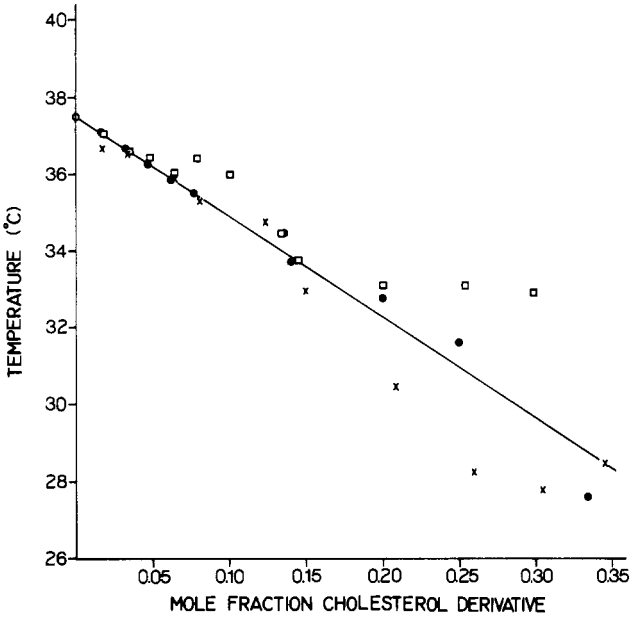

FIGURE 7: Effect of sterols on the gel to liquid-crystalline phase transition temperature of DEPE. Cholesterol (●); 5-cholesten-3β,7α-diol (□); cholestan-3β,5α,6β-triol (×).

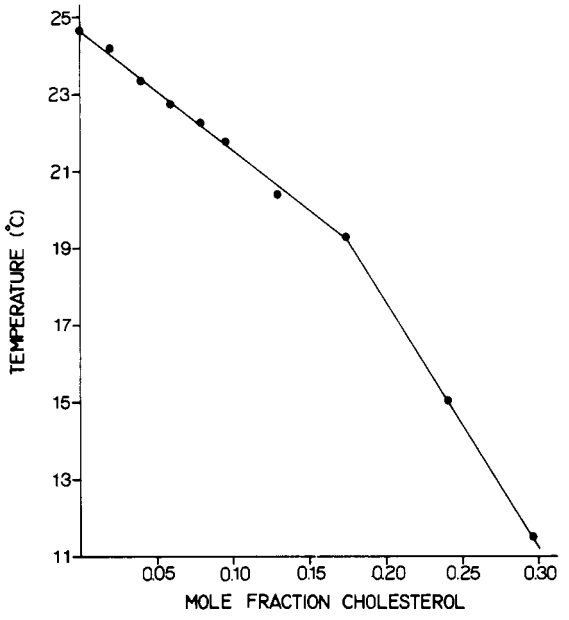

FIGURE 8: Effect of cholesterol on the gel to liquid-crystalline phase transition temperature of POPE.

sterol mole fractions. Furthermore, the temperature depression of the gel to liquid-crystalline transition caused by the sterols is not much less than the shifts they induce in the temperature of the bilayer to hexagonal phase transition (Figures 2 and 3). This is in contrast to many other additives which affect the bilayer to hexagonal phase transition to a much greater extent (Epand, 1985, 1986; Valtersson et al., 1985). The enthalpy of the gel to liquid-crystalline transition decreases markedly with increasing mole fraction of cholesterol (Figures 9 and 10) or of cholestan-3β,5α,6β-triol (Figure 9). The 5-cholesten-3β,7α-diol is less effective in reducing this transition enthalpy (Figure 9). The property of cholesterol to markedly reduce the gel to liquid-crystalline phase transition enthalpy has been previously observed with phosphatidylcholines (Mabrey et al., 1978; Estep et al., 1978) as well as with phosphatidylethanolamines containing saturated acyl side chains (Blume, 1980).

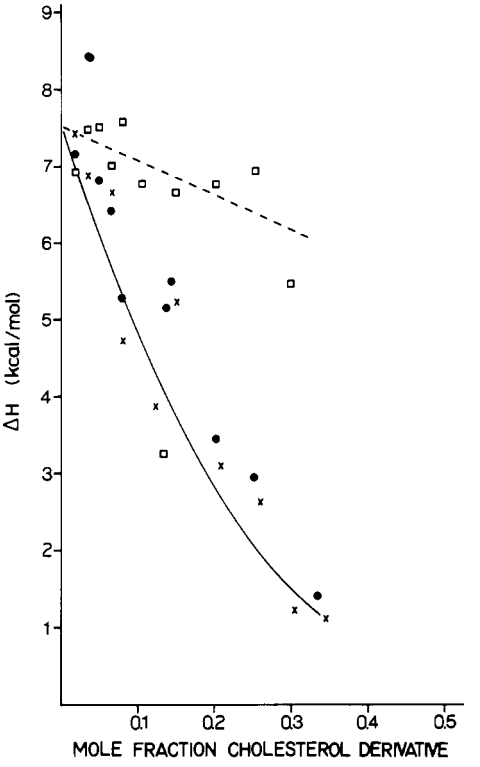

FIGURE 9: Effect of sterols on the enthalpy of the gel to liquid-crystalline phase transition of DEPE. Cholesterol (●); 5-cholesten-3β,7α-diol (□); cholestan-3β,5α,6β-triol (×).

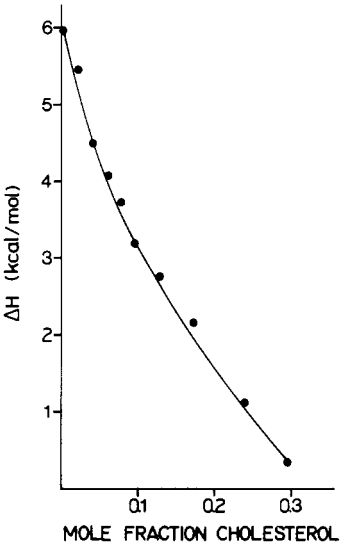

FIGURE 10: Effect of cholesterol on the enthalpy of the gel to liquid-crystalline phase transition of POPE.

The results of the calorimetric studies are summarized as phase diagrams (Figures 11–14).

Discussion

Cholesterol has previously been shown to promote hexagonal phase formation in mixtures of phosphatidylethanolamines and phosphatidylcholines (Tilcock et al., 1982). In this work, we demonstrate that at high mole fraction of cholesterol, this effect is reversed (Figures 2 and 3), and cholesterol stabilizes the bilayer phase of phosphatidylethanolamine. Factors which influence the bilayer to hexagonal phase equilibrium include molecular shape (Cullis & de Kruijff, 1979; Israelachvili et al., 1980; Wieslander et al., 1980), hydration (Seddon et al., 1983), and packing effects (Gruner, 1985). Because of its small head group, cholesterol is usually considered as a

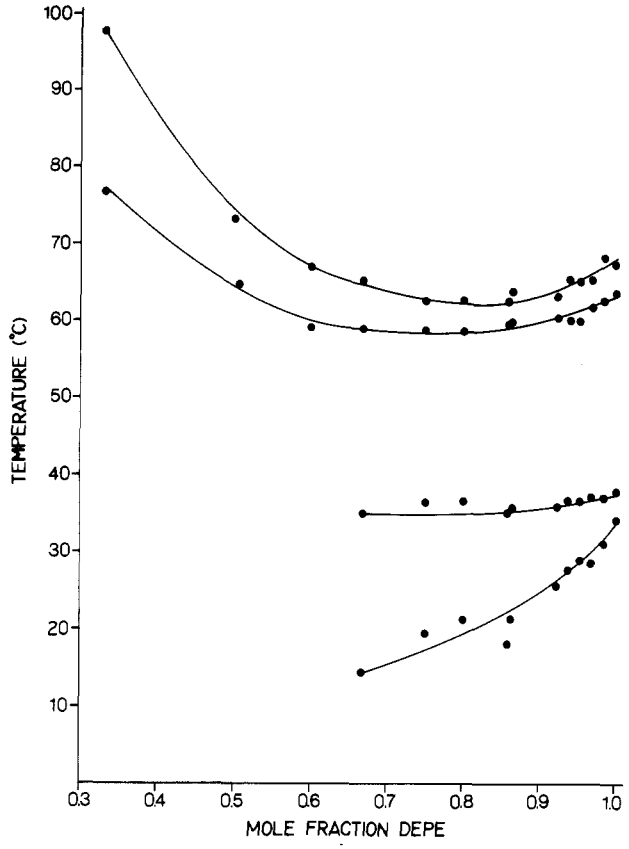

FIGURE 11: Phase diagram for DEPE–cholesterol.

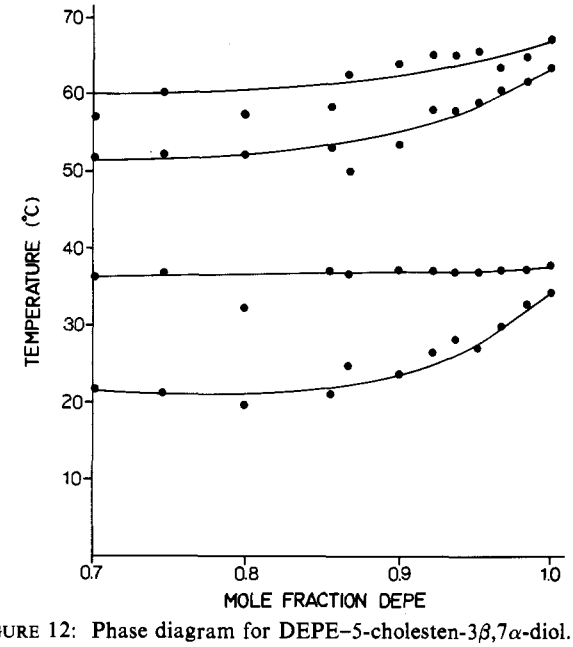

FIGURE 12: Phase diagram for DEPE–5-cholesten-3β,7α-diol.

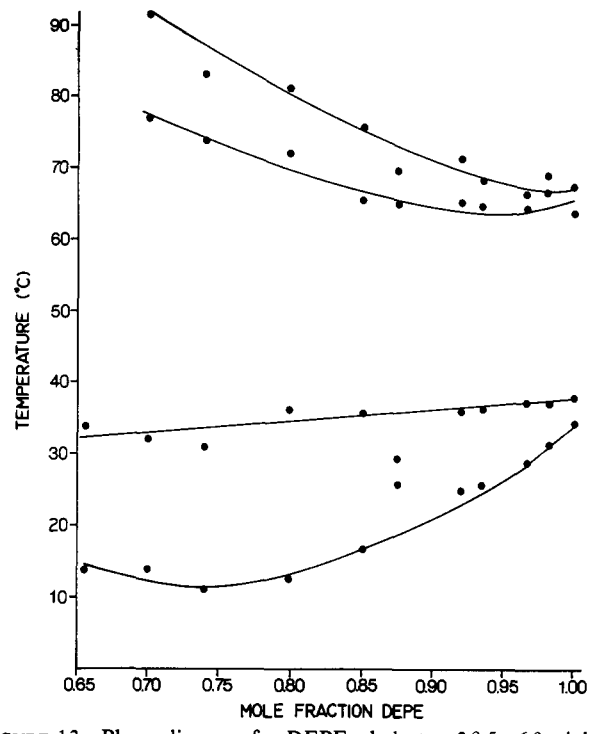

FIGURE 13: Phase diagram for DEPE–cholestan-3β,5α,6β-triol.

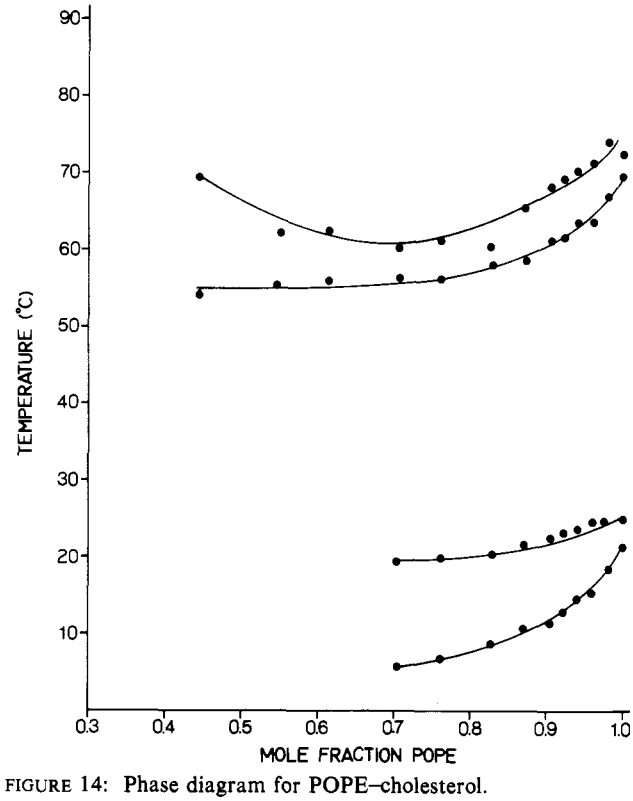

FIGURE 14: Phase diagram for POPE–cholesterol.

"cone-shaped" molecule and therefore promotes hexagonal phase formation. Cholesterol is also poorly hydrated, and this too favors hexagonal phase formation. This may be a particularly important factor in the induction of the hexagonal phase with bilayers containing phosphatidylcholines by cholesterol because of the greater degree of hydration of this phospholipid. However, cholesterol is not as long a molecule as DEPE or POPE. Therefore, it will not pack into a hexagonal phase arrangement as readily and will tend to destabilize the packing of hexagonal phase cylinders. This destabilization may account for the increased enthalpy required to convert the phosphatidylethanolamine bilayers to the hexagonal phase (Figures 5 and 6). Such an enthalpy increase is not observed with other additives (Epand, 1985, 1986). This packing effect also appears to depend on the nature of the phospholipid. The increase in the bilayer to hexagonal phase transition temperature at high cholesterol concentrations is greater with DEPE, a phospholipid with straighter and longer acyl chains compared to POPE.

The most notable feature of the effect of cholesterol on the bilayer to hexagonal phase transition of phosphatidylethanolamines is that marked changes occur only at relatively high mole fractions of cholesterol. About 10-fold higher

concentrations of cholesterol are required to lower the bilayer to hexagonal phase transition compared with diacylglycerols or eicosane (Epand, 1985). This is the case despite the fact that cholesterol has a very small head group, only a single hydroxyl group. We suggest that cholesterol is not completely miscible with the liquid-crystalline state of phosphatidylethanolamines. This is indicated by the fact that the upper temperature limit of the gel to liquid-crystalline transition of DEPE is almost completely independent of the addition of cholesterol (Figure 11) or 5-cholesten-3β,7α-diol (Figure 12). Such phase behavior is indicative of liquid–liquid immiscibility. In addition, the bilayer to hexagonal phase transition does not broaden significantly below 0.25 mole fraction of cholesterol (Figures 11 and 14). The fact that cholesterol does not decrease the cooperativity of this transition at these lower mole fractions indicates that it is phase separated from the phospholipid. This would also explain why, despite its cone shape and dehydrating effects, cholesterol does not markedly lower the bilayer to hexagonal phase transition temperature of phosphatidylethanolamines. The relative miscibilities of cholesterol and 5-cholesten-3β,7α-diol with DEPE must also be considered. Since 5-cholesten-3β,7α-diol has a larger head group and is more hydrated than cholesterol (two hydroxyl groups vs. one), one would anticipate a priori that it would be less effective in lowering the bilayer to hexagonal phase transition temperature. However, the reverse is the case (Figure 2). We propose that although 5-cholesten-3β,7α-diol is not completely miscible with DEPE, as indicated by the phase diagram (Figure 12, horizontal liquidus line), it is more miscible than cholesterol with DEPE. This increased miscibility is a result of the increased polar nature of this oxysterol, giving it a degree of hydration closer to that of the phospholipid.

Many oxysterols have cytotoxic effects (Smith, 1981). It is possible that some of these effects are a result of changes in the membrane distribution of these substances compared with cholesterol as well as their more potent effects on bilayer stability and presumably also bilayer curvature.

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