# Induction of Lateral Phase Separations in Binary Lipid Mixtures by Alcohol†

Elizabeth S. Rowe*
*Veterans Administration Medical Center, Kansas City, Missouri 64128, and University of Kansas Medical School, Kansas City, Kansas 66103*

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ABSTRACT: It has previously been shown that alcohol has different effects on the gel to liquid-crystal phase transition of phosphatidylcholines (PC's) and phosphatidylethanolamines (PE's) [Rowe, E. S. (1985) *Biochim. Biophys. Acta 813*, 321-330]. In this investigation, the thermotropic properties of binary PE-PC mixtures were studied in the presence of ethanol in order to determine whether the differential interactions of alcohol with PC and PE would lead to lateral phase separations. Phase diagrams of the dilaurylphosphatidylethanolamine-dipalmitoylphosphatidylcholine [PE(12:0)-PC(16:0)] system were constructed in the presence and absence of ethanol. It was shown that lateral phase separations occur in the gel phase over a certain composition range in the presence of 100 mg/mL ethanol. In the absence of alcohol these two lipids are miscible in both the gel and liquid-crystal states. The data suggest that in the presence of ethanol these lateral phase separations involve the coexistence of regular bilayer gel and the fully interdigitated gel phase, which has previously been shown to occur in pure PC(16:0) under these conditions [Simon, S. A., & McIntosh, T. J. (1984) *Biochim. Biophys. Acta 773*, 169-172]. The biological implications of these findings are discussed.

The role of lipid physical properties in the function of membranes is not understood. Although many if not most membrane functions are carried out by proteins, the lipids clearly have composition-dependent roles in various membrane functions. In recent years through studies on model membrane systems, it has been demonstrated that there are a number of distinct physical states, i.e., phases, that are accessible to various lipids under physiological conditions. [For reviews, see Chapman (1975), Lee (1977a,b, 1983), Cullis et al. (1983), Keough and Davis (1984), and McElhaney (1982).] In many cases the difference in free energies between these states is relatively small, so that the balance between the phases can be altered by small changes in temperature or concentrations of small molecules. The accessibility of these phases differs among individual lipids with lipid class, chain length, and degree of unsaturation. As these lipid phases become identified and characterized, it becomes more apparent that phase-state fluctuations, composition-dependent phase distributions, and lateral phase separations may be important biological mechanisms in membrane function. [For reviews, see Grant (1983) and Jain (1983).]

There are several lipid phases that have been identified and studied. In addition to the well-characterized gel and liquid-crystal bilayer states, there is a nonbilayer inverted hexagonal phase that can occur in PE's[1] [Cullis & De Kruijff, 1979; reviewed in Cullis et al. (1983)] and several low-temperature forms of the gel phases [see Silvius (1986)]. The most recently identified phase of interest is a fully interdigitated gel phase that occurs in phosphatidylglycerols (PG's) and phosphatidylcholines (PC's) with equal chain lengths in the presence of various amphipathic small molecules (Ranck et al., 1977; McDaniel et al., 1983; McIntosh et al., 1983; Simon & McIntosh, 1984) and an antibotic (Ranck & Tocanne, 1982a,b). Myelin basic protein induces a fully interdigitated gel state in PG (16:0) (Boggs et al., 1981; Boggs & Ranjaraj, 1985). Increased pressure induces an interdigitated state in PC(18:0) and PC(16:0) (Braganza & Worcester, 1986), and the ether-linked PC(16:0) exists in an interdigitated state at temperatures below its melting transition (Ruocco et al., 1985). In view of the fact that many of the properties of the interdigitated gel state are similar to those of the normal bilayer gel states, it is possible that this state is more prevalent than previously thought.

We have been systematically studying the interactions of alcohols with synthetic lipids in order to elucidate the role of lipid physical properties in the mechanisms of general anesthesia and/or intoxication and to gain some insight into the role of lipid properties and lipid composition in membrane function. We have previously shown that short-chain alcohols interact differently with PC's and PE's, particularly at relatively high alcohol concentrations (Rowe, 1983, 1985). At low alcohol concentrations, the transition midpoints for the main transition of both PE's and PC's are shifted to lower temperature, with linear dependence on alcohol concentration. This effect has been interpreted in terms of the thermodynamics of freezing point depression, which involves preferential interactions of the alcohol with the liquid-crystal state over the gel state and was found to be nonspecific with regard to the PC or PE head groups. However, at higher alcohol concentrations, there is a second interaction of the alcohols with the PC's that is characterized by a break in the $T_m$ effect and marked hysteresis in the main transition. In contrast, there is no such second effect on PE of the higher alcohol concentrations; the transition midpoint continues decreasing, and the transition remains reversible throughout the range of alcohol concentration studied (Rowe, 1983, 1985). The high alcohol effect was shown by X-ray diffraction to be due to the induction of the interdigitated gel state (Simon & McIntosh, 1984). Interdigitation has not been detected in any PE's to date.

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* Address correspondence to this author at the Veterans Administration Medical Center, 4801 Linwood Blvd., Kansas City, MO 64128.

[1] Abbreviations: PE, phosphatidylethanolamine; PC, phosphatidylcholine; PE(12:0), dilaurylphosphatidylethanolamine; PC(16:0), dipalmitoylphosphatidylcholine; PG, phosphatidylglycerol; EDTA, ethylenediaminetetraacetic acid.

PC and PE are the two most abundant membrane lipid classes, and their distribution is quite different in cell membranes. The physical properties of these lipid classes have some important differences [see Hauser et al. (1981), Boggs (1984), Lis et al. (1982), and Boggs et al. (1986)]. The properties of mixtures of PC and PE must be understood in order to elucidate their function and distribution in cell membranes.

This study was undertaken in order to determine whether the differential effects of alcohols on PC's and PE's would result in lateral phase separations of these lipids at alcohol concentrations where interdigitation is induced in PC's but not in PE's. It was desired to study a lipid pair in which miscibility in both the gel and liquid-crystal phases would occur in the absence of alcohol. It was also considered desirable to begin with a pair in which the PC was the higher melting of the two. For these reasons, after several pairs were surveyed, the pair PC(16:0)–PE(12:0) was selected. Our results demonstrate that ethanol clearly induces lateral phase separations in the mixtures of these two lipids at certain lipid ratios and alcohol concentrations.

## Materials and Methods

*Lipids.* The lipids were obtained from Avanti, Inc., Birmingham, AL, and Sigma and used as supplied after verification of purity by thin-layer chromatography. Lipid suspensions were hand-shaken multilamellar vesicles prepared as described previously (Bangham et al., 1967; Rowe, 1982a). The binary mixtures were prepared by mixing appropriate amounts of the chloroform stock solutions prior to preparing the suspensions. Lipid concentrations of the stock solutions were determined by phosphorus analysis according to Bartlett (1957). The total lipid concentration in the samples was approximately 0.55 mg/mL. Samples were prepared in 0.1 M NaCl and 0.001 M EDTA. Sucrose (16%) was added prior to the experiments to prevent settling and to improve the optical properties of the solutions (Rowe, 1982b).

*Spectrophotometry.* The phase transitions were followed by optical density at 400 nm as described previously (Rowe, 1982a, 1983). The change in optical density (turbidity) that accompanies the gel to liquid-crystal phase transition is a light-scattering change due to the change in refractive index increment of the lipid as the lipid density changes during melting (Yi & McDonald, 1973). A similar change in optical density occurs in both PC's and PE's during the main transition from the gel to the liquid crystal (Rowe, 1985).

The phase transitions were followed at 400 nm with the Varian/Cary 219 spectrophotometer, which is interfaced with an Apple IIe computer. The Cary is equipped with a built-in thermistor, so that the temperature and optical density data can be read directly into the computer. The temperature in the cuvettes was controlled by circulating water through jacketed cuvettes from an external programmable bath, and the thermistor was placed in a cuvette connected in series with the sample cuvettes in the Cary sample compartment. The scan rates were approximately 0.75 deg/min. The accumulation of data into the Cary, including data averaging over temperature intervals of 0.02–0.1 deg and storage to disk for subsequent analysis and plotting was carried out with our own BASIC software. The routine for nonlinear least-squares variable-point smoothing and derivative calculation was based on the Savitzky and Golay (1964) method and used a BASIC algorithm generously provided by Dr. R. Khalifah.

## Results

The transition curves of a series of binary mixtures of PE(12:0) and PC(16:0) were measured in the absence and presence of 100 mg/mL ethanol, by following the optical density at 400 nm as described above. Figure 1 shows the transition curves for the series of these mixtures in the absence of any alcohol. The lipid composition of each mixture is indicated in the figure legend. Figure 2 shows the first derivatives of selected curves from Figure 1. As seen in these two plots, all of these transitions are sharp and relatively symmetrical. It is interesting to note that at the lowest PE(12:0) content examined, the pretransition that occurs in pure PC(16:0) has already disappeared.

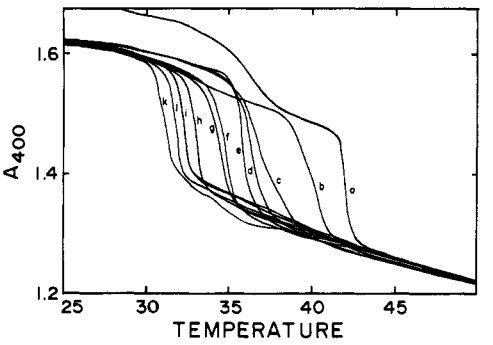


FIGURE 1: Melting transitions of PE(12:0)–PC(16:0) mixtures followed by absorbance at 400 nm. Mole fraction of PE(12:0): (a) 0.0, (b) 0.11, (c) 0.21, (d) 0.38, (e) 0.51, (f) 0.62, (g) 0.71, (h) 0.79, (i) 0.85, (j) 0.91, and (k) 1.0.

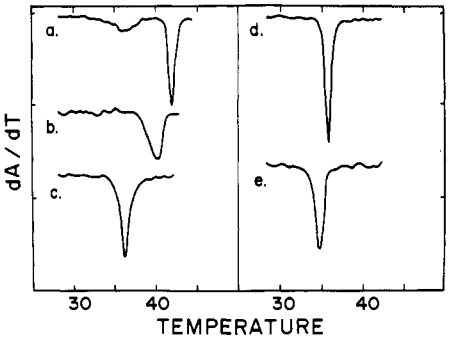


FIGURE 2: First-derivative curves for heating transitions of PE(12:0)–PC(16:0) mixtures from Figure 1. Mole fraction of PE(12:0): (a) 0.0, (b) 0.11, (c) 0.38, (d) 0.51, and (e) 0.62.

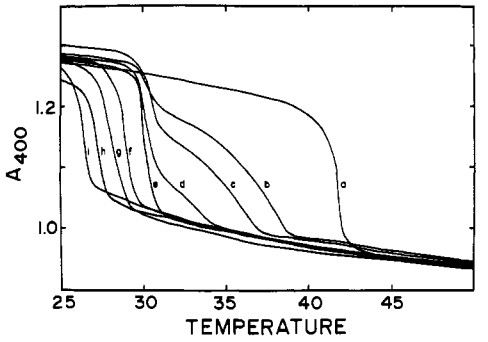


FIGURE 3: Melting transitions of PE(12:0)–PC(16:0) mixtures in the presence of 100 mg/mL ethanol followed by absorbance at 400 nm. Mole fraction of PE(12:0): (a) 0.0, (b) 0.11, (c) 0.21, (d) 0.38, (e) 0.51, (f) 0.62, (g) 0.71, (g) 0.79, and (i) 0.85.

Figure 3 shows the transition curves of the same mixtures in the presence of 100 mg/mL ethanol, and Figure 4 shows the first derivatives of selected curves from Figure 3. As seen here, from 0.11 to 0.40 mole fraction of PE(12:0) there is a break in the transition curves. Figure 4 clearly shows that in the presence of 100 mg/mL ethanol there are two transitions in the binary mixtures with mole fraction of PE(12:0) between 0.11 and 0.40. For all of the mixtures above 0.40 mole fraction of PE(12:0), single sharp transitions were observed.

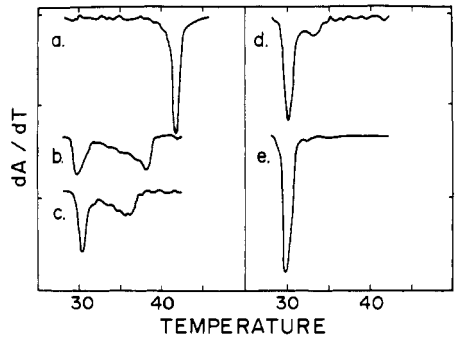


FIGURE 4: First-derivative curves for selected heating transitions from Figure 3. Mole fraction of PE(12:0): (a) 0.0, (b) 0.11, (c) 0.21, (d) 0.38, and (e) 0.51.

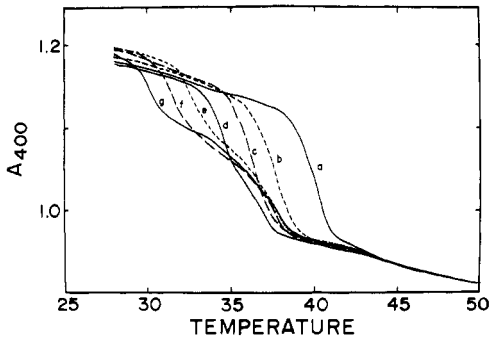


FIGURE 5: Melting transitions of a mixture of PE(12:0) and PC(16:0) containing 0.11 mole fraction of PE(12:0) as a function of ethanol concentration. Ethanol concentration in mg/mL: (a) 0, (b) 40, (c) 60, (d) 70, (e) 80, (f) 90, and (g) 100.

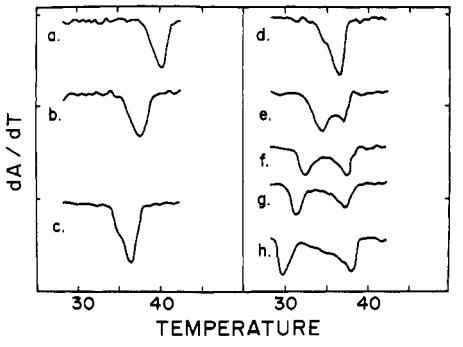


FIGURE 6: First derivatives of heating transitions of 0.11 mole fraction of PE(12:0) in PC(16:0) from Figure 5. Ethanol concentration in mg/mL: (a) 0.0, (b) 40, (c) 50, (d) 60, (e) 70, (f) 80, (g) 90, and (h) 100.

The induction of lateral phase separations by ethanol was further investigated by studying the ethanol dependence of this effect on one particular binary mixture. Figure 5 shows the effect of ethanol on the melting transition of the mixture containing 0.11 mole fraction of PE(12:0). The first derivatives of these curves are shown in Figure 6. It is seen in Figure 6 that a shoulder in the transition appears at 50 mg/mL ethanol and becomes fully resolved by 70 mg/mL ethanol. In Figures 5 and 6 it can be seen that the relative proportion of the higher melting material increases as the concentration of ethanol is increased.

Figure 7 shows the temperature maxima of the two transitions from the first derivatives of Figure 6 as a function of ethanol concentration. As seen here, there is a linear decrease in melting temperature up to approximately 50 mg/mL ethanol where the two transitions are first resolved. Above this concentration, the melting temperature of the higher melting fraction increases with ethanol concentration. The temperature of the maximum continues to decrease for the lower melting

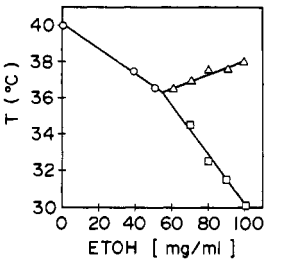


FIGURE 7: Temperature of first-derivative maxima from data in Figure 6 as a function of ethanol concentration.

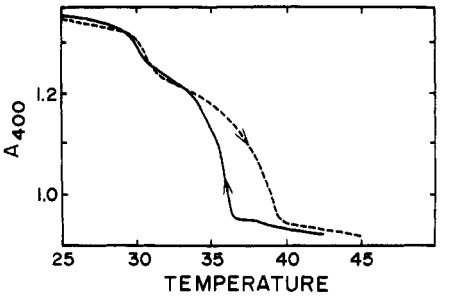


FIGURE 8: Heating and cooling transition of mixture containing 0.11 mole fraction of PE(12:0) in the presence of 100 mg/mL ethanol.

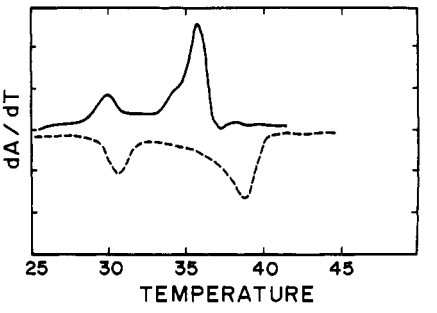


FIGURE 9: First derivatives of heating and cooling transitions from Figure 8. Solid line is cooling curve, and dashed line is heating transition.

portion but with an increase in the negative slope, reflecting the apparent concurrent change in composition of the lower melting material.

The reversibility of these transitions was also examined. All of the transitions shown in the preceding figures are heating scans, and no detectable rate dependence was observed; however, longer time frame metastability can only be detected by comparing heating and cooling scans. Figure 8 shows a typical heating and cooling experiment on a mixture with mole fraction of PE(12:0) of 0.11 containing 100 mg/mL ethanol. The first derivatives for these curves are shown in Figure 9. As seen here, there is a marked hysteresis for the higher melting transition, whereas the lower melting transition appears to be reversible. Similar results were obtained on the other compositions and alcohol concentrations.

*Phase Diagrams.* Phase diagrams for the PC(16:0)–PE(12:0) system in the presence and absence of ethanol were constructed from the data shown. The onset and completion temperatures were corrected for the finite width of the transitions of the pure lipids as described by Mabry and Sturtevant (1976).

Figure 10 shows the phase diagram for these lipids with no ethanol. The points and solid lines represent the data obtained, and the dashed lines represent the expected diagram for ideal mixing in both gel and liquid-crystal phases, calculated as described by Lee (1977b) and with the values of $\Delta H$ for PE(12:0) of 4.0 kcal/mol (Van Dijck et al., 1976) and for PC-

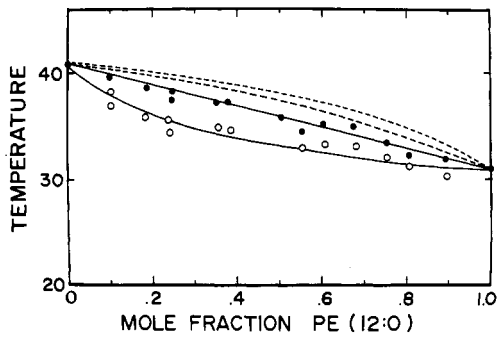


FIGURE 10: Phase diagram of PE(12:0)–PC(16:0) system constructed as described in the text.

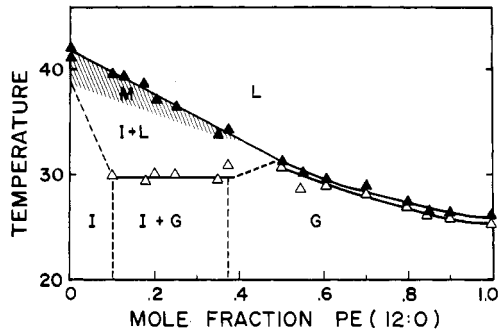


FIGURE 11: Phase diagram of PE(12:0)–PC(16:0) system in the presence of 100 mg/mL ethanol constructed as described in the text.

(16:0) of 8.6 kcal/mol (Mabry & Sturtevant, 1976). As seen here, although the mixture is not perfectly ideal, it clearly exhibits miscibility in both the gel and liquid-crystal phases.

Figure 11 shows the phase diagram for this mixture in the presence of 100 mg/mL ethanol. The triangles represent the onset and completion temperatures of the total transition, and the shaded area represents the region where the hysteresis is observed (i.e., the upper boundary of the shaded region represents the completion of the heating transitions, and the lower boundary of the shaded region represents the onset of the cooling transition). The dashed lines give the approximate boundaries of the regions of the phase diagram.

The phase diagram given in Figure 11 is a typical peritectic binary diagram in which limited solid–solid solubility is present [see Reisman (1970)]. The three-phase line at 30 °C from approximately 0.1 to 0.4 mole fraction of PE(12:0) indicates the coexistence of the two gel phases labeled I and G and the liquid-crystal phase L. At compositions below the beginning of the three-phase line, and temperatures below the solidus, we have only the I phase, which has variable amounts of PE(12:0) dissolved in it up to approximately 0.1 mole fraction of PE(12:0), the solubility limit. Between 0.1 and 0.4 mole fraction of PE(12:0), at and below 30 °C, we have a mixture of the I phase, saturated with PE(12:0), and the G phase, saturated with PC(16:0); the relative amounts of these two saturated solid solutions are given by the relative distances along the three-phase line. At compositions above approximately 0.4 mole fraction of PE(12:0) and at temperatures below the solidus, we have only the G phase, with variable amounts of PC(16:0) dissolved in it, decreasing from the solubility limit of approximately 0.6 mole fraction of PC(16:0) [i.e., 0.4 mole fraction of PE(12:0)].

The structural identity of the two gel phases must be inferred from other data. The I phase behaves similarly to pure PC(16:0) in the presence of 100 mg/mL ethanol, exhibiting two characteristics that have been previously attributed to the interdigitated phase (Simon & McIntosh, 1984; Rowe, 1985); the midpoint temperature of its transition exhibits a break as a function of alcohol concentration (Figure 7), and marked hysteresis occurs in its melting transition. The G phase behaves normally and is most likely the $L_{\beta}'$ or $L_{\beta}$ phase, similar to the gel phase adopted by PE's or by PE–PC mixtures high in PE content (Silvius, 1986). Thus, it appears that the interdigitated phase can accommodate up to 0.1 mole fraction of PE and the regular bilayer gel remains stable with as much as 0.6 mole fraction of PC, with this particular lipid pair in the presence of 100 mg/mL ethanol. However, the maximum amount of PE that can be accommodated in the interdigitated phase for this lipid pair appears to be a function of the concentration of the alcohol present, as seen in Figures 5–7; presumably, further variations in the relative amounts of PE that can be accommodated in the interdigitated phase will also depend on the particular lipids involved and the ligands used.

## Discussion

The most important finding resulting from this study is the demonstration that alcohol induces gel-phase lateral phase separations in a binary mixture of PC and PE; one of the phases appears to be the fully interdigitated gel phase described by Simon and co-workers (Simon & McIntosh, 1984; McDaniel et al., 1983; McIntosh et al., 1983) for PC's and characterized in this laboratory (Rowe, 1983, 1985). These results demonstrate that the difference in the properties of the PC and PE lipid classes can lead to considerably greater effects on the membrane distribution of lipids than has previously been recognized, when a third component with differential interactions with the two lipids is added.

In order to demonstrate that the lateral phase separations were induced by the addition of the ethanol, it was necessary to determine the degree of miscibility of the two lipids in the absence of ethanol. Our results indicate that they are fully miscible throughout the entire composition range. The phase diagram given in Figure 10 is qualitatively similar to those obtained on the binary mixture PE(14:0)–PC(18:0) that has been studied in two laboratories (Mabry & Sturtevant, 1976; Blume & Ackerman, 1974). This system was also discussed by Lee (1978), and it was interpreted to reflect nonideal mixing but not immiscibility. This system is similar to ours in that the PE is the lower melting of the pair, and it is one of a few found in the literature where this is the case. The more commonly studied PE–PC mixtures are those in which the PC is the lower melting of the pair. When the PC is the lower melting of the pair, there is some evidence of gel-phase immiscibility in some cases [see Silvius (1986), Blume et al. (1982), Mabry and Sturtevant (1976), Luna and McConnell (1978), and Arnold et al. (1981)]. The gel-phase immiscibility of PC–PE mixtures where the PE is the higher melting one has been attributed to the self-association of the PE through transient hydrogen bonding of the head groups in the gel phase (Boggs, 1984). This same H-bonding capability of PE has been used to explain the higher melting temperatures of PE's compared to PC's with the same chain lengths, the lack of tilt of the PE acyl chains in the gel phase, and the greater hydration of the PC head groups [see Hauser et al. (1981), Lis et al. (1982), and Boggs et al. (1986)].

The induction of the lateral phase separations in PE-(12:0)–PC(16:0) mixtures in the presence of ethanol, seen in the phase diagram in Figure 11, is a consequence of the PC's ability to form the interdigitated state, which the PE evidently does not share. The tendency of the PC's to form the interdigitated state is now well established. It can be induced in PC's by a variety of amphipathic molecules (McDaniel et al., 1983; McIntosh et al., 1983) and several short-chain alcohols (Rowe, 1985). It is interesting to note that interdigitation

occurs in PC(16:0) and PC(18:0) at increased pressure in the absence of any additives (Braganza & Worcester, 1986) and the di-16:0 ether-linked PC exists in the interdigitated state at normal pressures below the melting transition (Ruocco et al., 1985). The formation of the interdigitated state in PC's has been discussed in terms of the improved van der Waals contacts in the interdigitated state compared to the ordinary gel and the reduction in the head group crowding or charge repulsions that lead to the chain tilt in the ordinary gel phase (McIntosh et al., 1983; McDaniel et al., 1983). The role of the amphipathic inducing molecule has been discussed in terms of replacing water molecules on the PC surface with an amphipathic and more bulky molecule, forcing apart the head groups and creating potential voids in the acyl chain region (i.e., disrupting the van der Waals interactions in the acyl chains). At the same time the amphipathic molecule provides a more hydrophobic environment for the methyl groups at the acyl chain termini that are presumably at or near the bilayer surface in the interdigitated state (McDaniel et al., 1983; McIntosh et al., 1983; Simon & McIntosh, 1984). The pure PE's may not form interdigitated states because PE's already have better van der Waals interactions in the gel state as reflected in the lack of tilt of the acyl chains and the higher melting temperature. In addition, they have less water bound, apparently due to intermolecular hydrogen bonding, and thus, perhaps, fewer potential binding sites for the amphipathic molecules to the membrane surface.

It is interesting to note that the laterally segregated gel phases are not pure PE or PC; instead, both phases contain both PE and PC. This finding is consistent with the considerations discussed above concerning the possible reasons that the interdigitated phase forms in pure PC and not pure PE. From the point of view of the discussion above, it seems reasonable that the PC head group bulkiness and high levels of hydration are still present when there is some PE present in the PC. At the same time, for the particular lipid pair and ligand used here, the regular gel is stabilized by a mole fraction of PE(12:0) of only 0.4. This is consistent with the concept that a major influence in the formation of the interdigitated state is relief of the crowding of the PC head groups; apparently this amount of the PE(12:0) with its smaller head groups is sufficient to stabilize the bilayer gel relative to the interdigitated gel. Further study of the dependence on alcohol concentration of the amount of PE(12:0) required to stabilize the regular gel will contribute to a greater understanding of this mechanism.

The coexistence of interdigitated and noninterdigitated phases in the same sample raises the question of whether these two phases exist in the same lamella or not. While the size or extent of the regions or patches of lipid in each phase cannot be determined from our results, they suggest that the lateral phase separations exist on a microscopic rather than a macroscopic level. The most compelling evidence that the lateral phase separations are microscopic rather than macroscopic comes from the reversibility of the transitions: upon cooling from the fully miscible liquid-crystal phase, the two gel phases are formed as the cooling takes place, with the I phase forming first, at the higher temperature, followed by the fully reversible G phase. The results obtained were independent of scan rate and stable for repetitive scans, and the same results were obtained whether the transitions were measured within a few minutes of adding the alcohol or hours or even days later. Thus, while it seems reasonable to expect that the regions of each phase would be relatively large, total segregation of the two phases into macroscopically separated regions such as separate layers of the vesicles or even separate vesicles seems to be ruled out.

The conclusion that noninterdigitated and interdigitated phases can coexist in a single vesicle or liposome raises some interesting questions about the properties of these hybrid membranes, which deserve further study. Presumably, these membranes would be variable in thickness. The properties of the membrane at the interfaces between the two phases are expected to be unusual also. For example, one could predict that there is unusual exposure of hydrophobic acyl chains at these interfaces that could affect membrane fusion. Another property that might be affected by these interfaces is membrane permeability.

*Biological Considerations.* Several biological considerations are raised by the results reported here. Perhaps the most potentially important finding is that it is possible for interdigitated and noninterdigitated lipids to coexist in a membrane. Because the interdigitated state has many properties similar to the related bilayer gel state (McDaniel et al., 1983; McIntosh et al., 1983; Rowe, 1983; Simon & McIntosh, 1984), it is quite possible that its existence is more prevalent than previously recognized. A great many lipids have not been subjected to X-ray diffraction analysis, which is thus far the only direct means of detecting this phase. Other lipids have been shown to exhibit some of the other characteristics of this phase, particularly the hysteresis in melting transitions. For example, hysteresis has been observed in cerebrosides and certain sphingomyelins (Estep et al., 1980; Barenholz et al., 1983; Skarjune & Oldfield, 1982; Ruocco et al., 1981; Freire et al., 1980). Therefore, it is quite possible that interdigitated states can occur in biological membranes either naturally or as a result of a foreign perturbant. Our results show that interdigitated regions can occur in membranes that may be largely noninterdigitated. Thus, we have demonstrated the feasibility of interdigitated lipids occurring in limited or specialized regions in membranes, where they could have major functional impact even if present in small amounts.

The functional significance of small (or large) amounts of interdigitated lipids occurring in biological membranes includes several possibilities. For example, it is known that the two "leaflets" of the classical bilayer can be largely uncoupled (Sillerud & Barnett, 1982). A region of interdigitated lipid could provide a mechanism of coupling between the two sides of the bilayer. Similarly, it could provide a region where small or large molecules could traverse the membrane more easily. Also, as mentioned elsewhere, the boundaries between interdigitated and noninterdigitated regions may have unique properties that could have their own unique role in various membrane functions or membrane protein functions.

With regard to the possible mechanisms of intoxication and anesthesia, while we have shown that ethanol induces lateral phase separations, it occurs in these particular lipids at alcohol concentrations well above any physiologically possible concentrations. Nevertheless, the concentrations of alcohol needed to induce the interdigitated state in pure PC's varies with chain length or temperature (Rowe, 1983), and other parameters that may affect it such as degree of unsaturation have not been investigated. Thus, it cannot be ruled out that such effects of alcohol could occur under physiological conditions, even though it seems unlikely. In addition, there are several other drugs that have been shown to induce interdigitation, as well as an antibiotic and a protein, as discussed above, so it is likely that there are many more compounds that can also induce interdigitation. Therefore, it appears that induction of interdigitated states in specialized membrane regions cannot be

ruled out as having a possible role in the mechanisms of drug action.

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**Registry No.** PE(12:0), 18285-71-7; PC(16:0), 2644-64-6; EtOH, 64-17-5.

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