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The Effects of Ethanol on the Thermotropic Properties of Dipalmitoylphosphatidylcholine

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SUMMARY

The specific effect of ethanol on several aspects of the gel-to-liquid crystal transition of dipalmitoylphosphatidycholine was investigated using two spectrophotometric techniques, one probe method and one direct method. Ethanol shifts the phase-transition temperature to lower temperature, demonstrating that ethanol interacts preferentially with the fluid phase. Thermodynamic analysis of the melting point depression leads to a calculated membrane:buffer partition coefficient of 6.25 (mole fraction units) or 0.15 mole of ethanol per kilogram of lipid:mole of ethanol per liter of solution. Careful evaluation of the transition cooperativity with temperature resolution of ±0.1° shows that there is no reduction in transition cooperativity, and thus no reduction in size of the cooperative lipid clusters due to ethanol. The implications of these findings for the mechanism of action of ethanol in terms of current theories of anesthetic mechanisms are discussed.

INTRODUCTION

The molecular mechanisms of general anesthesia are unknown at present. It has long been recognized that a wide variety of structurally unrelated small organic molecules act as general anesthetics with potencies which are correlated with their lipid solubilities over a wide range of concentrations (for review see ref. 1). The general hypothesis is that these anesthetics act by dissolving in the lipid portion of the membranes and altering the function of some critical membrane events, probably carried out by membrane proteins. The present theories of anesthesia are based on specific effects of anesthetics on particular aspects of physical lipid properties (2–6). Ethanol is considered as a general anesthetic; however, few studies have been done specifically on ethanol, primarily because of its low membrane solubility. Ethanol is particularly suited for studies of anesthetic mechanisms because of the large body of information about its chronic and acute effects in experimental animals. For example, it has been shown that both the physical properties (7, 8), and the lipid composition (9, 10) of synaptosomal membrane preparations from mice chronically maintained on ethanol diets are altered relative to those from pair-fed controls.

In the present investigation we have examined in detail the effect of ethanol on the phase equilibria of DPPC[1] with particular emphasis on the effect of ethanol on the transition temperature and the transition cooperativity. The study of transition coperativity is particularly important from the point of view of present theories of general anesthesia. The results of these investigations are then evaluated from the perspective of the predictions of the present theories of anesthesia to attempt to distinguish among them.

MATERIALS AND METHODS

Materials. DPPC was purchased from Calbiochem (San Diego, Calif.) and found to be free of impurities by thin-layer chromatography in chloroform-methanol-water (35:65:5) on silica gel plates. Water was deionized, passed over a charcoal filter, and then distilled. Organic solvents were distilled.

Lipid samples were hand-shaken multilamelar liposomes prepared according to the method of Bangham *et al.* (11). The chloroform solution of lipid was shell-dried under a stream of nitrogen, and warm buffer (0.05 M Tris (pH 7.4), 0.001 M EDTA, and 0.15 M NaCl) was added and the sample vortexed. The suspension was incubated above the transition temperature for 2 hr, with intermittent vortexing. Individual samples prepared from stock suspensions by the addition of sucrose or ethanol were incubated above the transition temperature for 1 hr. The lipid concentrations in the suspensions ranged from 0.13 mg/ml to 0.6 mg/ml. Lipid concentrations were determined by phosphorus analysis according to the method of Bartlett (12).

c-PnA and *t*-PnA were obtained from Molecular Probes, Inc. (Evanston, Ill.). Stock solutions in methanol

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[1] The abbreviations used are: DPPC, dipalmitoylphosphatidylcholine; PnA, parinaric acid; *t*-PnA, *trans*-parinaric acid (9, 11, 13, 15, all *trans*-octadecatetraenoic acid); *c*-PnA, *cis*-parinaric acid (9, 11, 13, 15, *cis*-, *trans*-, *trans*-, *cis*-octadecatetraenoic acid).

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at concentrations of 1.0–0.5 mg/ml and containing butylated hydroxytoluene (0.01 mg/ml) were flushed with nitrogen and stored in the freezer, and were found to be stable by spectral criteria for several months. The absorption spectra were analyzed according to the criteria of Sklar *et al.* (13). When PnA was used in a lipid sample, the sample was flushed with nitrogen before adding the *t*-PnA to the sample in stoppered and jacketed cuvettes, and the *t*-PnA was added immediately prior to the experiments. Spectra of the *t*-PnA were measured at the end of the experiments, and were generally found to be unchanged during the experiment.

Spectrophotometry. The work described here was performed using the Cary 219 spectrophotometer equipped with first derivative, timer, wavelength programmer, cell programmer, and temperature readout accessories, as well as the digital interface port and printer. By this arrangement it is possible to make sequential measurements automatically at three wavelengths on several samples and to print out the digital absorption and temperature data. The temperature was controlled by water circulated through jacketed cuvettes from a Lauda K2/RD refrigerated bath, and the temperature was monitored by the built-in thermistor which was immersed in a jacketed cuvette hooked in series with the sample cuvettes. The heating rate could be varied between 0.2°/min and 1.5°/min. In general, two samples were followed simultaneously; this improved the temperature resolution by permitting comparison of two samples at temperatures which were identical to within 0.05 degree, even though the absolute temperature value is only correct to within 0.1 degree. In some experiments where signal averaging was required to increase precision (for example, for the derivative calculations), the temperature was changed in steps of 0.1° rather than continuously. At each step 10 readings were taken for averaging.

RESULTS

Phase equilibria followed by absorption. The primary parameter used for following the phase equilibria of DPPC in the present study was the change in optical density due to the light-scattering change which accompanies the phase transition. This method has the advantage over other optical methods of providing a direct measure of a lipid property; thus it does not require the addition of any perturbant which could disturb the effect of interest, and it does not require any assumptions about the location of a probe molecule. The physical basis for the change in optical density comes primarily from the change in the density of the lipid during the transition which leads to a change in refractive index increment and hence a light-scattering change; bilayer anisotropy changes also contribute (14).

Effect of sucrose on the phase transition. In order to study the phase transition of multilamellar suspensions of lipid it is useful to add an inert substance which will neutralize the density difference between the lipid and the solvent and prevent the settling of the suspension during the course of the experiment. Deuterium oxide, sucrose, and glycerol are suitable for this purpose. Stirring of the cuvette is a possibility which we also explored. Each of these alternatives prevented problems due to the settling of the suspensions without altering the temperature or cooperativity of the main transition. An additional effect was observed with sucrose and glycerol, which is described in detail elsewhere (15). In summary, we found that the appearance of the transition curve when followed by optical density is considerably affected by using a solvent with a relatively large index of refraction. Figure 1 shows two transition curves of DPPC followed by optical density at 400 nm. In one curve, the solvent contains 16.7% surcrose, whereas in the other the solvent contains only buffer and ^2H_2O. As shown here, the effect of the sucrose is to lower the absolute optical density, reduce the optical contribution of the pretransition, and increase the optical contribution of the main transition. No real thermodynamic effect of sucrose on the main transition has been found (16), and we have included appropriate controls under each of our conditions to show that sucrose is not affecting our results. Because of the improvement in the optical data obtained, and to prevent settling of the suspensions, most of our data were obtained in the presence of 16.7% sucrose.

Reversibility. In any thermodynamic study it is necessary to establish the existence of thermodynamic equilibrium under the conditions of interest. To demonstrate that the state of the lipid was dependent only on the conditions and not on the pathway of achieving them, several experiments were performed in which the equilibrium temperature was approached in both directions. As has been observed by many others, the pretransition has extremely slow kinetics so that thermodynamic studies of this transition are difficult. For example, we found that recovery of the optical density at 25° after heating to 50° and cooling required more than 2 hr. We have restricted our present study to the main transition because this is the transition of relevance to our interest in anesthetic mechanisms.

In some cases we had observed a slight discrepancy in the transition midpoints from heating and cooling scans performed at our usual scan rates. That this was a kinetic

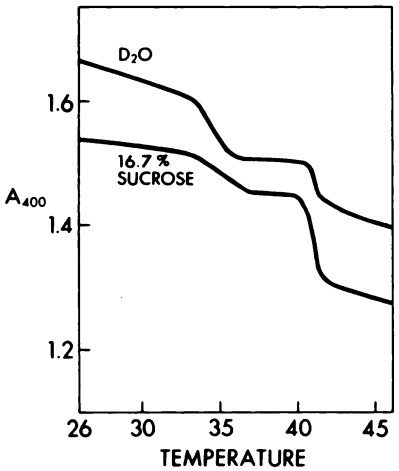

FIG. 1. *Effect of sucrose on optical properties of DPPC during the phase transition*

The solvent densities of each solution were the same; in the *upper curve* ^2H_2O was added and in the *lower curve* 16.7% sucrose was added to prevent settling of the liposome suspensions. The lipid concentration was 0.55 mg/ml.

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effect was established by the following experiment (data not shown). A sample was divided in half; one half was equilibrated at 25°, and the other at 50° for at least 30 min. These two samples were then placed in cuvettes which had been pre-equilibrated at 41.6° in the spectrophotometer, and the optical density was monitored until a constant reading was obtained. Both samples reached the same final optical density; however, the one which was cooled from 50° required at least 15 min to reach this equilibrium value, whereas the sample being heated from 25° reached equilibrium within 2 min, or approximately as fast as the temperature in the sample equilibrated. Both samples were then taken through a complete heating and cooling curve, in which they behaved identically. This experiment demonstrates that the transition is reversible, but that it takes longer for the sample to reach equilibrium in a cooling experiment than in a heating curve. Therefore we have choosen to use heating scans for most of our experiments, after verifying the reversibility of the transition under each set of conditions.

Effect of ethanol on phase transition temperature. In Fig. 2 are shown transition curves of DPPC followed by absorbance at 400 nm, in the presence and absence of ethanol. As seen here, the presence of ethanol in the solution reduces the temperature of the transition midpoint. From thermodynamic principles the observation that the melting temperature is lowered by ethanol leads to the conclusion that ethanol is more soluble in the fluid phase than in the solid or gel phase. The presence of ethanol also reduces the optical density of the lipid suspensions; this effect is not understood at the present time.

Figure 3 shows a plot of transition midpoint as a function of aqueous ethanol concentration (assumed to be equal to total ethanol concentration). The plot is linear at ethanol concentrations up to 50 mg/ml, where it shows an upward curvature.

Membrane:buffer partition coefficient. The obseration that ethanol reduces the phase transition temperature leads to the necessary conclusion that ethanol is more soluble in the fluid-phase lipid than in the solid-phase lipid. Analysis of this temperature shift in terms of the thermodynamics of freezing point depressions leads to an estimate of the difference in solubility of ethanol in the

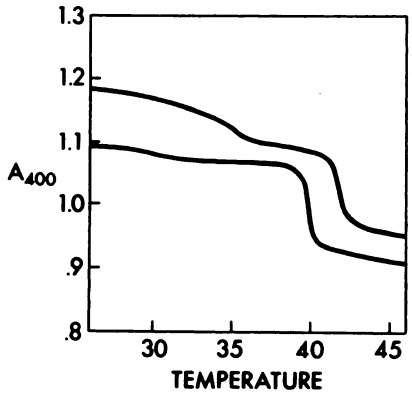

FIG. 2. *Effect of ethanol on phase transition of DPPC*
Upper curve, no ethanol; *lower curve*, ethanol 50 mg/ml. The lipid concentration was 0.45 mg/ml.

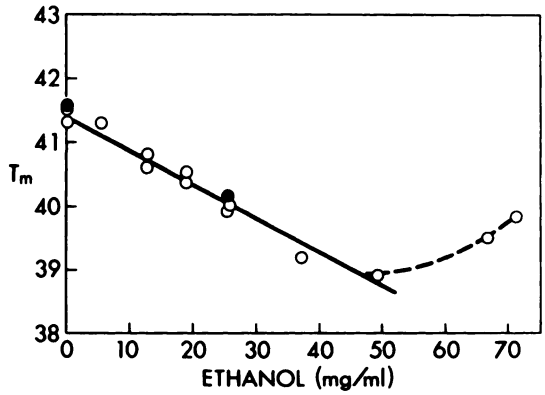

FIG. 3. *Transition midpoint as a function of ethanol concentration*
○, Samples contained 16.7% sucrose; ●, no sucrose.

fluid and solid phases. From these data the membrane:buffer partition coefficient can be calculated. The linearity of the plot in Fig. 3 indicates that the membrane:buffer partition coefficient is independent of the aquaeous concentration over the linear range. The membrane concentration is obtained by the relationship:

$$\ln X_L = \frac{-\Delta H}{R}\left(\frac{1}{T_m} - \frac{1}{T_0}\right) = \ln (1 - X_s) \cong X_s$$

where X_L is the mole fraction of lipid in the membrane, X_s is the mole fraction of solute dissolved in the membrane so that $X_L + X_s = 1$, ΔH is the enthalpy of the transition, and T_0 and T_m are the transition midpoints for the pure lipid and the lipid containing alcohol, respectively, in degrees Kelvin. Rearranging and simplifying we have

$$X_s = \frac{\Delta H \Delta T}{R T_0^2} \quad (1)$$

The partition coefficient is then

$$K_p = \frac{X_{s,\,memb}}{X_{s,\,soln}} = \frac{\Delta H \Delta T}{R T_0^2 X_{s,\,soln}} \quad (2)$$

Using the values of ΔH = 8.6 kcal/mole (17) with T_m = 41.4°, the partition coefficient calculated using the slope of Fig. 3 is 6.2 (mole fraction units). This corresponds to 0.15 mole of ethanol per kilogram of lipid:mole of ethanol per liter of solution.

Phase equilibria observed by PnA absorption. In addition to the direct measurements of the phase transition by absorption changes due to the lipid light-scattering change, measurements were also made using *c*-PnA and *t*-PnA probes, and following changes in their absorption as a function of temperature. *c*-PNA and *t*-PnA are naturally occurring fatty acids containing four conjugated double bonds which give rise to their useful spectroscopic properties. These two membrane probes have been extensively characterized by Sklar and co-workers (13). The *cis*- and *trans*-isomers of these fatty acids have similar spectra, but they behave differently when inserted into phospholipids. The *cis*-isomer partitions equally into the solid and fluid phases, whereas the *trans*-isomer prefers the solid phase by a factor of 3:1 to 5:1 (18). This property of *t*-PnA makes it a useful probe for the presence

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of small amounts of the solid-phase lipid. We have made use of this property of *t*-PnA to attempt to detect any changes in phase transition cooperativity of DPPC due to ethanol.

A portion of the absorption spectrum of *t*-PnA is shown in Fig. 4, in the presence of lipid. The absorption bands of *t*-PnA shift approximately 2 nm to lower wavelength as the lipid melts, again due to the change in polarizability of the lipid as the density decreases. Figure 4 shows the absorption of a typical example containing *t*-PnA at two temperatures, illustrating the relative magnitudes of the PnA absorption, the turbidity due to the lipid, and the change in each during the phase transition. We have found that the most advantageous means of following the *t*-PnA change is to measure the difference in optical density between two wavelengths near each other on either side of the absorption maximum. As the maximum shifts, there is a large change in this difference, and the change in background turbidity is removed from the measurement, since it is virtually the same at all temperatures. The calculation of the parameter is illustrated in Fig. 4. Figure 5 shows an example of a phase transition followed by this method, also showing the ΔA data for a blank containing no PnA.

PnA has no absorption at 400 nm, so it is possible to monitor transitions simultaneously on a single sample both by the absorption at 400 due to the lipid light-scattering change and also by the changes in PnA absorption. Figure 6 shows a control experiment in which the transition was followed at 400 nm for lipid in the presence and absence of PnA. These curves demonstrate that PnA has no effect on the transition in the amounts used here.

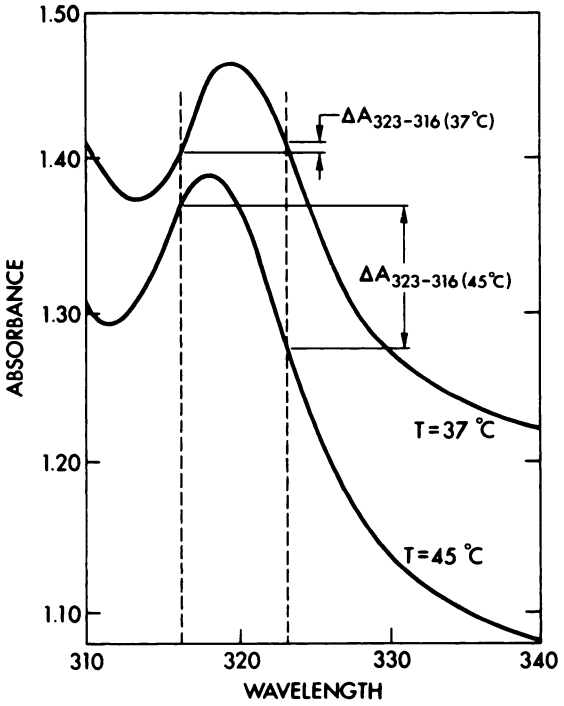

FIG. 4. *Absorption spectra of t-PnA in DPPC above and below the phase transition*

The DPPC concentration was 0.55 mg/ml; the lipid:PnA ratio was 130. $A_{323-316}$ was calculated by subtracting A_{316} from A_{323} as shown.

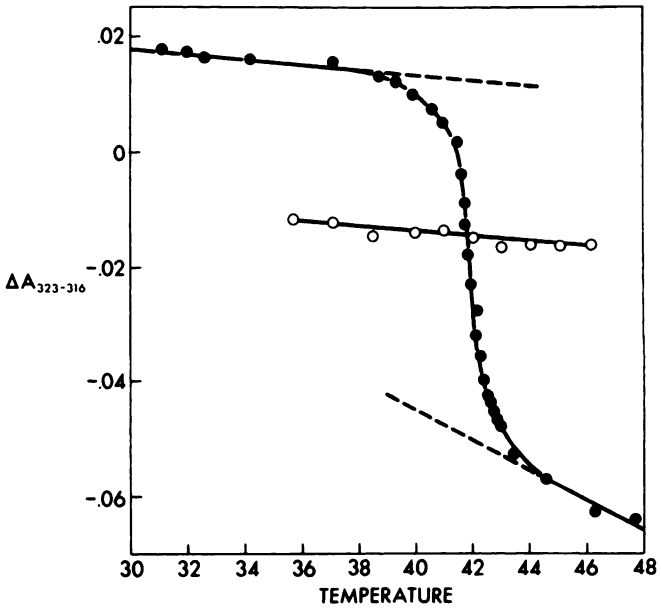

FIG. 5. *DPPC phase transition followed by ΔA parameter*

○, Samples contained no PnA. Each point represents an average of ten readings.

Figure 7 shows normalized transition curves of DPPC followed simultaneously by A_{400} and by the ΔA parameter for *t*-PnA. It is seen that the midpoint of the transition appears at approximately 0.2° higher temperature when observed by the *t*-PnA probe than by A_{400}. This is a consequence of the preferential interactions of the *t*-PnA with the solid-phase lipids.

Effect of ethanol on transition cooperativity. The steepness of the lipid-phase transition can be related to the cooperativity of the transition, which is a measure of the preferential interactions of the molecules in each phase. Some anesthetics decrease the cooperativity of lipid-phase transitions, and this effect has been suggested by one group to be the primary physical effect which leads to anesthesia (2). For this reason we have undertaken a careful examination of the effect of ethanol on the transition cooperativity of DPPC.

The cooperativity of the phase transition of DPPC was examined by constructing a graph of the first derivative

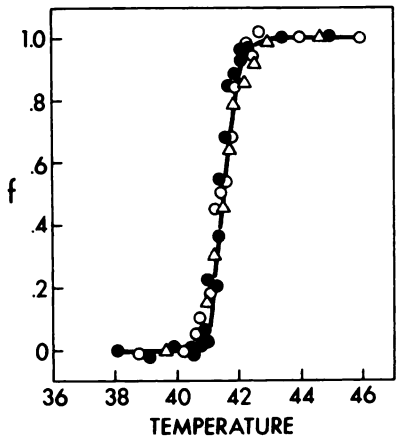

FIG. 6. *Normalized transition curves of DPPC in presence of c-PnA (○), t-PnA (●), and no PnA (△), followed by A_{400}*

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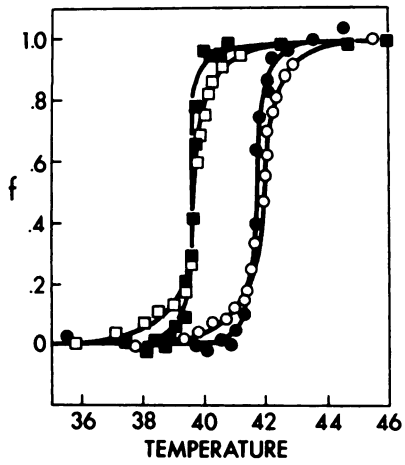

FIG. 7. *Normalized transition curves of DPPC in the presence (□, ■) and absence (○, ●) of ethanol (50 mg/ml), followed by A_{400} (■, ●) and by $\Delta A_{323-316}$ (□, ○)*

of the transition parameter with respect to temperature. For these experiments the temperature was changed in steps, and 10 data points were recorded at each temperature and averaged; the step size in the transition region was 0.1°. These points were then used to calculate the point-to-point slope of the data from Fig. 7 (before normalization).

The derivative curves of DPPC in the presence and absence of ethanol, using both the A_{400} and the ΔA parameters, are shown in Fig. 8. The transition width expressed by the width at half-height of these plots is related to the transition cooperativity. Relying on the A_{400} data, it is clear by inspection that there is no broadening of the transition by ethanol. Consideration of the temperaure of the maximum for each curve demonstrates the incongruity of the transitions observed by ΔA and A_{400}; both in the presence and absence of ethanol the *t*-PnA reports the transition midpoint at approximately 0.2° higher temperature than the true midpoint based on

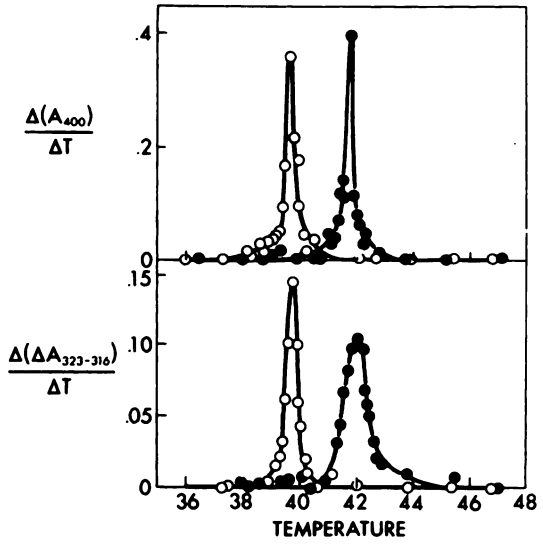

FIG. 8. *First derivative with respect to temperature of data from Fig. 7 (before normalization)*

Upper curves, A_{400}; lower curves, $\Delta A_{323-316}$. ●, No ethanol.

A_{400}. The discrepancy between the midpoint of the two parameters is due to the preference of tPnA for gel-phase lipid; it would be expected to increase in the presence of a perturbant which broadened the transition.

DISCUSSION

Thermodynamic treatment of phase transitions. The thermodynamic aspects of lipid-phase transitions have been discussed in several recent reviews (19–21). The bilayer lipid-gel to liquid-crystal phase transition can be approximated to a first-order transition similar to the melting of a solid. The thermodynamic analysis of the transition temperature shift caused by the presence of solute molecules to estimate the amount of solute dissolved in the lipid has been used by several authors (22–24).

The shift in transition temperature to lower temperature leads to the conclusion that the solute interacts preferentially with the fluid phase; however, the thermodynamics alone does not provide a mechanism of this interaction. The conclusion that the membrane concentration calculated from Eq. 1 approximates the actual amount of ethanol dissolved in the membrane relies on additional data. For example, Hill (25) showed that the amount of octanol bound to DPPC as determined by direct measurement was similar to the amount calculated for DPPC by transition temperature shift. In addition, it has been shown that the membrane:buffer partition coefficients for the *n*-alcohols increase with increasing chain length, as expected for a hydrophobic interaction (e.g., see refs. 25 and 26).

Partition coefficient. The partition coefficient determined by the shift of the transition temperature of DPPC was 6.25 (mole fraction units), or 0.15 mole of ethanol per kilogram of lipid:mole of ethanol per liter of solution, as compared with the value for DMPC of 0.07 mole of ethanol per kilogram of lipid:mole of ethanol per liter of solution determined previously (24). In using the thermodynamic treatment to determine partition coefficients, it must be noted in Eq. 2 that the value determined is dependent upon the ΔH for the phase transition. There have been some discrepancies in literature values for this parameter.

The partition coefficient determined by the shift of the transition temperature of DPPC can be compared with published literature values. Literature values for partitioning of ethanol into various membranes range from 0.062 mole of ethanol per kilogram of membrane:mole of ethanol per liter of solution to 0.14 mole of ethanol per kilogram of membrane:mole of ethanol per liter of solution with one anomalously high value of 0.44 mole of ethanol per kilogram of membrane:mole of ethanol per liter of solution (1, 22–27). With a few exceptions, these values were obtained by extrapolation of the membrane:buffer partition coefficient or octanol:water partition coefficient of the higher alcohols. Two values determined by transition temperature shift of DPPC were 0.12 and 0.062 mole of ethanol per kilogram of membrane:mole of ethanol per liter of solution (refs. 22 and 23, respectively); in both of these studies the concentration of ethanol used was not given, so it is not certain that these experiments were in the range where the effect

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is linear (Fig. 3). One direct-binding study of the binding of radiolabeled ethanol to dimyristoylphosphatidylcholine (27) gave a value of 0.44 mole of ethanol per kilogram of membrane:mole of ethanol per liter of solution; however, this value relies on a possibly erroneous correction to the data which assumes that a water layer inaccessible to sucrose would also be inaccessible to ethanol. Without this correction, this value would be close to the other literature values for dimyristoylphosphatidylcholine.

Cooperativity. The effects of perturbants on the cooperativity of the phase transition may have important biological consequences. One of the present theories of general anesthetic mechanisms holds that the "cluster" size of regions of coexisting phases is a critical parameter in the function of membrane proteins (2). Using optical methods, calculations of absolute cluster size require several assumptions. Comparison of the cooperativity of a lipid transition under two sets of conditions requires fewer assumptions and appears to be justified in the present study. For these comparisons we chose to consider the transition width, as determined by the width at half-height of the derivative curves of Fig. 8. We have found that the cooperativity of the phase transition of DPPC is not decreased by the addition of ethanol, as seen by comparison of the transition width of the transition observed by A_{400} in the presence and absence of 1.1 M ethanol. This is in agreement with Jain and Wu (28), who found that *n*-alcohols up to butanol, and some other classes of organic molecules, did not broaden the transition of DPPC, as measured by differential scanning calorimetry. Our data appear to be sufficiently accurate to eliminate transition broadening as a mechanism of action for ethanol, at least with respect to DPPC.

Anesthetic mechanisms. Several theories of general anesthesia have been suggested, based on the observations that a wide variety of simple, structurally unrelated organic molecules have anesthetic potencies which are correlated with their membrane solubility or the octanol:water partition coefficient. However, superimposed on a pattern of similarity are also differences among the various substances, and these differences have been considered by some to rule out the existence of a general unitary mechanism of action for these molecules (4). At the least, these differences require the detailed examination of particular anesthetics before concluding that any particular anesthetic has all of the properties of the general class. The general theories differentiate between lipid order or microviscosity effects (4), changes in phase distribution or lateral phase separations (3), and cooperative fluctuation between phases by critical clusters of lipids (2). Our study of ethanol-DPPC interactions gives no data on the question of membrane microviscosity; however, others have shown that ethanol does increase the fluidity of membranes (e.g., ref. 8). These changes in fluidity are extremely small at clinical concentrations, and no molecular mechanism has been proposed to explain how such a small fluidity effect could substantially change the function of a membrane protein. A more physically plausible proposal is that of Trudell (3), in which the presence of lateral-phase separations in the vicinity of some critical membrane protein is thought to be the crucial physical lipid property. The shift of phase equilibria, eliminating the lateral phase separations, is then the cause of the change in function of the proteins. The physical rationale for this hypothesis is that the maximal lateral compressibility of the bilayer in the presence of coexisting gel and fluid phases is required for the protein activity. Mountcastle *et al.* (2) have taken this concept further, and focused on the dynamic nature of the coexisting clusters of lipids in each phase; these authors maintain that it is the local fluctuation in density accompanying these cooperative cluster phase transitions which is critical for the proper functioning of some membrane proteins. According to their hypothesis, the important aspect of anesthetic action is the reduction of transition cooperativity caused by the anesthetic which inactivates a protein by decreasing the magnitude of the local fluctuations.

Our study of ethanol-DPPC interactions demonstrates that ethanol shifts the phase equilibria to lower temperatures with no detectable change in transition cooperativity. The shift in phase equilibria at constant temperature at clinical concentrations would not be enough to change the phase distribution by very much. Biological membranes contain a wide variety of lipids, and it is possible that ethanol will have a greater effect on lipid mixtures within lipid classes, lipid-cholesterol combinations, or protein-lipid interactions. Another possibility is that the recently recognized non-bilayer states which are accessible to some phospholipids have a role in the mechanism of anesthesia (30). The change in lipid composition of chronic alcoholic mice relative to controls suggests that ethanol may have different interactions with different lipids. In addition, our finding that ethanol interacts preferentially with fluid-phase lipids as compared with gel-phase lipids suggests that ethanol may interact preferentially with the fluid regions of biological membranes. It remains to be determined whether the degree of fluidity of the fluid phase has a significant influence on the alcohol-membrane interaction. Further work on the lipid specificity of ethanol-membrane interactions is planned.

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REFERENCES

1. Seeman, P. The membrane actions of anesthetics and tranquilizers. *Pharmacol. Rev.* **24**:583–654 (1972).
2. Mountcastle, D. B., R. L. Biltonen, and M. J. Halsey. Effect of anesthetics and pressure on the thermotrophic behavior of multilamellar dipalmitoylphosphatidylcholine liposomes. *Proc. Natl. Acad. Sci. U. S. A.* **75**:4906–4910 (1976).
3. Trudell, J. R. A unitary theory of anesthesia based on lateral phase separations in nerve membranes. *Anesthesiology* **46**:5–10 (1977).
4. Richards, C. D., K. Martin, S. Gregory, C. A. Keightley, T. R. Hesketh, G. A. Smith, G. B. Warren, and J. C. Metcalfe. Degenerate perturbations of protein structure as the mechanism of anesthetic action. *Nature (Lond.)* **276**:775–779 (1978).
5. Eyring, H., J. W. Woodbury, and J. S. D'Arrigo. A molecular mechanism of general anesthesia. *Anesthesiology* **38**:415–424 (1973).
6. Hill, M. W. The Gibbs free energy hypothesis of general anesthesia, in *Molecular Mechanisms in General Anesthesia* (M. J. Halsey, J. A. Sutton, and R. A. Miller, eds.). Churchill-Livingston, Edinburg, 132–144 (1974).
7. Chin, J. H., and D. B. Goldstein. Drug tolerance in biomembranes: a spin label study of the effects of ethanol. *Science (Wash. D. C.)* **196**:684–685 (1977).
8. Chin, J. H., and D. B. Goldstein. Effects of low concentrations of ethanol on

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the fluidity of spin-labelled erythrocyte and brain membranes. *Mol. Pharmacol.* **13**:435–441 (1977).
9. Chin, J. H., L. M. Parson, and D. B. Goldstein. Increased cholesterol content of erythrocyte and brain membranes in ethanol-tolerant mice. *Biochim. Biophys. Acta* **513**:358–363 (1978).
10. Littleton, J. M., and G. J. John. Synaptosomal membrane lipids of mice during continuous exposure to ethanol. *J. Pharm. Pharmacol.* **29**:579–580 (1977).
11. Bangham, A. D., J. De Gier, and G. D. Greville. Osmotic properties and water permeability of phospholipid liquid crystals. *Chem. Phys. Lipids* **1**:225–245 (1967).
12. Bartlett, G. R. Phosphorus assay on column chromatography. *J. Biol. Chem.* **234**:466 (1957).
13. Sklar, L. A., B. S. Hudson, M. Petersen, and J. Diamond. Conjugated polyene fatty acids as fluorescent probes: spectroscopic characterization. *Biochemistry* **16**:813–818 (1977).
14. Yi, P. N., and R. C. MacDonald. Temperature dependence of optical properties of aqueous dispersions of phosphatidylcholine. *Chem. Phys. Lipids* **11**:114–134 (1973).
15. Rowe, E. S. Effect of sucrose on the optical properties of dipalmitoylphosphatidylcholine. *Biochim. Biophys. Acta* **685**:105–108 (1982).
16. Suurkuusk, J., B. R. Lentz, Y. Barenholz, R. L. Biltonen, and T. E. Thompson. A calorimetric and fluorescent probe study of the gel-liquid crystalline phase transition in small single-lamellar dipalmitoylphosphatidylcholine vesicles. *Biochemistry* **15**:1393–1401 (1976).
17. Lentz, B. R., E. Freire, and R. L. Biltonen. Fluroescence and calorimetric studies of phase transitions in phosphatidylcholine multilayers: kinetics of the pre-transition. *Biochemistry* **17**:4475–4480 (1978).
18. Sklar, L. A., G. P. Miljanich, and E. A. Dratz. Phospholipid lateral phase separation and the partition of *cis* parinaric acid and *trans* parinaric acid among aqueous, solid lipid, and fluid lipid phases. *Biochemistry* **18**:1707–1716 (1979).
19. Lee, A. G. Lipid phase transitions and phase diagrams. I. Lipid phase transitions. *Biochim. Biophys. Acta* **472**:237–281 (1977).
20. Lee, A. G. Lipid phase transitions and phase diagrams. II. Mixtures involving lipids. *Biochim. Biophys. Acta* **472**:285–344 (1977).
21. Chapman, D. Phase transitions and fluidity characteristic of lipids and cell membranes. *Q. Rev. Biophys.* **8**:185–235 (1975).
22. Hill, M. The effect of anaesthetic-like molecules on the phase transition in Smectic mesophases of dipalmitoyllecithin. I. The normal alcohols up to C=9 and three inhalation anaesthetics. *Biochim. Biophys. Acta* **356**:117–124 (1974).
23. Kamaya, H., S. Kaneshina, and I. Ueda. Partition equilibrium of inhalation anesthetics and alcohols between water and membranes of phospholipids with varying acyl chain-lengths. *Biochim. Biophys. Acta* **646**:135–142 (1981).
24. Rowe, E. S. Membrane:buffer partition coefficient for ethanol in dimyristoylphosphatidylcholine. *Alcoholism Clin. Exp. Res.* **5**:259–263 (1981).
25. Hill, M. W. Partition coefficients of some anesthetic-like molecules between water and smectic mesophases of dipalmitoylphosphatidylcholine. *Biochem. Soc. Trans.* **3**:149–152 (1975).
26. Seeman, P., S. Roth, and H. Schneider. The membrane concentrations of alcohol anesthetics. *Biochim. Biophys. Acta* **225**:171–184 (1971).
27. Katz, Y., and J. M. Diamond. Thermodynamic constants for nonelectrolyte partition between dimyristoyl lecithin and water. *J. Membr. Biol.* **17**:101–120 (1974).
28. Jain, M. K., and N. Y.-M. Wu. Effect of small molecules on the dipalmitoyl lecithin liposomal bilayers. III. Phase transition in lipid bilayer. *J. Membr. Biol.* **34**:157–201 (1977).
29. Pang, K.-Y. Y., L. M. Braswell, L. Chang, T. J. Sommer, and K. W. Miller. The perturbation of lipid bilayers by general anesthetics: a quantitative hypothesis. *Mol. Pharmacol.* **18**:84–90 (1980).
30. Cullis, P. R., and B. DeKruijff. Lipid polymorphism and the functional roles of lipids in biological membranes. *Biochim. Biophys. Acta* **559**:399–420 (1979).

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