Interactions between Anesthetics and Lipid Mixtures. Normal Alcohols†

A. G. Lee

ABSTRACT: The effects of normal alcohols up to 1-dodecanol on phase transitions in phosphatidylcholines and phosphatidylethanolamines have been studied using chlorophyll a as fluorescent probe. With the phosphatidylcholines, alcohols up to octanol cause a lowering of the transition temperature, and a broadening of the transition, whereas for dipalmitoylphosphatidylethanolamine, only a lowering of the transition is observed. The lowering of the phase transition temperature in dipalmitoylphosphatidylcholine by butanol and hexanol is close to that expected for ideal behavior, but the behavior of the longer chain alcohols becomes less ideal. The effects of these alcohols on mixtures of lipids have been studied, and they illustrate the care necessary if these plots of temperatures of onset and completion of gel phase formation are to be called "phase diagrams". The effect of 1-octanol on mixtures of lipids is to increase the proportion of lipid present in the liquid-crystalline state. In contrast, 1-decanol causes an increase in the phase transition temperature for dimyristoylphosphatidylcholine, although it lowers the transition temperature for dipalmitoylphosphatidylcholine, and 1-dodecanol raises the transition temperature for both of these phosphatidylcholines, although it lowers that for dipalmitoylphosphatidylethanolamine. Dodecanol appears to behave in these lipid bilayer membranes as a lipid with a phase transition temperature of ca. 55 °C. Anesthesia is discussed as a phenomenon of liquidus extension: alcohols up to 1-octanol increase the proportion of lipid in the liquidus state and result in anesthesia, whereas the longer alcohols do not, and result in catalepsy.

A wide variety of different types of drug have the ability to block conduction of the nervous impulse, including the gaseous general anesthetics, alcohols, organic acids and bases, certain substituted quaternary ammonium ions, and biotoxins. All of these compounds produce local anesthesia by blocking the sodium current in nerve (Ritchie, 1975) but, with such a wide variety of different compounds, it seems unlikely that there could be a single blocking mechanism. In general terms, there are two models to explain the nerve conduction block. In the first, the local anesthetic combines with a specific receptor in the nerve membrane, and in the second the anesthetic interacts nonspecifically with the membrane, probably with the lipid component. It is clear that toxins such as tetrodotoxin and saxitoxin combine specifically with the sodium channels in nerve, but with substituted quaternary ammonium ions such as procaine it is not yet clear whether interaction with a specific receptor or a nonspecific interaction with the membrane is of importance (Ritchie, 1975). As an approach to this problem, spectroscopic studies of the interaction between anesthetics and lipid bilayers have been performed, but mainly with complex mixtures of lipid such as egg yolk lecithin and lipid from beef brain white matter (Seeman, 1972; Colley and Metcalfe, 1972; Paterson et al., 1972; Trudell et al., 1973; Johnson et al., 1973; Jacobson and Wobschall, 1974). All of these results point to an increase in "fluidity" on the addition of anesthetics, but it is difficult to be certain of the molecular changes responsible for this increasing fluidity. Although all of the above experiments were carried out at ca. 20 °C, at which temperature the lipids will be in the liquid-crystalline state, recent experiments (Lee et al., 1974; Ting and Solomon, 1975) have been interpreted as meaning that clusters of relatively densely packed lipid are present in bilayers of unsaturated lipids as well as freely dispersed lipid. The primary effect of the added anesthetic could then be to break up these relatively ordered lipid clusters, rather than effecting the mobility of the freely dispersed lipid.

The only studies of the effects of alcohols on chemically defined lipids seem to be the study by Hill (1974) using light scattering and that by Jain et al. (1975) using differential scanning calorimetry. Both observed that addition of alcohols up to octanol caused a lowering of the temperature of the gel to liquid-crystalline phase transition for dipalmitoylphosphatidylcholine, and Jain et al. (1975) also reported on asymmetrical broadening of the transition. Here we report studies of the effects of alcohols on phosphatidylcholines and phosphatidylethanolamines, and on mixtures of these lipids.

Experimental Section

Dipalmitoylphosphatidylcholine and dimyristoylphosphatidylcholine were obtained from Koch-Light and dipalmitoylphosphatidylethanolamine was from Fluka. Chlorophyll a was purified by column chromatography on powdered sugar columns by the method of Strain and Svec (1966). Chlorophyll a concentrations were estimated from absorption spectra, using the extinction coefficients given by Strain and Svec (1966).

Samples were prepared by dissolving lipids plus chlorophyll a (1.6×10^{-9} mol) in chloroform in 10-ml stoppered flasks and evaporating to dryness under a stream of nitrogen and in a vacuum desiccator. Buffer (4 ml of 0.01 M Tris-HCl, pH 7.2, and 0.1 M NaCl) was added and the mixture shaken on a Vortex mixture. For all systems reported here the lipid-chlorophyll molar ratios were equal at 400:1. For experiments with the alcohols up to 1-octanol, the appropriate amount of the alcohol was added to the final aqueous solution. For 1-decanol and 1-dodecanol, the alcohols were added to the original lipid-chlorophyll mixture in chloroform.

Fluorescence measurements were made on an Aminco Bowman SPF fluorimeter. Samples were continuously stirred during the measurements, and temperatures were measured

† From the Department of Physiology and Biochemistry, University of Southampton, Southampton S09 3TU, England. *Received November 20, 1975.*

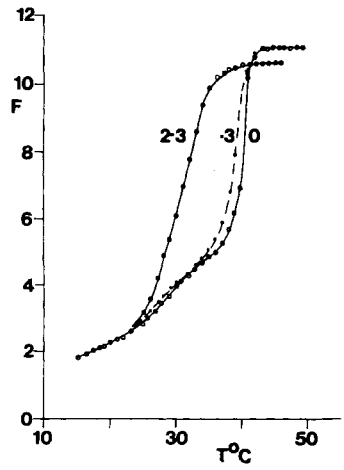

FIGURE 1: Fluorescence intensity vs. temperature for chlorophyll a incorporated into liposomes of dipalmitoylphosphatidylcholine, and the effect of addition of 0.3 and 2.3 mM octanol.

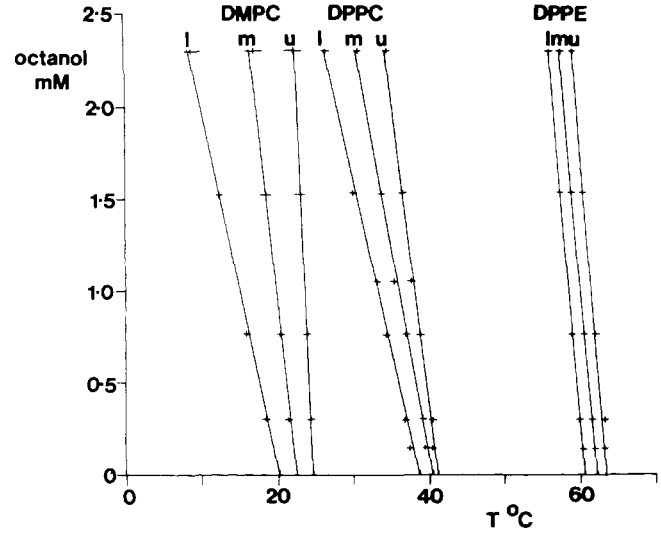

FIGURE 2: Effect of octanol on the phase transition temperatures for dimyristoylphosphatidylcholine (DMPC), dipalmitoylphosphatidylcholine (DPPC), and dipalmitoylphosphatidylethanolamine (DPPE). u refers to the upper end of the transition, l to the lower end, and m to the midpoint.

by a thermocouple. Fluorescence was excited at 420 nm and recorded at 670 nm.

Results

Effects of Alcohols up to 1-Octanol on Single Lipids. Previous studies (Lee, 1975b,c) have shown that chlorophyll a incorporated into liposomes at a chlorophyll–lipid molar ratio of 1:400 does not affect the observed temperature of the gel to liquid-crystalline phase transition. Further, plots of the fluorescence intensity of chlorophyll a in liposomes as a function of temperature show abrupt decreases in magnitude at temperatures corresponding to the phase transition, as a result of the formation of nonfluorescent aggregated chlorophyll a species. Figure 1 shows the effect on the fluorescence of chlorophyll a incorporated into liposomes of dipalmitoylphosphatidylcholine caused by the addition of 1-octanol. The main gel to liquid-crystalline transition is shifted to lower temperatures, but the effect is somewhat complex. The upper transition temperature, corresponding to the onset of gel-phase formation, and the lower transition temperature, corresponding to the completion of gel-phase formation, are given experimentally by the intersections of the straight lines which can be drawn through the three distinct portions of each fluorescence–temperature curve. Figure 2 shows that addition of 1-octanol has a greater effect on the lower transition temperature, corresponding to the completion of gel-phase formation, than on the upper transition temperature, corresponding to the onset of gel-phase formation. Also shown in Figure 2 is the effect on the transition temperature, defined as the midpoint of the fluorescence transition curve.

Hill (1974) has presented a thermodynamic analysis of the lowering of lipid transition temperatures by added alcohols. If the alcohol can partition into lipid in the liquid-crystalline phase, but is completely excluded from lipid in the gel phase, then it is possible to derive the following relationship for the dependence of the depression of the transition temperature on the aqueous concentration expressed as the fraction of saturation:

$$\frac{\delta \Delta T}{\delta r} = \frac{RT^2 PS}{Q} \quad (1)$$

where r is the fraction of saturation given by

$$r = C_w/S \quad (2)$$

where C_w is the aqueous concentration of solute, S is the concentration at aqueous saturation, and P is the partition coefficient of the alcohol between the water and lipid phases

$$P = C_m/C_w \quad (3)$$

Equation 1 is of interest since, for ideal mixing, it can be shown that

$$PS = 2 \quad (4)$$

Table I gives the values of PS obtained in this work from plots of midpoint transition temperatures against concentration for alcohols up to 1-octanol. For all the alcohols, plots of temperature against concentration of alcohol were linear for at least a drop of 6 °C in transition temperature, although with 1-butanol plots became nonlinear at higher concentrations, with increasing alcohol concentrations producing smaller effects on the transition temperature. For all the alcohols, the effect on the temperature representing the onset of gel-phase formation was less than that on the temperature of completion of gel-phase formation, as shown for 1-octanol in Figure 2.

Also as shown in Figure 2, the addition of 1-octanol to dimyristoylphosphatidylcholine and to dipalmitoylphosphatidylethanolamine had a smaller effect on the midpoint transition temperatures than for dipalmitoylphosphatidylcholine. However, the most noticeable difference was that for dimyristoylphosphatidylcholine, the effect on the temperature of the end of the transition range was very much greater than on the temperature of the beginning of the transition, whereas for dipalmitoylphosphatidylethanolamine, effects were identical on both, in other words, addition of 1-octanol caused a considerable increase in the width of the transition for dimyristoylphosphatidylcholine, but had no effect on the width of the transition for dipalmitoylphosphatidylethanolamine.

Addition of alcohols affected the pretransition (Figure 1). The pretransition appeared at ca. 29 °C in dipalmitoylphosphatidylcholine and was little affected up to a concentration of 1.05 mM 1-octanol, but at 1.5 mM octanol, the pretransition was no longer apparent. Similar effects were seen with the other alcohols, the pretransition disappearing at the concentrations of alcohol causing about a 5 °C decrease in the main transition temperature. Addition of alcohols up to 1-octanol had no effect on the fluorescence intensity for chlorophyll a

TABLE I: The Effect of Alcohols up to 1-Octanol on the Lipid-Phase Transition.

Lipid	Probe	Alcohol	Solubility in Water[a] (mol/mol)	$\Delta T/r$ (°C)	PS[b]	Concn (M) for a 5 °C Drop in T_c[c]
Dipalmitoylphosphatidylcholine	Chlorophyll a	Butanol	1.78×10^{-2}	43	2.1	1.3×10^{-1}
		Pentanol	4.50×10^{-3}	40	2.0	3.3×10^{-2}
		Hexanol	1.10×10^{-3}	31	1.5	6.7×10^{-3}
		Heptanol	2.79×10^{-4}	29	1.4	3.0×10^{-3}
		Octanol	8.09×10^{-5}	19	1.0	1.2×10^{-3}
	ANS	Octanol		22	1.1	1.1×10^{-3}
	DPOT	Octanol		13	0.7	1.7×10^{-3}
Dimyristoylphosphatidylcholine	Chlorophyll a	Octanol		11	0.4	2.0×10^{-3}
Dipalmitoylphosphatidylethanolamine	Chlorophyll a	Octanol		10	0.5	2.2×10^{-3}

[a] Bell (1973). [b] Transition data from Hinz and Sturtevant (1972). [c] The midpoint transition temperature.

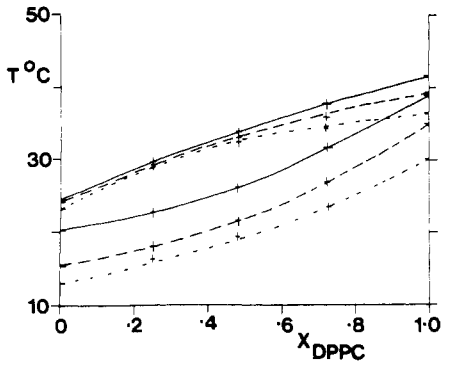

FIGURE 3: The effect of octanol on the temperatures of onset and completion of gel-phase formation in mixtures of dimyristoylphosphatidylcholine and dipalmitoylphosphatidylcholine. (Solid line) No octanol; (broken line) 0.8 mM octanol; (dashed line) 1.5 mM octanol.

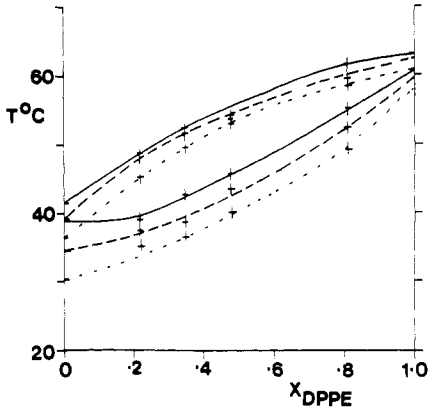

FIGURE 4: The effect of octanol on the temperatures of onset and completion of gel-phase formation in mixtures of dipalmitoylphosphatidylcholine and dipalmitoylphosphatidylethanolamine. (Solid line) No octanol; (broken line) 0.8 mM octanol; (dashed line) 1.5 mM octanol.

incorporated into lipid bilayers above the phase transition temperature.

Studies with 1-anilino-8-naphthalenesulfonate (ANS[1]) and 1,8-diphenyl-1,2,5,7-octatetraene (DPOT) also indicate a lowering of the transition temperature due to the addition of alcohols: data are presented in Table I.

Effects of Alcohols up to 1-Octanol on Lipid Mixtures. As described elsewhere (Lee, 1975b), plots of fluorescence intensity of chlorophyll a as a function of temperature when incorporated into lipid mixtures provide a series of temperatures corresponding to the onset and completion of gel-phase separation. These can then be plotted to give what can be loosely termed as "phase diagrams". The effects in mixtures of phosphatidylcholines and phosphatidylethanolamines are shown in Figures 3-5.

Effects of 1-Decanol and 1-Dodecanol. It is convenient to first consider the effect of 1-dodecanol.

The addition of dodecanol to dipalmitoylphosphatidylcholine causes an increase in transition temperature. The water solubility of dodecanol is so low (2.3×10^{-5} mol/l.; Bell, 1973) that if $PS \simeq 2$, then ca. 95% of the dodecanol will be partitioned into the lipid phase. The effect of the dodecanol on the transition temperature is therefore presented in Figure 6 in the form of a "phase diagram", plotting transition temperatures against mole fraction of dodecanol in the lipid phase, assuming that all the dodecanol has partitioned into the lipid. At a mole fraction of 0.66 dodecanol, the sample was observed to start to coagulate, and at a mole fraction of 0.75 dodecanol, coagulation was so extensive that accurate fluorescence measurements were no longer possible, even with stirring. That the effect of added dodecanol is on the lipid and not due to a specific interaction between the alcohol and chlorophyll a was shown by studies using other probes. Thus, for example, with 1,8-diphenyloctatetraene as probe, the transition with dodecanol present at a mole fraction of 0.66 was centered at ca. 47 °C, comparable to that observed with chlorophyll a.

A second effect of added dodecanol was an increased fluorescence intensity for chlorophyll a under these experimental conditions where both the quantities of chlorophyll a (1.56×10^{-9} mol) and lipid (6.1×10^{-7} mol) were kept constant. If the added dodecanol dispersed freely in the membrane, then the concentration of chlorophyll a would be effectively reduced within the membrane, and if it is assumed that the addition of

[1] Abbreviations used: ANS, 1-anilino-8-naphthalenesulfonate; DPOT, 1,8-diphenyl-1,2,5,7-octatetraene.

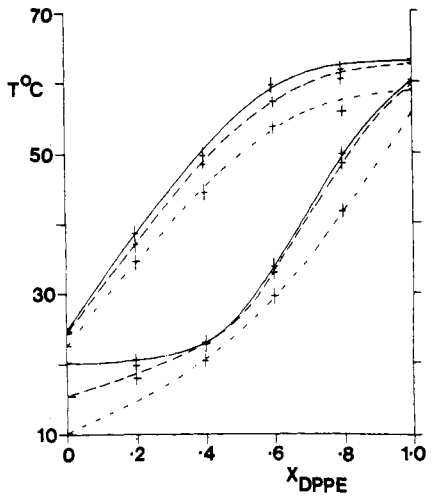

FIGURE 5: The effect of octanol on the temperatures of onset and completion of gel-phase formation in mixtures of dimyristoylphosphatidylcholine and dipalmitoylphosphatidylethanolamine. (Solid line) No octanol; (broken line) 0.8 mM octanol; (dashed line) 2.3 mM octanol.

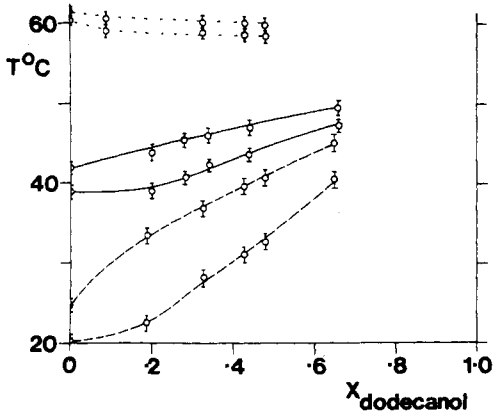

FIGURE 6: Temperatures of onset and completion of gel-phase formation for mixtures of dodecanol and (solid line) dipalmitoylphosphatidylcholine; (broken line) dimyristoylphosphatidylcholine; or (dotted line) dipalmitoylphosphatidylethanolamine.

two dodecanol molecules to the bilayer is equivalent to the addition of one phospholipid, then the increase in fluorescence intensity caused by addition of dodecanol is very close to that observed previously for variation in concentration caused by changing the amount of lipid (Lee, 1975c). A third effect of added dodecanol was to remove the pretransition.

The effect of addition of dodecanol to dimyristoylphosphatidylcholine was also to cause an increase in transition temperature whereas addition to dipalmitoylphosphatidylethanolamine caused a slight decrease (Figure 6). The effect of addition of dodecanol to a mixture of dipalmitoylphosphatidylcholine and dipalmitoylphosphatidylethanolamine is shown in Figure 7.

The effect of addition of 1-decanol is more difficult to quantitate since with a solubility of 3.2×10^{-4} mol/l. (Bell, 1973), if $PS \simeq 2$, then about 50% of the decanol will be partitioned into the bilayer. Plots of concentration of alcohol against transition temperature will be nonlinear because of depletion of the aqueous phase of the alcohol into the lipid phase. However, the important observation shown in Figure 8 is that, whereas addition of 1-decanol to dipalmitoylphosphatidylcholine caused a lowering of the transition temperature, addition to dimyristoylphosphatidycholine caused an increase in transition temperature. The effect of decanol on

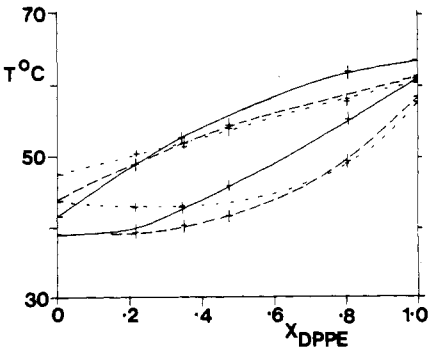

FIGURE 7: The effect of addition of dodecanol to mixtures of dipalmitoylphosphatidylcholine and dipalmitoylphosphatidylethanolamine at molar ratios of dodecanol of (solid line) zero, (broken line) 0.2, and (dashed line) 0.44.

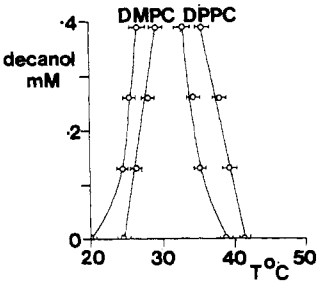

FIGURE 8: The effect of 1-decanol on the temperatures of onset and completion of gel-phase formation in dimyristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatidylcholine (DPPC).

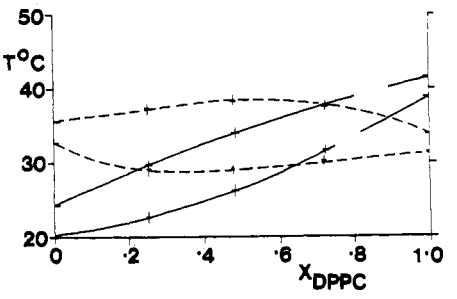

FIGURE 9: The effect of 1-decanol on the temperatures of onset and completion of gel-phase formation in mixtures of dimyristoylphosphatidylcholine and dipalmitoylphosphatidylcholine with (solid line) no decanol; (broken line) 0.8 mM decanol.

the phase diagram for a mixture of these two lipids is shown in Figure 9.

Discussion

In previous studies it has been shown that chlorophyll a is a suitable and convenient fluorescent probe for phase transitions in lipid bilayers. When incorporated into lipid bilayers in the liquid-crystalline state, chlorophyll a is present as a mixture of a monomeric, fluorescent form and oligomeric, nonfluorescent forms. Addition of alcohols up to 1-octanol to dimyristoylphosphatidylcholine, dipalmitoylphosphatidylcholine, or dipalmitoylphosphatidylethanolamine in the liquid-crystalline state produces no significant change in fluorescence intensity. This means that the ratio of monomeric and oligomeric forms of chlorophyll must be little affected, suggesting that the addition of these alcohols has relatively little effect on the fluidity of the lipid bilayer in the liquid-crystalline state. This is consistent with other observations. Thus it has been estimated that the "microviscosity" at the

center of a bilayer of dimyristoylphosphatidylcholine in the liquid-crystalline state is about equal to that in 1-decane at 31 °C, and that the viscosity near the glycerol head group of the lipid is only approximately 15 times greater than that for 1-decane (Lee, 1975a; Lee et al., 1976). Further, for 1-decanol, ^{13}C nuclear magnetic relaxation data (Doddrell and Allerhand, 1971) show that rates of rotation about C-C bonds are less than in 1-decane (Levine et al., 1974) so that, apart from a disordering effect, addition of these alcohols might be expected to have only relatively slight effects on membrane viscosity. In the case of unsaturated lipids, however, the effects of alcohols might be more marked. It has been suggested elsewhere (Lee et al., 1974) that in bilayers of unsaturated lipids in the liquid-crystalline phase, small quasicrystalline clusters of molecules are present in the liquid phase together with freely dispersed molecules. With increasing temperature, these clusters gradually break up to give monomeric, freely dispersed molecules. It might be expected that addition of alcohols to such a system would also lead to break up of the cluster.

Therefore, although the addition of alcohols up to 1-octanol seems in general to have relatively little effect on the fluidity of lipids in the liquid-crystalline state, it has a very marked effect on the temperature of the transition of the lipid from a liquid-crystalline state to the gel state. As shown in Figure 2, the effect on the transition for the phosphatidylcholines is rather complex. The alcohols have less effect on the temperature at which the onset of gel-phase formation occurs than on the temperature representing the completion of gel-phase formation. This results in an increase in the width of the phase transition, as observed by Jain et al. (1975) using differential scanning calorimetry. The calorimetric results and the fluorescence data reported here are in close agreement. Thus Jain et al. (1975) report that 1.14 mM 1-octanol causes a doubling of the width of the phase transition for dipalmitoylphosphatidylcholine, and the fluorescence data of Figure 2 show a doubling of the width of the transition at 1.15 mM. These effects presumably arise because the phase transition in the phosphatidylcholines is not a simple first-order transition (Lee, 1975a). For dipalmitoylphosphatidylethanolamine, on the other hand, alcohols up to 1-octanol appear to have an equal effect on both the temperatures of onset and completion of gel-phase formation.

Hill (1974) has shown that, for ideal mixing, values of PS can be obtained from such data and that the value of the product should be two, where P is the partition coefficient and S is the concentration at aqueous saturation. Values of PS obtained in this work are listed in Table I, using a transition temperature defined as the midpoint of the transition. For 1-butanol in dipalmitoylphosphatidylcholine, the product PS is very close to the ideal value of 2 and, from the solubility of 1-butanol in water, the partition coefficient can be estimated to be 118, which can be compared with the value of 119 for dimyristoylphosphatidylcholine at 25 °C (Katz and Diamond, 1974).

The partitioning of the alcohols appears to become less ideal as the chain length increases. This is in contrast to the results obtained by Hill (1974) using light scattering, who found that, although partitioning of the odd-numbered chain length alcohols was nonideal, that of the even-numbered chain length alcohols up to 1-octanol was close to that expected for ideal behavior. The reason for this difference has not been established. Seeman et al. (1971) have determined partition coefficients into erythrocyte ghost membranes, and their data show that the product PS decreases from 1-pentanol to 1-octanol.

The effects of alcohols up to 1-octanol on mixtures of lipids can be plotted in the form of "phase diagrams". As discussed elsewhere (Lee, 1975a), these diagrams are experimentally simply diagrams showing the temperatures of onset and completion of gel-phase lipid formation, and labeling them phase diagrams represents an extrapolation from a microscopic system to a macroscopic concept which may or may not be justified. Because of changes in cooperativity of the phase transition, the width of the transition may appear to be greater in the microscopic, lipid system, than it would be in a macroscopic system. At a temperature at which both gel and liquid-crystalline phases are present, the compositions of the gel and liquid-crystalline phases read off from the "phase diagram" would then appear to be more dissimilar than in reality they are. Similarly, there are are possible errors in estimating the relative proportions of gel and liquid-crystalline phase lipids from these diagrams. Nevertheless, the results for the single lipids show that alcohols do affect the lipid transition temperatures, and that effects on mixtures of lipids are similar. It seems reasonable, therefore, to treat these diagrams as if they were phase diagrams, but an example of the care that is sometimes necessary will be discussed below.

The primary effect of 1-octanol on the diagram for mixtures of dimyristoylphosphatidylcholine and dipalmitoylphosphatidylcholine is to shift both the upper, fluidus, curve and the lower, solidus, curve to lower temperatures (Figure 3). The shape of the phase diagram also changes because of the greater effect of octanol on the width of the phase transition for dimyristoylphosphatidylcholine. The overall effect is to increase the amount of fluid lipid present. Thus for an equimolar mixture at 25 °C, all the lipid is in the gel phase in the absence of 1-octanol, but in the presence of 0.8 mM octanol, 25% of the lipid is in the fluid state. Similarly, at 30 °C, 40% of the lipid is in the fluid state in the absence of octanol, but 63% is fluid in the presence of 0.8 mM octanol. The significance of these results for theories of anesthesia will be described below.

The phase diagrams for mixtures of dipalmitoylphosphatidylethanolamine and dimyristoylphosphatidylcholine or dipalmitoylphosphatidylcholine are more complex. Both show a eutectic with composition and melting point close to that of the pure phosphatidylcholine, indicated by a horizontal solidus line (Findlay, 1951). In the gel phase, therefore, rather than forming a simple solid solution, there is segregation of lipids to form separate regions enriched in either phosphatidylcholine or phosphatidylethanolamine. Addition of 1-octanol to either of these mixtures results in the expected lowering of the fluidus and solidus curves (Figures 4 and 5). Addition of alcohol also removes the horizontal portion of the solidus curve in these diagrams and could be taken to show that the lipids are now completely miscible in the gel phase: such a conclusion is, however, probably not justified for the following reasons. As shown in Figure 2, addition of 1-octanol to dipalmitoylphosphatidylethanolamine causes no increase in width for the phase transition: effects on the temperatures of onset and completion of gel-phase formation are equal. Similarly, it is observed that for mixtures containing a mole fraction of dipalmitoylphosphatidylethanolamine of 0.2 or greater, addition of 1-octanol has an almost equal effect on the onset and completion temperatures. This is not so for the pure phosphatidylcholines, where the effect on the temperature of completion of gel-phase formation is much more marked, and it is the large effect on these temperatures for the phosphatidylcholines which results in the disappearance of the horizontal portion of the phase diagram. Indeed, the experimentally determined transition temperatures for mixtures with a mole fraction of ca. 0.2 of dipalmitoylphosphatidylethanolamine are considerably higher

than expected from extrapolation to the pure phosphatidylcholine and are more consistent with a horizontal portion of the solidus curve and thus with gel-phase immiscibility. These results, therefore, seem to indicate some of the dangers inherent in labeling these diagrams as phase diagrams. Nevertheless, although the region to the left of a mole fraction of ca. 0.2 dipalmitoylphosphatidylethanolamine is difficult to interpret, that to the right seems fairly straightforward. In particular, addition of octanol again results in the formation of increasing amounts of fluid-phase lipid. Thus, for example, in mixtures of dimyristoylphosphatidylcholine and a mole fraction of 0.6 of dipalmitoylphosphatidylethanolamine at 35 °C, the percentage of fluid-phase lipid is 6% in the absence of octanol and 20% in the presence of 2.3 mM octanol.

In contrast to the effects on lipids and lipid mixtures with alcohols up to 1-octanol, addition of 1-dodecanol causes an increase in phase transition temperature for dimyristoyl- and dipalmitoylphosphatidylcholines (Figure 6). There are two obvious explanations for these effects. The first is that a 2:1 complex is formed between the phospholipid and dodecanol, analogous to the postulated complexes between alkyl alcohols and sulfates (Kung and Goddard, 1964). However, the relative effects on the phosphatidylcholines and phosphatidylethanolamines would then be difficult to interpret. The alternative explanation is that dodecanol is acting effectively as another lipid, with a phase transition at ca. 55 °C, since this would explain why the phase transition temperatures for the phosphatidylcholines are raised whereas that of dipalmitoylphosphatidylethanolamine is lowered (Figure 6). It would also account for the observed increase in fluorescence intensity for chlorophyll a on addition of dodecanol. Lastly, if dodecanol is simply acting as another lipid, then it would be expected that addition of 1-octanol to the mixtures would cause a decrease in transition temperature. This is in fact observed, the effects of 1-octanol being linear up to 2.3 mM octanol, with a slope for both the upper and lower transition temperatures similar to the upper transition temperature for dipalmitoylphosphatidylcholine alone. The only unexpected aspect of these observations is that dodecanol would be acting in the bilayer with an effective transition temperature of ca. 55 °C, whereas its bulk melting point is 24 °C. However, there appears to be no simple relationship between the bulk melting point and the behavior of the compound in a bilayer, at a surface pressure that has been estimated to be 31–35 dyn/cm (Demel et al., 1975).

Consistent with the above explanation is the behavior of 1-decanol, which causes an increase in phase transition temperature for dimyristoylphosphatidylcholine but a decrease in phase transition temperature for dipalmitoylphosphatidylcholine, consistent with an effective transition temperature of ca. 30 °C for decanol in the lipid bilayer. The effects of decanol and dodecanol on two mixtures of lipids are shown in Figures 7 and 9. The effects are as expected from the effects on single lipids, with a lowering of transition curves on the right-hand side of the diagrams and a raising of them on the left-hand side.

Fluidus-Phase Lipids and Anesthesia. Much recent work on reconstituted lipid–protein systems has shown conclusively the extreme sensitivity of membrane proteins to the physical state of the surrounding lipid. Thus the ATPase activity of the calcium transport protein from sarcoplasmic reticulum has been shown to be very low below 24 °C when the surrounding lipid is dimyristoylphosphatidylcholine, but when the surrounding lipid is dipalmitoylphosphatidylcholine, the protein is active only down to 30 °C (Warren et al., 1974). Similarly, Dufourcq et al. (1975) have shown that, when the cytochrome b_5 system is reconstituted with dipalmitoylphosphatidylcholine, there is a conformational change in the temperature range 30–40 °C. Finally Strittmatter and Rogers (1975) have studied the interaction between cytochrome b_5 and cytochrome b_5 reductase in liposomes of dimyristoylphosphatidylcholine and observed a marked reduction in reaction rate at ca. 24 °C, attributable to the formation of gel-phase lipid with a concomitant reduction in the rate of protein diffusion and collision. In these systems, Arrhenius plots when the lipid is in the liquid-crystalline state are not unusual, and marked effects only occur when gel-phase lipid is formed.

There is now strong evidence that, in bacteria at the optimal growth temperature, lipids are present both in the gel and in the liquid-crystalline phase. In mammalian cells the situation is likely to be more complex because of the presence of cholesterol, but again it is likely that domains of lipid of differing fluidity are present (reviewed in Lee, 1975a).

It is generally believed that the primary effect of the alcohol anesthetics is on the lipid component of the membrane, and that major direct effects on membrane proteins are irreversible and occur only at higher, lytic concentrations of the alcohols (Seeman, 1972). It has been observed that anesthesia either occurs when a certain critical concentration of anesthetic is reached within the membrane, or when a particular molar volume of anesthetic is achieved within the membrane (Smith, 1974). These ideas, however, represent correlations between the relative potencies of the anesthetics, rather than providing a molecular model for anesthetic action. They also leave the important "cut off" phenomenon unexplained: if the anesthetic potency is measured for a homologous series, the potency generally increases up to a particular member, at which point higher members of the series are found to be inactive. The studies reported here suggest that the major effects of the anesthetic alcohol on the lipid component of the membrane will be to decrease the proportion of lipid present as clusters in the liquid-crystalline phase, and to increase the proportion of lipid in the liquid-crystalline phase at the expense of that in the gel phase. The increase in proportion of fluid lipid will then result in increases in rates of action of membrane proteins and changes in the permeability properties of the membrane. As a secondary, and largely incidental effect, the volume of the membrane will also increase on addition of anesthetic alcohols since the volume occupied by a lipid in the liquid-crystalline phase is greater than that occupied by a lipid in the gel phase.

Direct evidence that the anesthetic alcohols act by increasing the proportion of lipid in the fluid phase is likely to be difficult to obtain for mammalian systems because of the high proportion of cholesterol present in most membranes. However, it has been observed that the reduction in the action potential in a toad single nerve fiber induced by application of a 3% ethanol solution can be reversed by cooling to 15 °C (Spyropolous, 1957) which is consistent with the above ideas.

An explanation for the "cutoff" effect in anesthesia can also be proposed. The results reported here show that whether or not an alcohol acts to fluidize a membrane depends both on the alcohol and on the lipids in the membrane. Whereas 1-octanol acts to increase the proportion of fluid lipid for all the mixtures studied here, 1-decanol and 1-dodecanol act to increase the amount of fluid lipid in some and decrease the amount in others. Interestingly, Lawrence and Gill (1975) have reported that, whereas octanol is an effective general anesthetic when administered to animals intravenously, intravenous doses of hexadecanol produce a cataleptic state. It is not sufficient for an alcohol or other molecule simply to dissolve in the lipid

phase of a membrane to cause anesthesia: the other requirement we suggest is that the proportion of lipid in a fluid state should be increased.

A simpler test of these ideas could be provided by studies of bacterial behavior. It has been suggested that behavior in *Rhodospirillum rubrum* depends on a membrane depolarization with consequent changes in permeability, perhaps to Ca^{2+} ions (Lee and Fitzsimons, 1976). Since it has been shown that anesthetics reversibly affect the motility of these bacteria (Lee, unpublished observations), and since their lipid composition is fairly simple, it should prove possible to provide a direct test of the theory.

References

Bell, G. H. (1973), *Chem. Phys. Lipids 10,* 1.

Colley, C. M., and Metcalfe, J. C. (1972), *FEBS Lett. 24,* 241.

Demel, R. A., Geurts van Kessel, W. S. M., Zwaal, R. F. A., Roelofsen, B., and Van Deenen, L. L. M. (1975), *Biochim. Biophys. Acta 406,* 97.

Doddrell, D., and Allerhand, A. (1971), *J. Am. Chem. Soc. 93,* 1558.

Dufourcq, J., Faucon, J. F., Lussan, C., and Bernon, R. (1975), *FEBS Lett. 57,* 112.

Findlay, A. (1951), The Phase Rule, 9th ed, rewritten by Campbell, A. N., and Smith, N. O., New York, N.Y., Dover Publications.

Hill, M. W. (1974), *Biochim. Biophys. Acta 356,* 117.

Hinz, H.-J. and Sturtevant, J. M. (1972), *J. Biol. Chem. 247,* 6071.

Jacobson, K., and Wobschall, D. (1974), *Chem. Phys. Lipids 12,* 117.

Jain, M. K., Wu, N. Y.-M., and Wray, L. V. (1975), *Nature (London) 255,* 494.

Johnson, S. M., Miller, K. W., and Bangham, A. D. (1973), *Biochim. Biophys. Acta 307,* 42.

Katz, Y., and Diamond, J. M. (1974), *J. Membr. Biol. 17,* 101.

Kung, H. C., and Goddard, E. D. (1964), *J. Phys. Chem. 68,* 3465.

Lawrence, D. K., and Gill, E. W. (1975), *Mol. Pharmacol. 11,* 595.

Lee, A. G. (1975a), *Prog. Biophys. Mol. Biol. 29,* 3.

Lee, A. G. (1975b), *Biochim. Biophys. Acta 413,* 11.

Lee, A. G. (1975c), *Biochemistry 14,* 4397.

Lee, A. G., Birdsall, N. J. M., Metcalfe, J. C., Roberts, G. C. K., and Warren, G. B. (1976), *Proc. R. Soc. London* (in press).

Lee, A. G., Birdsall, N. J. M., Metcalfe, J. C., Toon, P. R., and Warren, G. B. (1974), *Biochemistry 13,* 3699.

Lee, A. G., and Fitzsimons, J. T. R. (1976), *J. Gen. Bacteriol.* (in press).

Levine, Y. K., Birdsall, N. J. M., Lee, A. G., Metcalfe, J. C., Partington, P., and Roberts, G. C. K. (1974), *J. Chem. Phys. 60,* 2890.

Paterson, S. J., Butler, K. W., Huang, P., Labelle, J., Smith, I. C. P., and Schneider, H. (1972), *Biochim. Biophys. Acta 266,* 597.

Ritchie, J. M. (1975), *Br. J. Anaesth. 47,* 191.

Seeman, P. (1972), *Pharmacol. Rev. 24,* 583.

Seeman, P., Roth, S., and Schneider, H. (1971), *Biochim. Biophys. Acta 225,* 171.

Smith, E. B. (1974), in Molecular Mechanisms in General Anaesthesia, Halsey, M. J., Millar, R. A., and Sutton, J. A., Ed., Edinburgh, Churchill Livingstone.

Spyropolous, C. (1957), *J. Gen. Physiol. 40,* 848.

Strain, H. H., and Svec, W. A. (1966), in The Chlorophylls, Vernon, L. P., and Seely, G. R., Ed., New York, N.Y., Academic Press.

Strittmatter, P., and Rogers, M. J. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72,* 2658.

Ting, P., and Solomon, A. K. (1975), *Biochim. Biophys. Acta 406,* 447.

Trauble, H. (1971), *Naturwissenschaften 58,* 277.

Trudell, J. R., Hubbell, W. L., and Cohen, E. N. (1973), *Biochim. Biophys. Acta 291,* 321.

Warren, G. B., Toon, P. A., Birdsall, N. J. M., Lee, A. G., and Metcalfe, J. C. (1974), *Biochemistry 13,* 5501.