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Analysis of the Defect Structure of Gel-Phase Lipid†

A. G. Lee

ABSTRACT: The partitioning of the spin label 2,2,6,6-tetramethylpiperidinyl-1-oxy (Tempo) into phosphatidylcholine bilayers and the monomer–aggregate equilibrium for chlorophyll a incorporated into phosphatidylcholine bilayers have been interpreted in terms of the formation of defects in the gel-phase lipid, starting some 20 °C below the temperature of the main gel to liquid crystalline phase transition. By contrast, defects seem to be largely absent from bilayers of dipalmitoylphosphatidylethanolamine in the gel phase. The defect structure accounts for the continuous nature of the phase transition for phosphatidylcholines, and also for the increase in width of the transition caused by the addition of alcohols.

Biological membranes are often said to exist within the lipid-phase transition, in the sense that lipids are present both in the gel and in the liquid-crystalline phases (see Lee, 1975a; Cronan and Gelmann, 1975). Further, from the fatty acid compositions, determined especially for bacterial membranes (Cronan and Gelmann, 1975), it seems that at ambient temperatures many of the lipids will be within a few degrees of their respective phase-transition temperatures. It therefore becomes of importance to study the properties of lipids close to the phase transition, particularly since major changes in physical properties are expected in this region.

Ubbelohde (1965) has argued convincingly for the presence of premelting and prefreezing phenomena close to many solid–liquid transitions, where the premelting phenomena make the solid more "liquid-like" and the prefreezing phenomena make the liquid more "solid-like". Evidence has already been presented consistent with the presence of prefreezing phenomena in lipid bilayers, with the formation of quasicrystalline clusters of lipid present within a matrix of otherwise freely dispersed, liquid-crystalline, phase lipid (Lee et al., 1974b; Ting and Solomon, 1975; Bashford et al., 1976). Here, evidence is presented consistent with premelting phenomena in lipid bilayers in the crystalline or gel phase.

Of course, at all temperatures above absolute zero, a crystalline solid will contain defects, the number of defects increasing with increasing temperature, simply because the formation of defects is associated with an increase in entropy. The defects may simply be vacant sites in the otherwise regularly packed lattice, or they may adopt more complex arrangements. One such possibility is the formation of large-angle grain boundaries. A grain boundary is simply the boundary separating two crystals or grains that differ in orientation, and, as shown in Figure 1a, when two grains differ only slightly in orientation, packing at the grain boundary is not very different from bulk packing. However, when the difference in orientation of the two grains is more considerable

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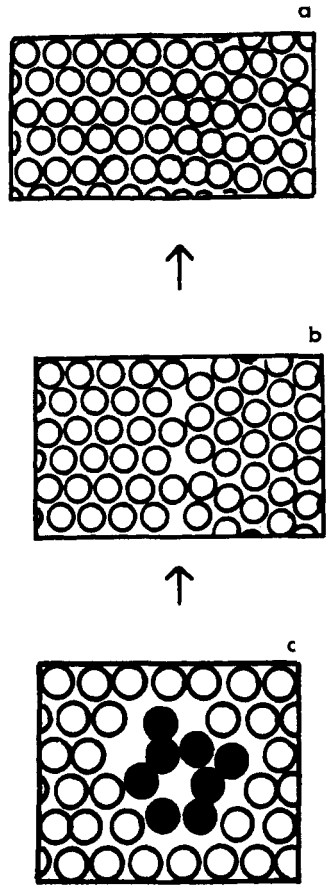

FIGURE 1: (a) A low-angle grain boundary. (b) A high-angle grain boundary, with vacancies. (c) A disordered region, in which eight molecules adopt a "liquid-like" arrangement with formation of only three vacancies.

(greater than ca. 15 or 20°), then the boundary appears to be an array of molecules and holes in roughly equal numbers per unit length (Figure 1b). Another possibility is that vacancies may aggregate to give a larger void, into which the surrounding molecules will tend to collapse, thus forming volumes of disorder, presumably of a random close-packed character in the Bernal sense (Bernal, 1964). As shown in Figure 1c, it is possible in this way for a considerable volume of gel to be transformed into a disordered aggregate with the creation of relatively few intrinsic vacancies.

In this paper, it is shown that the distribution of the spin label 2,2,6,6-tetramethylpiperidinyl-1-oxy (Tempo) between the aqueous and lipid phases present in an aqueous lipid dispersion can be interpreted in terms of premelting phenomena. The equilibrium between monomeric and aggregated forms of chlorophyll a incorporated into liposomes can be interpreted in similar terms. The data so obtained provides a natural explanation for the permeability properties of liposomes as a function of temperature, and can also account for the observation that a number of lipophilic drugs increase the width of the phase transition in phosphatidylcholines.

The presence of vacancies and grain boundaries also provides an explanation for the finite width of the phase transition for phosphatidylcholines (Lee, 1975a). Since the liquid-crystalline phase is more disordered than the gel phase, transition to the liquid-crystalline phase in close proximity to such defects is likely to occur more readily and at lower temperatures than for the transition in the bulk, fully ordered, gel-phase regions.

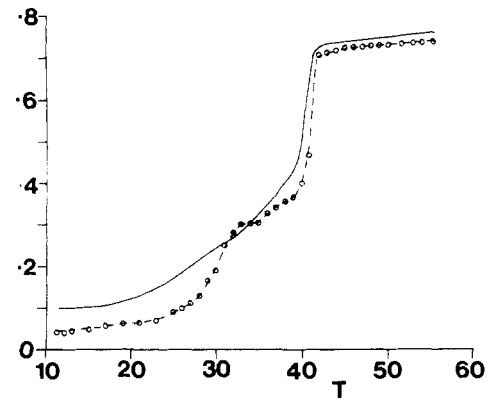

FIGURE 2: The spectral parameter f vs. temperature for Tempo partitioning into dipalmitoylphosphatidylcholine (broken line) and the fluorescence intensity (arbitrary units) vs. temperature for chlorophyll a incorporated into dipalmitoylphosphatidylcholine (solid line).

Experimental Procedure

Sources of chemicals and experimental methods were as in Lee (1976a). Fluorescence measurements were made on an Aminco Bowman SPF Fluorimeter, and electron spin resonance measurements on a Varian E3 spectrometer.

Results

Thermal Effects in Single Lipids. The electron spin resonance spectrum of Tempo in an aqueous lipid dispersion consists of a superposition of the spectra of Tempo in an aqueous and a lipid environment. The amplitudes B and F of the high-field signals are approximately proportional to the amount of Tempo dissolved in the lipid and in the aqueous phase, respectively (Shimshick and McConnell, 1973; Lee et al., 1974b). The parameter f, given by $f = B/(F + B)$, is then approximately equal to the fraction of spin label dissolved in the lipid. In Figure 2, the Tempo solubility parameter f is plotted against temperature for an aqueous dispersion of dipalmitoylphosphatidylcholine: the plot agrees with that previously published by Shimshick and McConnell (1973). Clearly, there is an abrupt decrease in f at 40.5 °C, corresponding to the temperature of the liquid crystalline to gel transition. Below this main transition there is a second, broader, transition, probably corresponding to the pretransition observed with differential scanning calorimetry (Hinz and Sturtevant, 1972).

A plot of the fluorescence intensity of chlorophyll a incorporated into liposomes of dipalmitoylphosphatidylcholine as a function of temperature has a shape very similar to that for Tempo solubility (Figure 2). There is an abrupt decrease in fluorescence intensity at 40.5 °C, which can be attributed to the formation of nonfluorescent, aggregated, chlorophyll species, with a further decrease at the temperature of the pretransition (Lee, 1975b,c).

The pretransition in phosphatidylcholines has been attributed to a change in orientation of the lipid fatty acid chains with respect to the bilayer surface (Rand et al., 1975). There is, however, no obvious reason why a rearrangement of lipid fatty acid chains to a perpendicular orientation should, as such, lead to an increased Tempo solubility or to an increase in the proportion of monomeric chlorophyll a. On the other hand, if the pretransition leads to an increased number of vacancies, or equivalently to an increased proportion of disordered lipid, then the above observations would naturally follow. This possibility can be analyzed further.

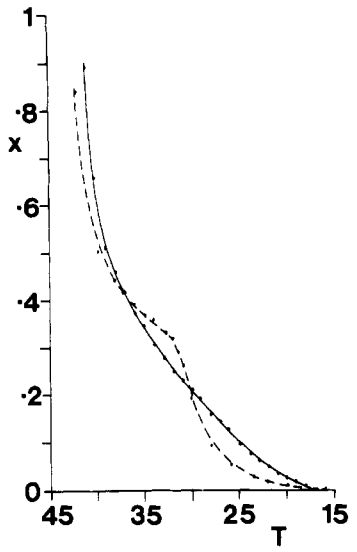

FIGURE 3: Variation in the mole fraction x of dipalmitoylphosphatidylcholine in a liquid crystalline-like state below the main phase transition, estimated from fluorescence (solid line) and ESR (broken line) measurements.

If it is assumed that the increase in Tempo solubility or in chlorophyll a fluorescence is due to the formation of lipid in a liquid crystalline-like phase below the transition temperature, then the value of the measured parameter P_t at some temperature, t, is given by

$$(1 - x)P_g + xP_l = P_t \qquad (1)$$

where x is the fraction of lipid in the liquid crystalline-like phase, and P_g and P_l are the values for the measured parameter in the gel and liquid crystalline phases, respectively, extrapolated to the temperature t. Thus, x, is given by

$$x = \frac{P_t - P_g}{P_l - P_g} \qquad (2)$$

Figure 3 shows the variation of x with temperature calculated in this way. The more complex response of Tempo partitioning to temperature can be attributed to its binding in the glycerol backbone region of the lipid: it will therefore be somewhat sensitive to changes in configuration of the lipid head group (see later).

Two sources of this "premelting" have to be considered: impurity effects and vacancy formation. These can be distinguished by their characteristic temperature dependencies. If the premelting is due to impurities, then they will have their greatest effect if Raoult's law is obeyed (see below). The presence of a mole fraction n/N of impurity soluble in the liquid crystalline phase but not in the gel phase will lower the transition temperature from that of the pure lipid, T_t^0, to T_t, where T_t is given by

$$T_t^0 - T_t = \left(\frac{R(T_t^0)^2}{\Delta H_t}\right)\frac{n}{N} \qquad (3)$$

where ΔH_t is the enthalpy of transition. At some temperature T_x below T_t, a fraction $(1 - x)$ of the lipid is in the gel phase, and the impurity concentration in the liquid crystalline phase is n/Nx, since the gel phase is assumed not to dissolve the impurity. Therefore,

$$T_t^0 - T_x = \left(\frac{R(T_t^0)^2}{\Delta H_t}\right)\frac{n}{Nx} \qquad (4)$$

Thus, a plot of $1/x$ vs. T_x would be a straight line. In Figure 4a, the data obtained from the chlorophyll a experiments are plotted in this way, and, clearly, are nonlinear. Further, to account for the slope of the line at high temperatures, a mole fraction of 16% impurity would be required: thin-layer chromatographic analysis shows the sample to be essentially pure.

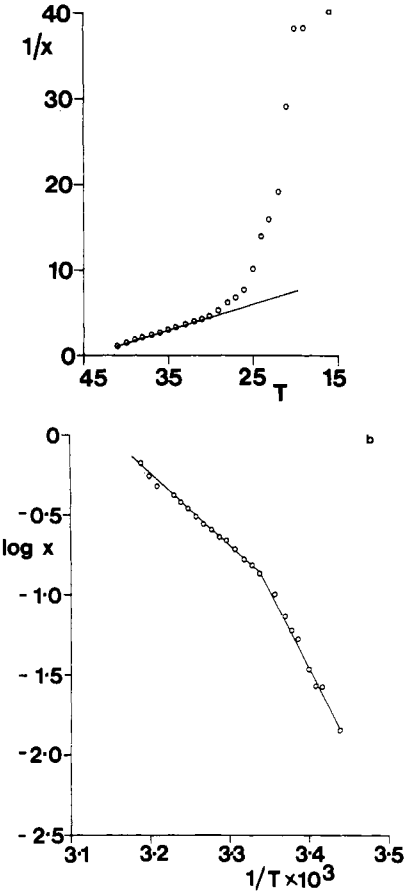

FIGURE 4: (a) A plot of $1/x$ vs. temperature. (b) A plot of log x vs. $1/$ temperature (see text).

A suitable test for premelting due to vacancy formation is not so straightforward, but one has been suggested by Ubbelohde (1965). Ubbelohde has suggested that the concentration of lattice flaws might follow an equation of the form

$$n/N = C \exp(-E/RT) \qquad (5)$$

where E is the activation energy for vacancy formation. If each vacancy corresponds to an increase δx in the proportion of lipid in the liquid-crystalline phase, then

$$\ln x = \ln (\delta xNC)(-E/RT) \qquad (6)$$

A plot of log x against $1/T$ will then be linear. Figure 4b shows that the data from the chlorophyll a experiments plotted in this way consists of two straight lines, intersecting at ca. 27 °C, the temperature corresponding to the beginning of the pretransition. From the slopes of the lines, an activation energy for defect formation can be estimated as ca. 46 kcal/mol below 27 °C and 22 kcal/mol above 27 °C. Similar results are obtained from the Tempo data, except that the pretransition is more marked (see Figure 2), and that two breaks are seen in the plots, at 26 and 32 °C.

Premelting effects in dimyristoylphosphatidylcholine are apparently very similar to those in dipalmitoylphosphatidylcholine, since the shapes of the Tempo solubility curves

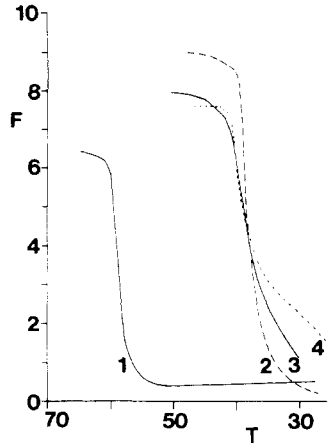

FIGURE 5: Plots of fluorescence intensity vs. temperature for chlorophyll a incorporated into: curve 1, dipalmitoylphosphatidylethanolamine; curve 2, dipalmitoylphosphatidylcholine plus 30 mol % dodecane; curve 3, dipalmitoylphosphatidylcholine plus 30 mol % decane; curve 4, dipalmitoylphosphatidylcholine.

(Shimshick and McConnell, 1973) and chlorophyll a fluorescence curves (Lee, 1975b,c) are similar for the two lipids. A quantitative analysis is not possible however, since it is not possible experimentally to achieve a low enough temperature to estimate a value of the measured parameter characteristic of the "pure" gel phase.

The experimental temperature–fluorescence intensity plot for chlorophyll a incorporated into bilayers of dipalmitoylphosphatidylethanolamine (Figure 5) is very different from that for the phosphatidylcholines. Any premelting effects are very small and occur within just a few degrees of the gel to liquid crystalline transition.

Effects of Long Chain Molecules. The premelting effects reported above are affected by the addition of a variety of long-chain molecules. Figure 5 shows the effect of addition of decane and dodecane to bilayers of dipalmitoylphosphatidylcholine. Although the alkanes have only a very small effect on the temperature of the gel to liquid crystalline phase transition, the addition of decane largely removes the pretransition and with dodecane there is an even more marked reduction in premelting effects. Thus, in dipalmitoylphosphatidylcholine alone at 31 °C, ca. 25% of the bilayer is present in a liquid crystalline-like form, whereas addition of 12 mol % dodecane reduces this to 7 mol %, and 25 mol % dodecane removes all the liquid crystalline-like form. It has been shown elsewhere that the pretransition in dipalmitoylphosphatidylcholine is also removed by decanol, dodecanol (Lee, 1976a), and myristic acid (Lee, 1976b).

Depression of the Lipid-Transition Temperature. Hill (1974) has presented a thermodynamic analysis of the effect of alcohols on the temperature of the phase transition in dipalmitoylphosphatidylcholine, and a fair agreement between theory and experiment has been found (Hill, 1974; Lee, 1976a). However, an unexpected observation, found both by differential scanning calorimetry (Jain et al., 1975) and fluorescence techniques (Lee, 1976a), is that the longer-chain alcohols cause an increase in the width of the phase transition. As shown in Figure 6a for 1-octanol, the increase in width of the transition is the result of a smaller effect of octanol on the high-temperature end of the gel to liquid crystalline phase transition than on the low-temperature end. An analysis of this effect is possible in terms of a defect-solid model.

At equilibrium, the solute alcohol will partition between the aqueous and lipid phases. The solute within the lipid phase will

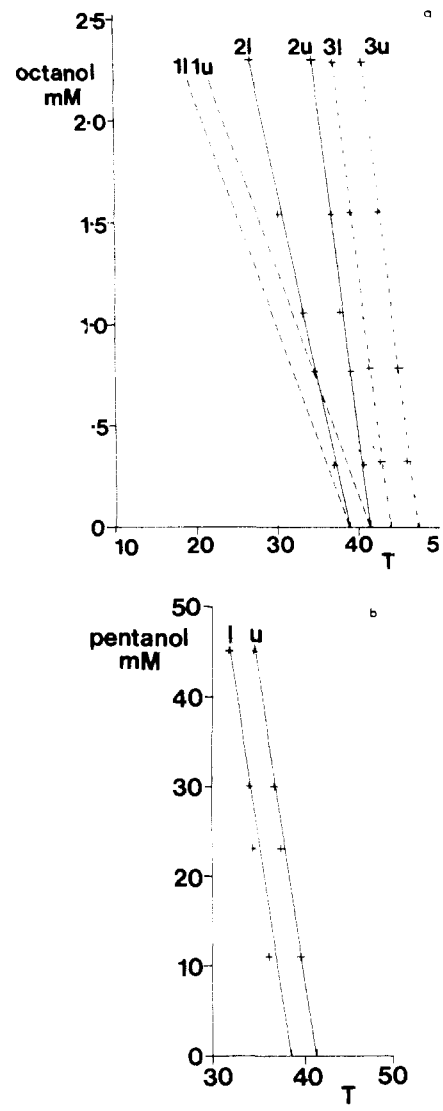

FIGURE 6: (a) The effect of 1-octanol on the temperatures of the upper (u) and lower (l) ends of the phase transition for: 2, dipalmitoylphosphatidylcholine; 3, dipalmitoylphosphatidylcholine plus 34 mol % dodecanol; 1, theoretical for dipalmitoylphosphatidylcholine, assuming Raoult's Law is obeyed. (b) The effect of pentanol on the temperatures of the upper (u) and lower (l) ends of the phase transition in dipalmitoylphosphatidylcholine.

be partly in regions of lipid in the liquid-crystalline phase and partly in regions in the gel phase. The simplest case is that considered by Hill (1974), where the solute is practically insoluble in gel-phase lipid, and the familiar relationship (eq 7) is obtained

$$\frac{1}{T_t} - \frac{1}{T} = \frac{R}{\Delta H_t} \ln N_{\text{lipid}}^{l} \tag{7}$$

where T_t is the transition temperature in the absence of solute and ΔH_t is the enthalpy of transition, and N_{lipid}^{l} is the mole fraction of lipid in the liquid-crystalline phase. For dilute solutions, where the depression of the transition temperature is small, eq 7 can be approximated as

$$\Delta T = T_t - T = \left(\frac{RT_t^2}{\Delta H_t}\right) N_{\text{solute}}^{l} \tag{8}$$

where N_{solute}^{l} is the mole fraction of solute in the liquid-crystalline phase. Hill (1974) has shown how it is possible to proceed from this equation to an equation for the slope of the de-

TABLE I: Effects of Straight-Chain Alcohols on the Phase Transition in Phospholipids.

Lipid	Alcohol	Upper end of transition		Lower end of transition	
		$\Delta T/r$	$\frac{N_{solute}^{g}}{N_{solute}^{l}}$	$\Delta T/r$	Apparent $\frac{N_{solute}^{g}}{N_{solute}^{l}}$
Dipalmitoylphosphatidylcholine	Pentanol	40.5	0	40.5	0
	Hexanol	29	0.3	32	0.2
	Heptanol	20	0.5	33	0.2
	Octanol	12	0.7	24	0.4
Dimyristoylphosphatidylcholine	Octanol	10	0.8	25	0.6
Dipalmitoylphosphatidylethanolamine	Octanol	9	0.8	9	0.8
Dipalmitoylphosphatidylcholine					
+ 34 mol % dodecanol	Octanol	12	0.7	13	0.7
+ 44 mol % dodecanol	Octanol	14	0.7	14	0.7

pression of the transition temperature as a function of the fraction of saturation, r, of the aqueous solution of solute

$$\frac{\delta \Delta T}{\delta r} = \left(\frac{RT_t^2}{\Delta H_t}\right) 2 \tag{9}$$

Figure 6b shows a plot of this equation for the effect of 1-pentanol on dipalmitoylphosphatidylcholine, using the alcohol-solubility data of Bell (1973) and the thermal-transition data of Hinz and Sturtevant (1972), and Figure 6a shows the corresponding line for 1-octanol. Clearly, for 1-pentanol experiment agrees closely with theory, whereas for 1-octanol agreement is poor.

For the short-chain alcohols, packing with the lipids in the gel state is likely to be poor, so that solid solution is unlikely, as assumed in the above calculation. For the longer-chain alcohols, however, packing is likely to be considerably better in a mixed bilayer in the gel state, and part of the deviation from theoretical expectation can be attributed to this cause. In this case, as discussed by Ubbelohde, it is reasonable to replace eq 7 by

$$\frac{1}{T_t} - \frac{1}{T} = \frac{R}{\Delta H_t} \ln \left(\frac{N_{lipid}^{l}}{N_{lipid}^{g}}\right) \tag{10}$$

where N_{lipid}^{g} is the mole fraction of lipid in the gel phase. If both N_{solute}^{l} and N_{solute}^{g} are small, then

$$R \ln \left(\frac{N_{lipid}^{l}}{N_{lipid}^{g}}\right) \simeq R(N_{solute}^{g} - N_{solute}^{l}) \tag{11}$$

so that

$$\Delta T \simeq \frac{RT_t^2}{\Delta H_t} N_{solute}^{l} \left(1 - \frac{N_{solute}^{g}}{N_{solute}^{l}}\right) \tag{12}$$

As assumed in the derivation of eq 9 (Hill, 1974), if interactions of the solute in the membrane and in the solute are the same then

$$RT \ln \left(\frac{N_{solute}^{l}}{N_{aqueous}}\right) S = RT \ln 2 \tag{13}$$

where S is the mole fraction at aqueous saturation and $N_{aqueous}$ is the mole fraction of solute in the aqueous phase. Thus, one obtains

$$\frac{\Delta T}{\delta r} \simeq \left(\frac{RT_t^2}{\Delta H_t}\right) 2 \left(1 - \frac{N_{solute}^{g}}{N_{solute}^{l}}\right) \tag{14}$$

When the ratio of solute partitioned into gel-phase lipid is very much less than that into liquid-crystalline phase lipid, then, of course, eq 14 reduces to eq 9. Fitting eq 14 to the experimental results gives a ratio of $N_{solute}^{g}/N_{solute}^{l}$, and the ratios obtained are listed in Table I. Also included is data for the effect of 1-octanol on mixtures of dipalmitoylphosphatidylcholine and dodecanol (see Figure 6b) assuming that the heat of transition is not appreciably affected by the addition of dodecanol.

The decreasing effects of the alcohols on the upper transition temperature with increasing chain length can be attributed to an increasing partitioning into the gel-phase lipid. It would, however, be unreasonable to attribute the decreased effect of the alcohols on the lower end of the transition to a decreased partitioning into the gel state, since it has been postulated that the upper end of the transition corresponds to transition between "bulk" gel and liquid-crystalline phases, whereas the lower end of the transition corresponds to this transition in the region of vacancies or dislocations: the alcohol would presumably be more soluble in the disordered gel-state regions. However, this observation suggests one made by Oldham and Ubbelohde (1940) on freezing in mixtures of long-chain ketones and paraffin. Experimentally, it seems that the R ln N_{lipid}^{g} term in eq 10 is having less than its expected effect; that is, that the entropy of mixing in the gel state is considerably less than that expected for a random distribution of solute and lipid. This would follow if the solute were to preferentially partition into the disordered regions of the gel state, previously postulated to be present. The depression of the transition temperature of the lipid in the region of the vacancies would then be greater than for the bulk lipid, and an increase in width of the transition with increasing solute concentration will result, as observed experimentally.

Discussion

Ubbelohde (1965) has presented a fairly complete picture of the molecular events involved in the melting of a solid. At all temperatures above absolute zero, a solid will contain defects which might be vacant sites or the more complex arrangements pictured in Figure 1b,c. The number of such defects will increase with increasing temperature. In proximity to these defects, transition to the liquid state will occur more readily, and thus at a lower temperature than for the melting of the bulk, fully ordered, material. The phase transition will then be continuous, occurring over a finite temperature range. The evidence presented here suggests that a very similar model can be presented for the gel to liquid-crystalline phase transition in lipid bilayers.

As discussed elsewhere (Lee, 1975a), the gel to liquid-crystalline phase transition in bilayers of phosphatidylcholines is found to be continuous, in the sense that it occurs over a range of temperatures, varying with sample history. Further, there are a variety of very marked pretransition effects. As shown here, the solubility of Tempo in bilayers of dipalmi-

toylphosphatidylcholine starts to increase at ca. 20 °C with a further marked increase at ca. 27 °C. As shown previously (Lee, 1975b), the fluorescence of chlorophyll a incorporated into lipid bilayers can be used to monitor changes within the bilayers, since the monomer–aggregate equilibrium for chlorophyll a is very sensitive to the environment. Chlorophyll a fluorescence in bilayers of dipalmitoylphosphatidylcholine also starts to increase at ca. 27 °C, corresponding to an increase in the proportion of monomeric, fluorescent, chlorophyll.

These results are consistent with the fomation of disordered, liquid crystalline-like, lipid within the gel matrix at a temperature ca. 20 °C below the main transition in phosphatidylcholines. Other evidence in favor of this conclusion can be found. Thus, Lippert and Peticolas (1971, 1972) report changes in the Raman spectra of sonicated aqueous dispersions of dipalmitoylphosphatidylcholine and dioleoylphosphatidylcholine that can be attributed to the loss of the all-trans extended structure of the fatty acid chain, starting at ca. 15 °C below the main transition, although the interpretation is complicated by the known increase in width of the transition caused by sonication (see Suurkuusk et al., 1976). Direct evidence for the presence of grain boundaries has come from dark-field electron micrqscopy (Hui et al., 1974; Hui and Parsons, 1975), in which lipid domains of different orientation were seen in the gel phase, each domain being typically several microns wide at 17 °C. This would correspond to very approximately one lipid in a thousand being in the surface boundary, and thus in a relatively disordered state. Areas of continuously striated pattern seen in freeze-fracture electron micrographs of liposomes (Ververgaert et al., 1973) may also correspond to domains.

The presence of vacancies is also consistent with the observations that addition of alcohols to phosphatidylcholine causes both a decrease in the temperature of the main transition and an increase in width. The data suggest that the longer-chain alcohols are at least partly miscible with the lipids in the gel state. Further, the alcohols will partition into the disordered lipids in the regions of vacancies, and there will have a greater effect on the transition temperature of the lipid than in the region of completely ordered gel-phase lipid. Plots of variation of amount of disordered lipid as a function of temperature show discontinuities at temperatures corresponding to the so-called pretransition. The pretransition has been attributed to a change in the orientation of the lipid fatty acid chains from being tilted with respect to the bilayer plane to being oriented perpendicular to the bilayer plane (Rand et al., 1975), thus allowing rotation about their long axes (Lee et al., 1974a, 1976). It has been suggested that the head-group conformation changes at the pretransition, from being parallel to the bilayer surface to being perpendicular to it (Levine et al., 1972). In the low temperature, folded-down, configuration, intermolecular interactions between the head groups are likely, and disruption of these by vacancy formation would be thermodynamically unfavorable.

Addition of long-chain molecules, such as decane, dodecane, decanol, and myristic acid, to phosphatidylcholines significantly reduces the amount of disordered lipid in the gel phase and removes the pretransition. The reason for this is not clear. Presumably, the abolition of the pretransition is the reflection of a high cooperativity. This would certainly account for its abolition by a wide variety of molecules, including many local anaesthetics (Papahadjopoulos et al., 1975; Lee, 1976c).

In contrast to the significant disordering usually observed in gel-phase phosphatidylcholines, for phosphatidylethanolamines there is no evidence for disordering until very close to the main gel to liquid-crystalline phase transition. This can probably be attributed to a strong interaction between the phosphatidylethanolamine head groups, with the positively charged amine group of one molecule interacting electrostatically with the negatively charged phosphate group of an adjacent molecule. Any marked disordering of the lipid would lead to a reduction in this electrostatic interaction, and so be thermodynamically unfavorable.

Significant disorder in the gel-phase lipid of a membrane would be expected to have important effects on the properties of the membrane. The most obvious of these is on the permeability properties of the membrane. Recent studies have shown that the permeability of bilayers of phosphatidylcholines to small molecules starts to increase ca. 5 °C below the main transition, and reaches a maximum at the transition temperature itself (Blok et al., 1975). This is expected from the ideas developed here. In the gel phase, with increasing temperature an increasing proportion of the lipid will be present in small areas of disorder. Eventually, the proportion of disordered gel-phase lipid will become so large that the gel phase becomes unstable and a gel to liquid-crystalline phase transition will occur. In the gel phase, the vacancies and dislocations present in an otherwise regular lattice provide favorable sites for the diffusion of small molecules. In the liquid-crystalline phase, however, the notion of regular sites becomes meaningless, and thus one can no longer speak of vacancies, etc. (Lee, 1975a). Lipids in the liquid-crystalline phase are less tightly packed than in the gel phase, but the disordered regions providing an easy route for diffusion have disappeared. Papahadjopoulos et al. (1973) have interpreted the maximum permeability observed at the temperature of the phase transition in similar terms.

The proportion of disordered lipid can be much affected by the addition of lipophilic molecules. Thus, the amount of disordered lipid is reduced by addition of dodecanol, and it is therefore of interest that Lawrence and Gill (1975) report that, whereas intravenous doses of octanol act as an effective general anaesthetic, hexadecanol produces a cataleptic state.

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Fluidity of the Lipids Next to the Acetylcholine Receptor Protein of Torpedo Membrane Fragments. Use of Amphiphilic Reversible Spin-Labels†

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ABSTRACT: Choline esters of spin-labeled fatty acids (long-chain acylcholines) were used to probe the hydrophobic environment of the acetylcholine receptor protein in membrane fragments from *Torpedo marmorata*. These spin-labels competitively inhibit the binding of [^{3}H]acetylcholine to the receptor site. Their inhibition constants (K_I) were close to 200 nM. At the high membrane concentration required for electron spin resonance (ESR) experiments, the apparent inhibition constants (K_I^{app}) differed from K_I determined by using dilute membrane concentration. This is due to the amphiphilic character of long-chain acylcholine. For most spin-labels used, only difference ESR spectroscopy provided reliable spectra corresponding to receptor-bound spin-labeled acylcholines. Acetylcholine receptor agonists and antagonists displaced the acylcholine from the receptor sites, whereas choline had only a weak effect. This produced a modification in the ESR spectra of the bound acylcholines and provided evidence that the acylcholines bound to the receptor sites in a specific manner. The interpretation of the spectra of receptor-bound spin-labels favored a strong barrier to the motion of the probe when attached to the middle of the acyl chain. However, when the probe was close to the methyl terminal of a stearoylcholine molecule a much greater fluidity was found. Short-range spin–spin interactions were created between spin-labels bound to the receptor site and spin-labels in a fluid phase. This indicates that lipids next to the receptor protein are not completely immobilized in spite of the semicrystalline organization of the proteins in the postsynaptic region.

The electric organ of Torpedo is an excellent source of membrane fragments rich in acetylcholine receptor protein (Cohen et al. 1972, Duguid and Raftery, 1973). X-ray diffraction and electron microscopy studies of these membrane fragments (Cartaud et al., 1973, Nickel and Potter, 1973, Dupont et al., 1974) have revealed a regular organization of the receptor protein in the plane of the membrane. If these proteins form crystalline arrays in the postsynaptic region, it is questionable whether the lipids present in that membrane can form fluid bilayers. Hence, it was important to determine whether such bilayers can exist in the vicinity of the acetylcholine receptor protein.

In order to study the lipid environment of the cholinergic receptor protein of Torpedo, we have used a spin-label method similar to that previously described to study mitochondrial and microsomal membrane-bound proteins (Devaux et al., 1975a,b). It was shown that if a spin-labeled fatty acid was linked to a polar group binding to specific membrane proteins, the ESR[1] spectra of the paramagnetic probe could provide valuable information on the state of the mobility of the hydrocarbon chains next to the proteins, as well as on the depth that the proteins penetrated into the hyprophobic part of the membrane.

† From the Biophysique Moléculaire, GPS-ENS Tour 23, Université Paris VII, 75221 Paris Cedex 05, France. *Received July 14, 1976.* This investigation was supported by research grants from the Délégation à la Recherche Scientifique et Technique and the Centre National de la Recherche Scientifique.

‡ Supported by the DGRST.

[1] Abbreviations used are: ESR, electron spin resonance; TETRAM, *O,O*-diethyl *S*-(*N,N*-diethylamino)ethyl phosphorothiolate.