*Biochimica et Biophysica Acta*, 448 (1976) 220–230


BBA 77443

# PHOSPHOLIPID PHASE TRANSITIONS

## EFFECTS OF *n*-ALCOHOLS, *n*-MONOCARBOXYLIC ACIDS, PHENYL ALKYL ALCOHOLS AND QUATERNARY AMMONIUM COMPOUNDS

A. W. ELIASZ[a], D. CHAPMAN[a] and D. F. EWING[b]

[a]*Chemistry Department, Chelsea College, University of London, London, SW3 6LX and* [b]*Chemistry Department, Hull University, Hull (U.K.)*

(Received June 4th, 1976)

SUMMARY

The interactions of a series of alcohols, acids and quaternary ammonium salts with a phosphatidylcholine-water model biomembrane (dipalmitoyl phosphatidylcholine) system have been studied using differential scanning calorimetry. In particular the effects of these molecules upon the lipid endothermic phase transitions were investigated over a range of concentrations. A variety of effects was observed. (a) Those molecules which shift or broaden the main lipid transition can also remove the pretransition endotherm. (b) *n*-Alcohols and *n*-monocarboxylic acids containing the same number of carbon atoms have very similar effects at molar concentrations up to 40 %. Those molecules containing 12 or more carbon atoms raise the main lipid phase transition whilst those molecules containing 10 or less carbon atoms lower this transition temperature. (c) The phase diagram of stearoyl alcohol in the phosphatidylcholine-water system shows the formation of lipid-alcohol complexes. (d) Alkyl trimethyl ammonium bromides showed behaviour which differs considerably from *n*-alcohols and *n*-carboxylic acids of the same chain length. (e) Other alkyltrialkyl and tetraalkylammonium bromides show that a variety of effects on the lipid phase transition can be obtained. (f) With the homologous series of phenylalkyl alcohols from benzyl alcohol to 4-phenyl butanol increasing the number of methylenes between the terminal OH and the benzene ring leads to greater interaction between solute and bilayer.

The range of different effects obtained with the compounds studied offers a means for introducing various degrees and types of perturbation into membrane systems.

## INTRODUCTION

Phospholipid molecules and particularly the phosphatidylcholines are important components of cell membranes and have been widely used in model biomembrane systems. Recent experiments of the 1,2-dipalmitoyl phosphatidylcholine-water system

which forms an array of lipid bilayers [1] have shown that the main characteristic endothermic phase transition of the lipid can be shifted to higher or lower temperatures and sometimes removed dependent upon interactions of the lipid with other components, e.g. metal ions, polypeptides or proteins [2] as well as various drug molecules such as anaesthetics [3], drugs acting on the central nervous system [4, 5] and membrane-expanding drugs. [6]

In the present study we examine a series of alcohols, acids and quaternary ammonium salts to see how the interaction with the 1,2-dipalmitoyl phosphatidylcholine-water system changes within a homologous series as well as simulating the characteristics of certain drug molecules which interact with biomembranes.

We have concentrated on homologous series of molecules each having one polar group in order to see the range of effects which can be obtained. The molecules can be divided into two classes: those having an uncharged polar group $R_1$-OH, $R_1$-$CO_2H$ and those having a charged polar group $R_1$-$N^+$-$(R_2)_3$. If $R_1$ is an alkyl chain only, then an important orientation of the introduced molecule in the lipid bilayer is with $R_1$ situated amongst the hydrocarbon chains and with the polar group situated at the lipid-water interface. For sufficiently long alkyl chains this will be the preferred orientation in the bilayer. This will cause least disruption to the packing of the lipid hydrocarbon chains and at the same time provide maximum hydrophobic interaction between the lipid and the "impurity" molecule.

## MATERIALS AND METHODS

1,2-Dipalmitoyl phosphatidylcholine was obtained from Koch Light Limited, batches were checked for purity by thin-layer chromatography and used without further purification. The *n*-monocarboxylic acids and the *n*-alkyl alcohols (> 99.5 % pure) were obtained either from Fluka A.G. or Koch Light and also used without further purification. Benzyl alcohol, 2-phenyl ethan-1-ol and 3-phenyl propan-1-ol were obtained from Koch Light respectively and were all puriss grade. 4-Phenyl butan-1-ol was a gift from Dr. Aveyard of Hull University and was > 98 % pure by gas-liquid chromatography. The quaternary ammonium salts were either purchased from Eastman Kodak, obtained from Unilever Research (Port Sunlight) or synthesised from the appropriate alkyl bromide and trialkylamine.

Samples for differential scanning calorimetry were prepared by one of three methods. (i) The dipalmitoyl phosphatidylcholine and the second component to be added were mixed in dry (95:5 v/v) benzene/methanol and the solvent removed by freeze drying. The samples were then made up in excess water 3:1 by weight and mixed on a bench vibrator above the lipid transition temperature. To obtain reproducible results careful mixing was found to be important. This method was used with the $C_{18}$-$C_{12}$ acids and alcohols, and the quaternary ammonium bromides except for the $C_1$-$C_5$ tetraalkyl ammonium bromides.

(ii) Where the second component was a liquid at or close to room temperature, it was added to the lipid/water mixture and the resulting mixture dispersed by mixing with a bench vibrator above the lipid transition temperature. Thus method was used for $C_{10}$ and $C_8$ alcohols and acids and the $\omega$-phenyl alkan-1-ols.

(iii) The tetramethyl to tetrapentyl ammonium bromides were added as aqueous solutions to the dry lipid followed by the usual dispersion procedure.

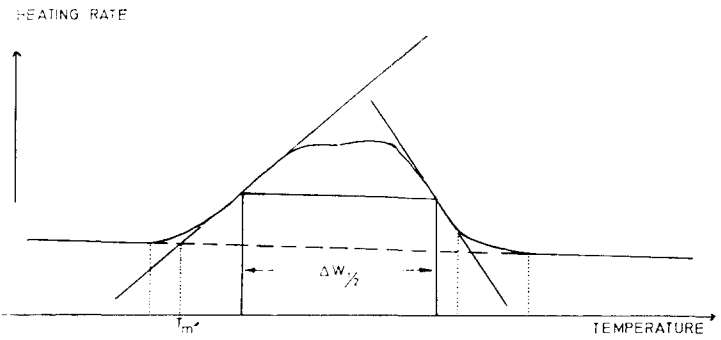


Fig. 1. The method of determining the onset of transition temperature ($T_c$) and width at half-height ($\Delta W_{\frac{1}{2}}$) from a calorimetric heating curve.

Calorimetric studies were carried out on a Perkin-Elmer DSC-2 differential scanning calorimeter with a heating or cooling rate of 5 °C per min. The heats of transition were calculated from the area under the curves which was determined by use of a fixed arm planimeter. For sharp transitions it is customary to use $T_c$, as defining the onset of the transition (Fig. 1). The accurate measurement of the onset temperature of broad transitions can however be difficult. The heats of transition were found to be reproducible to better than 10 % except where transitions were very broad, the widths of transition at half height were reproducible to better than 10 %, and the onset temperatures of the transitions were reproducible to better than 0.5 °K. The X-ray diffraction experiments were carried out using a Nelson diffractometer using Co radiation.

## RESULTS

### *n-Monocarboxylic acids and alcohols*

Heating curves for octanol-dipalmitoyl phosphatidylcholine and stearoyl alcohol-dipalmitoyl phosphatidylcholine, all in excess water and over a range of relative concentrations, are shown in Figs. 2 and 3.

The onset temperatures, and the widths of transition at half height are shown in Figs. 4 and 5. The values of the heats of transition of pure dipalmitoyl phosphatidylcholine obtained were found to be in good agreement with previous studies [4, 10] (Main lipid transition 8.2 kcal/mol, and the pretransition 1.7 kcal/mol). It is clear that the monocarboxylic acids and alcohols of a given chain length exhibit similar behaviour. The homologues from $C_{12}$ to $C_{18}$ increase the main lipid transition temperature and the $C_{10}$ to $C_8$ homologues decrease the main lipid transition temperature.

The $C_8$ and $C_{10}$ homologues tend to lower (slightly) the heat of the main lipid endotherm, whereas the $C_{12}$ to $C_{18}$ homologues tend to slightly raise the heat of the main lipid endotherm.

The mole ratios used in plotting the results are added mole ratios, the assumption is made that all of the added alcohol or acid is associated with the lipid. The hydrated $C_8$ to $C_{18}$ acids and alcohols have distinct transitions and over the concentration

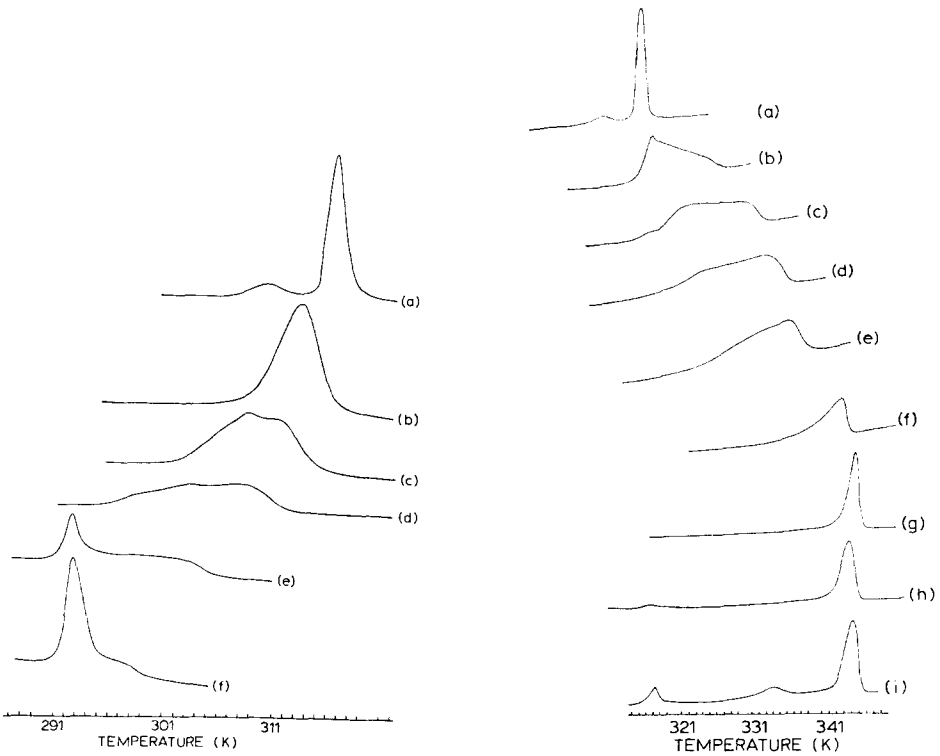


Fig. 2. Calorimetric heating curves for various mixtures of dipalmitoyl phosphatidylcholine and octanol in excess water. (Heating rate 5 °C/min). The mixtures contain the following mole ratios of octanol to dipalmitoyl phosphatidylcholine: (a) pure dipalmitoyl phosphatidylcholine; (b) 0.13; (c) 0.29; (d) 0.61; (e) 1.22; (f) 1.81.

Fig. 3. Calorimetric heating curves for various mixtures of dipalmitoyl phosphatidylcholine and stearoyl alcohol in excess water (Heating rate 5 °C/min). The mixtures contain the following mole ratios of stearoyl alcohol to dipalmitoyl phosphatidylcholine: (a) pure dipalmitoyl phosphatidylcholine; (b) 0.18; (c) 0.35; (d) 0.55; (e) 0.72; (f) 1.30; (g) 3.25; (h) 4.88; (i) 6.50.

range plotted in Figs. 4 and 5 no peaks corresponding to excess alcohol or acid were observed. Furthermore, the solubility of these alcohols and acids in water over the temperature range in question is low, and with the amounts of water added a saturated solution of acid or alcohol would account for less than 5% of the added acid or alcohol.

The width of transition at half height is taken as a qualitative measure of the extent to which the added acid or alcohol can widen the temperature range over which both fluid and gel phases can co-exist.

The plot of the phase diagram for stearoyl alcohol-dipalmitoyl phosphatidylcholine in excess water is shown in Fig. 6. X-ray diffraction measurements in regions I, IV and V show the presence of a lamellar phase. On the basis of the phase diagram we suggest that in region I the lamellae contain a solution of stearoyl alcohol in phosphatidylcholine. In region II a mixture of solid and fluid regions occurs whose compositions are given by the solidus and fluidus lines. Region III

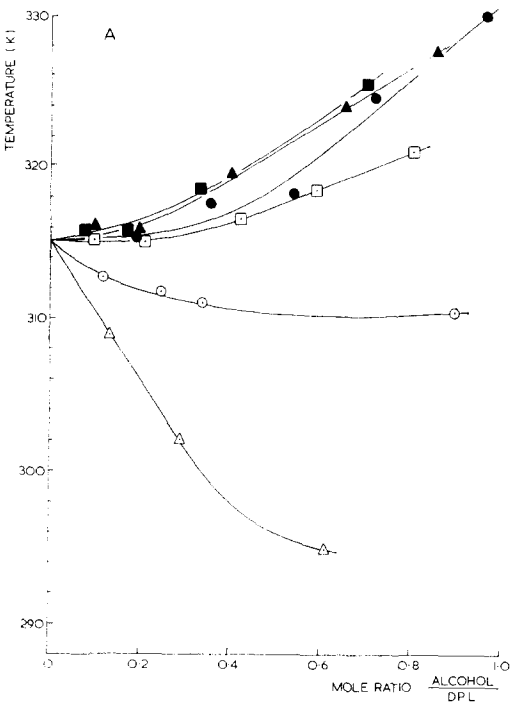


Fig. 4A. See opposite page for legend.

consists of stearoyl alcohol in phosphatidylcholine at approximately 1:1 molar composition as well as stearoyl alcohol which probably exists as small regions within the plane of the bilayer; regions IV and V contain various mixtures of solid solutions of phosphatidylcholine and stearoyl alcohol, and region V is bounded by a complex of stearoyl alcohol and phosphatidylcholine containing approximately 4 molecules of stearoyl alcohol to one of phosphatidylcholine. X-ray diffraction measurements on a mixture containing 76 mol% of stearoyl alcohol at 20 °C show it to be a lamellar phase having an inter-lamellar repeat distance of 73$\pm$ 3 Å and a short spacing diffraction peak at 4.16 Å which corresponds to closely packed hydrocarbon chains.

Above 80 mol% stearoyl alcohol the alcohol begins to separate out as a separate phase and two transitions characteristic of the alcohol hydrate are observed (fig. 3).

*ω-Phenyl alkan-1-ols*

The heating curves of the homologous series of ω-phenyl alkan-1-ols were obtained starting from benzyl alcohol. Increasing the number of methylene groups between the OH group and the benzene ring has a significant effect on the main lipid transition temperature. The main lipid transition broadens and less compound is required to produce a given lowering of the transition temperature. These results are summarised in Fig. 7.

*Quaternary ammonium compounds*

Several compounds of the type $R_1N^+$-$(R_2)_3Br$ (where $R_1$ and $R_2$ are normal

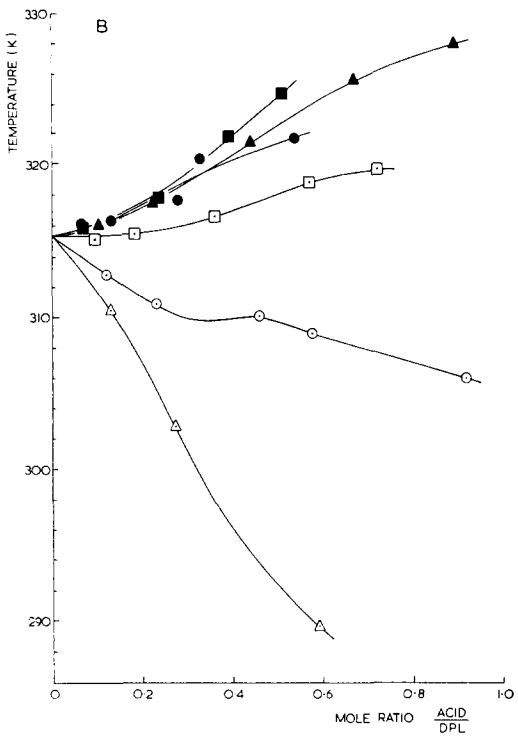


Fig. 4. (A) Onset transition temperatures for normal aliphatic alcohol-dipalmitoyl phosphatidylcholine/excess water mixtures plotted against the mole ratio of acid to dipalmitoyl phosphatidylcholine: ●—●, octadecanol (stearoyl alcohol); ■—■, hexadecanol (palmitoyl alcohol); ▲—▲, tetradecanol (myristoyl alcohol); ⊡—⊡, dodecanol (lauroyl alcohol); ⊙—⊙, decanol; ◬—◬, octanol. (B) Onset transition temperatures for normal carboxylic acid-dipalmitoyl phosphatidylcholine/excess water mixtures plotted against the mole ratio of acid to dipalmitoyl phosphatidylcholine: ●—●, octadecanoic acid (stearic acid); ■—■, hexadecanoic acid (palmitic acid); ▲—▲, tetradecanoic acid (myristic acid); ⊡—⊡, dodecanoic acid (lauric acid); ⊙—⊙, decanoic acid; ◬—◬, octanoic acid.

aliphatic chains) were studied. By varying $R_1$ and $R_2$ it was possible to obtain a wide variety of effects on the transition of the dipalmitoyl phosphatidylcholine $H_2O$ system.

A representative set of heating curves for mixtures of tetra hexyl ammonium bromide (see structure) with dipalmitoyl phosphatidylcholine in excess water are shown in Fig. 8.

With tetrapentyl and tetrahexyl ammonium bromides the pretransition initially broadens and shifts to lower temperatures as the relative amount of these compounds increase and disappears at higher concentrations. The main transition broadens followed by a decrease in the area of the main peak at the expense of a shoulder situated at lower temperatures. The heats of transition remained approximately unchanged. 200 mM solutions of tetramethyl-, tetraethyl-, and tetrapropylammonium bromide had no effect on the dipalmitoyl phosphatidylcholine endotherms. 200 mM tetrabutylammonium bromide removed the pretransition endotherm normally observed but had no effect on the main lipid endotherm.

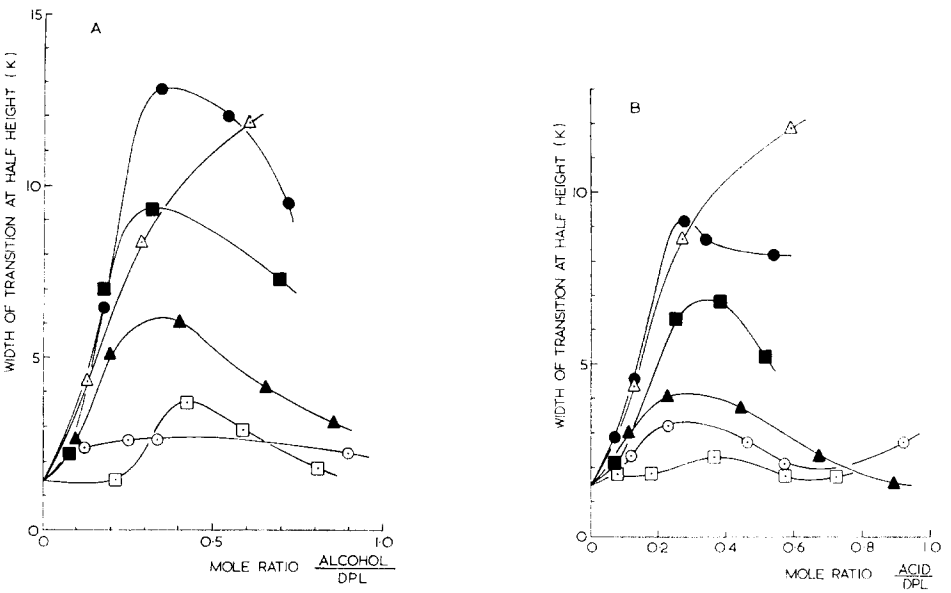


Fig. 5. (A) Effects of varying ratios of *n*-alcohols to dipalmitoyl phosphatidylcholine (DPL) on the width of the transition at half-height of the dipalmitoyl phosphatidylcholine-*n*-alcohol/excess water systems: ●—●, octadecanol (stearoyl alcohol); ■—■, hexadecanol (palmitoyl alcohol); ▲—▲, tetradecanol (myristoyl alcohol); ⊡—⊡, dodecanol (lauroyl alcohol); ○—○, decanol; △—△, octanol. (B) Effects of varying ratios of *n*-carboxylic acids to dipalmitoyl phosphatidylcholine on the width of the transition at half-height of the dipalmitoyl phosphatidylcholine-*n*-carboxylic acid/excess water systems: ●—●, octadecanoic acid (stearic acid); ■—■, hexadecanoic acid (palmitic acid); ▲—▲, tetradecanoic acid (myristic acid); ⊡—⊡, dodecanoic acid (lauric acid); ○—○, decanoic acid; △—△, octanoic acid.

In the homologous series of alkyl trimethylammonium bromides (with alkyl chains from $C_{18}$ to $C_8$) those compounds with chain length below $C_{16}$ initially lower the transition temperature. At higher concentrations the dispersions become clear. At the same time the transition becomes broad and the heat of transition decreases considerably. (These results are consistent with the quaternary ammonium salt initially penetrating the bilayer, then subsequently disrupting the bilayer to give mixed micelles, but this requires confirmation using X-ray techniques).

*Shifts of lipid transition temperature*

Assuming that all of the foreign molecules partition into the lipid bilayer above the main transition, and that there is a linear relationship between the amount of foreign substance in the bilayer above the transition and the amount of depression of the transition temperature, it is possible to use our data to calculate the concentration of solute (in moles of solute per mole of lipid) to cause a 1 °C depression of the transition temperature. The values were obtained from the results of two different concentrations of foreign molecule in the range 5–30 %. The results of measurements taken at two concentrations within this range were consistent to within 20 %.

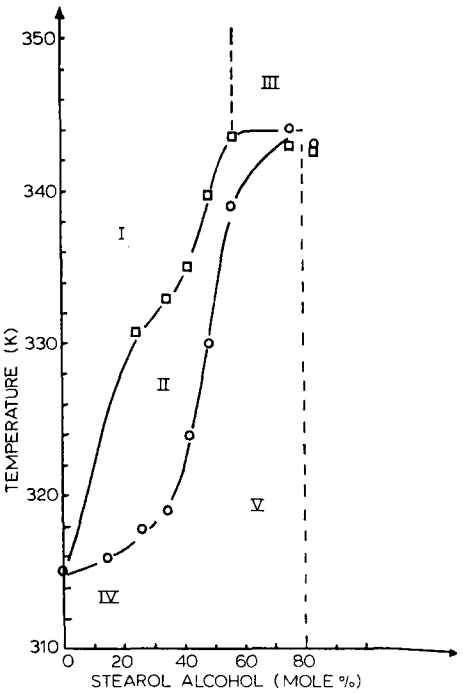


Fig. 6. The phase diagram for stearoyl alcohol and dipalmitoyl phosphatidylcholine in excess water: □—□, fluidus line; determined from cooling curves; ○—○, solidus line: determined from heating curves.

## DISCUSSION

It is now generally accepted that many biomembranes are built up on a bilayer structure of lipid. The lipid may be in a fluid condition or sometimes coexisting rigid and fluid regions occur [6]. It is also known that variations of the fluidity of the lipid bilayer can effect water permeability, or diffusion processes across and within the plane of the membrane [8, 9].

The dipalmitoyl phosphatidylcholine-water system forms spontaneously a convenient array of lipid bilayers which has been studied by many workers [10, 11]. The lipid undergoes two endothermic transitions which occur at 34.5 °C (the pretransition endotherm) and at 41.5 °C (the main transition). At the main transition the enthalpy is 8.7–9.6 kcal $mol^{-1}$ [12, 13]. This main transition has been associated with a "melting" of the lipid hydrocarbon chains. Recent X-ray studies show [14] that at the pretransition temperature that the chains move from a tilted configuration to a vertical configuration ($L\beta'$ to $L\beta$ forms using the Tardieu et al. convention ref. 15).

Our present study is concerned with the interaction of a range of "foreign" molecules with these lipid bilayer systems. It is clear that many of these molecules can remove the pretransition endotherm (Figs. 2 and 3). Similar effects have been observed with antidepressents [4], anaesthetics [16], proteins [17] and phorbol esters [18].

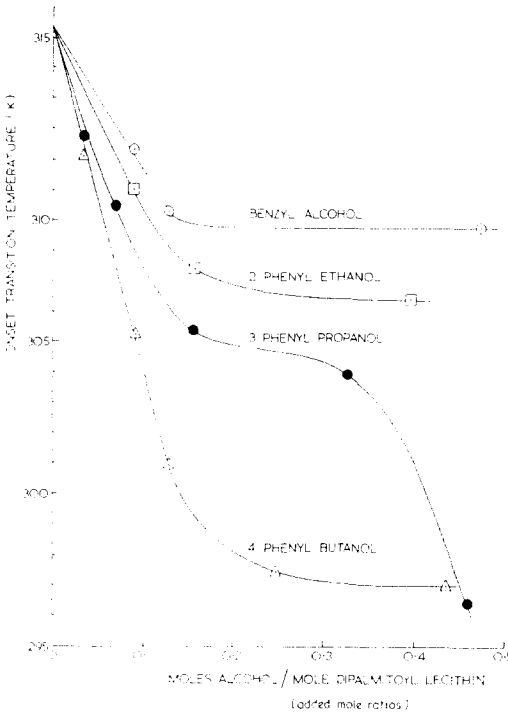


Fig. 7. Effects of varying ratios of $\omega$-phenyl alcohols to dipalmitoyl phosphatidylcholine on the onset of transition temperature of the dipalmitoyl phosphatidylcholine/$\omega$-phenyl alcohol/excess water systems.

It is also important to appreciate that the concentration and distribution of a foreign molecule can differ considerably below and above the main transition temperature. A relatively polar molecule remain close to the polar region below the transition (or even mainly in the aqueous region) and distribute itself partly into the hydrophobic region of the lipid above the transition temperature. The factors affecting the distribution of foreign molecules within the plane of the bilayer are important as they will determine various equilibria between the fluid and gel regions during the course of a transition. When the lipid is in the gel (or ordered chain condition) there may occur a mixture of solid solutions within the plane of the bilayer whereas in the fluid state the system may be monodisperse. During the course of the transition a series of disproportions between solid and fluid regions may occur [12, 19].

Recent studies [4, 5] have shown that many drug molecules can affect the main lipid transition and also broaden the range of the transition, i.e. it can enlarge the temperature range in which the two dimensional domains of fluid and gel phases coexist. It can be seen that the main transition is shifted to lower temperatures for the shorter chain fatty acids and alcohols but to higher temperatures with the longer chain derivatives and in some cases (Figs. 2 and 3) that the range of transition is increased.

Inspecting the dipalmitoyl phosphatidylcholine-stearoyl alcohol phase diagram [Fig. 6] shows that there are solid solution immiscibilities below the transition tem-

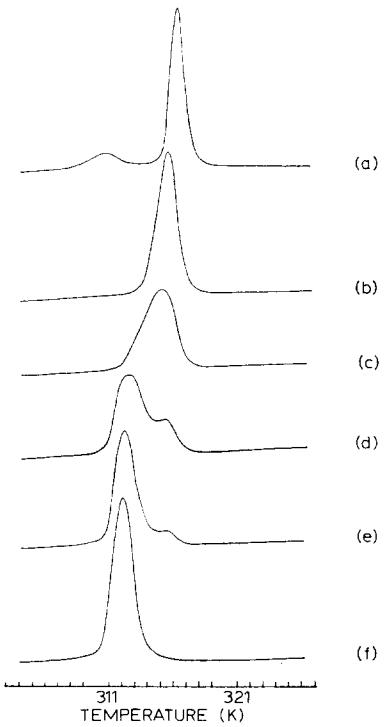


Fig. 8. Calorimetric heating curves for dipalmitoyl phosphatidylcholine/tetrahexylammonium bromide/excess water for various molar ratios of tetrahexylammonium bromide to dipalmitoyl phosphatidylcholine. Heating rate 5 °C per min. The following mole ratios of tetrahexylammonium bromide to dipalmitoyl phosphatidylcholine are shown: (a) pure dipalmitoyl phosphatidylcholine; (b) 0.02; (c) 0.04; (d) 0.09; (e) 0.11; (f) 0.18.

TABLE I

| Molecule | Concentration of solute to give a 1 °C depression |
|---|---|
| Decanoic acid | $5.0 \cdot 10^{-2}$ |
| Decanol | $6.0 \cdot 10^{-2}$ |
| Octanoic acid | $2.4 \cdot 10^{-2}$ |
| Octanol | $2.2 \cdot 10^{-2}$ |
| Benzyl alcohol | $4.7 \cdot 10^{-2}$ |
| 2-Phenyl ethan-1-ol | $3.85 \cdot 10^{-2}$ |
| 3-Phenyl propan-1-ol | $2.4 \cdot 10^{-2}$ |
| 4-Phenyl butan-1-ol | $1.7 \cdot 10^{-2}$ |

perature in the gel phase, and that the system separates out into a phosphatidylcholine-rich phase and an alcohol-rich phase within the plane of the bilayer. The alcohol-rich phase has a higher melting point than the phosphatidylcholine-rich phase.

The data for the other alcohols and acids showing the onset transition temperature as a function of mol% alcohol (Fig. 4) are effectively solidus curves of the partial phase diagrams for these systems. The data show that there is considerably less solid solution immiscibility as one goes to shorter chain lengths, and that $C_{10}$ and $C_8$ alcohols do not appear to show any solid solution immiscibility. Furthermore, the width of the transition at a given concentration of alcohol or acid decreases as the chain length decreases to $C_{10}$ (Fig. 5). This effect is probably due to a decrease in the stability of the alcohol-lipid and acid-lipid aggregates as the length of the acid or alcohol hydrocarbon chain decreases. These shorter chain acids and alcohols now begin to behave as classical solute impurities, however, there are differences, since octanol broadens the transition far more than decanol (and similarly for octanoic acid relative to decanoic acid). The fact that the change over point in the effect of these added alcohols and monocarboxylic acids upon the lipid phase transition occurs at about $C_{10}$ chain length is interesting.

If the fatty acid or alcohol introduced into the lipid bilayer is considerably shorter than sixteen carbons in length then the expected result of introducing such a molecule, anchored to the phosphate group and close packed in a hexagonal lattice with the lipid hydrocarbon chains, is to decrease the van der Waals interactions between the terminal methyl and methylene groups of the lipid hydrocarbon chains. This is expected to considerably lower the lipid main transition temperature.

Another effect to be considered however is that the presence of the alcohols (or acids) dispersed among the lipid molecules will be to affect the head group repulsions of the choline head group. This effect may counter the van der Waal chain interactions and tend to raise the main lipid transition temperature. Some recent theoretical calculations [20] have suggested that the head group repulsive interactions are approximately of the same magnitude as the attractive interactions of six methylene groups.

Similar experiments to ours have been made by Hui and Barton (21) with dimyristoyl phosphatidylcholine in excess water and a series of alcohols. These authors observed a change over point in the main transition temperature of the lipid which occurs at around the $C_8$ alcohol.

Previous studies using light scattering of some short chain alcohols with dipalmitoyl phosphatidylcholine-water systems also showed [6] a reduction in the main lipid transition temperature. The nature of the complexes which appear to be formed between dipalmitoyl phosphatidylcholine and alcohols and acids of a given chain length are probably associated with specific geometrical packing arrangements possible within the lipid crystalline bilayer structure. The calorimetric data suggests, 1-1 complexes for $C_{14}$ and $C_{12}$, acids and alcohols and a 4-1 complex for octadecanol with dipalmitoyl phosphatidylcholine. For an ideal system, in which the solute-membrane interactions and the solute-solute interactions are the same, Hill [6] has shown that the concentration of solute in the membrane required to produce a one degree depression is 0.0442. For systems which deviate considerably form this value, other interactions must be involved than those arising simply from dispersing a solute in a solvent. Our value for octanol is in broad agreement with that obtained

by Hill ($2.2 \cdot 10^{-2}$ compared with a value of $4 \cdot 10^{-2}$). We note a broadening of the transitions caused by some foreign molecules (e.g. octanol) which the light scattering technique does not observe. The phenyl substituted alcohols, benzyl alcohol and phenyl ethan-1-ol have values close to the theoretical values whereas 3-phenyl propan-1-ol and 4-phenyl butan-1-ol have values which are about half the theoretical value. Based on the data of Cater et al. [4] the two drugs desipramine and M IV show even greater deviations, the values for the concentration of solute required to produce a 1 °C depression (assuming that the drug is partitioned into the bilayer) is $1.1 \cdot 10^{-2}$ for desipramine and $0.70 \cdot 10^{-2}$ for M IV. The considerable deviations from ideality shown by these compounds suggest that more complex interactions are involved in these cases.

The alkyl trimethylammonium bromides contain a bulky polar group carrying a net positive charge. The repulsive interactions between the positive charges carried by these molecules are expected to favour dispersion throughout the lipid bilayer. The large size of the polar group makes it unlikely that the polar group will be anchored amongst the choline headgroups of the phosphatidylcholine but will project beyond the choline headgroup. This could explain why both hexadecyl- and octadecyl trimethylammonium bromide have little effect on the main lipid transition. If the trimethylammonium headgroup is located beyond or close to the choline headgroup of the phosphatidylcholine a much smaller length of alkyl chain will extend into the hydrocarbon chain region of the phosphatidylcholine molecules compared to *n*-alcohols or monocarboxylic acids of the same length. Consistent with this suggestion hexadecyl triethyl- and hexadecyl tributylammonium bromides have little effect on the main lipid transition whereas tetradecyl trimethylammonium bromides lowers the onset of the lipid transition temperature by $\approx 3$ °K at a concentration of 0.20 mol per mol dipalmitoyl phosphatidylcholine while dodecyl-, decyl- and octyl trimethylammonium bromide lower the onset of transition temperature by 5–8 °C at a similar concentration. At higher concentrations of the shorter ($< C_{16}$) chain length salts a combination of electrostatic and packing effects appear to make a micellar configuration the more stable phase. When the trimethylammonium group in decyl trimethylammonium bromide is replaced by a tributyl group, then at concentrations at which decyl trimethylammonium bromide appears to bring about micelle formation, decyl tributylammonium bromide gives rise to a sharp transition whose enthalpy is close to that for pure dipalmitoyl phosphatidylcholine and has a transition temperature $> 10$ °C below that for the pure lipid. This behaviour is similar to that shown by the antidepressant desipramine [4].

The effects observed with tetrahexyl ammonium (see Fig. 9) bromide are similar to those observed by Chapman et al. [2] with uranyl ions, and may imply that a titration of the lipid head groups by the tetrahexylammonium ion is occurring with the formation of a complex having its own transition temperature. The fact that titration shows an approximate stoichiometry of 6:1 and that the transition of the 6:1 complex remains sharp is consistent with the bilayer structure being maintained. It is, however, possible that a separate phase containing the lipid and quaternary salt is being produced, not necessarily as a lamellar phase. (This requires further X-ray studies).

The homologous series from benzyl alcohol to 4-phenyl butanol illustrates a different effect. Benzyl alcohol over the concentration range studied has very little

effect on the width of the transition, whereas 3-phenyl propanol, 4-phenyl butanol and 2-phenyl ethanol have a marked effect on the shape and width of the transition. Benzyl alcohol may be behaving as an ideal solute but the same does not seem to apply to the other members of the series.

*Biological relevance*

The *n*-alcohols have been added to a variety of biological membrane systems. The results of these investigations have been summarised in a recent review by Fourcans and Jain (22) and have included such diverse systems as motility of *Paramecium*, gut contractility of guinea pig, β-galactoside permease of *Escherichia coli* and membrane expansion and $Ca^{2+}$ binding by erythrocytes. In these investigations the effects of $C_1$-$C_8$ alcohols were studied, occasionally $C_{10}$ was also examined. In all the systems which were affected by a series of *n*-alcohols, a correlation was observed between the ability of the alcohols to modify lipid-lipid interactions and their effect on a given function. When the effects of *n*-alcohols on the uptake of *O*-nitrophenyl-galactose by lactose permease of *E. coli* at 0 °C were studied, the ability to stimulate uptake reached a maximum for nonanol and was considerably less for decanol. This was attributed to smaller perturbation in the lipid chains on intercalation of the longer chain alcohol. The work reported here is consistent with this observation. Our results suggest that still longer alcohols would have an even smaller stimulatory effect. If one could incorporate long chain alcohols into biological membranes one would expect them to have a rigidifying effect equivalent to lowering the temperature of the membrane.

Similar studies do not appear to have been carried out using unionised medium chain length fatty acids ($C_6$-$C_{10}$), although the present work would indicate that unionised $C_8$ and $C_{10}$ fatty acids should perturb lipid-lipid interactions to a similar extent as the respective alcohols. Possible reasons are that at pH values close to 7 both ionised and unionised species will be present and this could complicate matters because ionised and unionised forms will probably have different effects. Secondly, in many organisms the levels of free fatty acids are very low as these organisms metabolise fatty acids rapidly or convert them to other forms such as triglycerides. Nevertheless, one might expect that in circumstances where the local concentration of free fatty acids of medium ($C_6$-$C_{10}$) and longer ($C_{12}$-$C_{18}$) lengths became high, serious disturbances of physiological function could occur. Two critical areas of the mammalian body where high concentrations of free fatty acids could have serious consequences are the heart and the brain.

The pharmacological properties of the alkyl trimethylammonium halides have been widely studied, [23, 24] and it has been observed that there is an optimal chain length for pharmacological activity. Jacob's [23] work on the ganglionic stimulant activity of the series $CH_3(CH_2)_n N^+Me_3I^-$ using dog blood pressure as assay revealed a maximum for $n = 4,5$ and negligible activity for $n = 7,8$. A possible explanation for these results is that above a certain chain length the molecules may be so strongly oriented by the hydrocarbon chains in the bilayer that their interaction with the active site may be hindered. Conversely for molecules such as $C_{16}$ acyl CoA, which possess a very polar terminal group, inhibition of the mitochrondrial diphosphate carrier may require orientation of the molecule by lipid hydrocarbon chains of the membrane for correct interaction with the site of inhibition. Molecules of shorter chain length will not be oriented so well and so will not inhibit the enzyme [24].

ACKNOWLEDGEMENTS

We wish to acknowledge with gratitude financial support for this research from the Ministry of Defence and the Nuffield Foundation. Part of this research was carried out whilst A. W. E. was in receipt of a CASE Science Research Council postgraduate award with Reckitt and Colman Limited and D.C. was a Senior Wellcome Trust Research Fellow. We wish to thank Professor N. B. Chapman (Hull University) and Professor H. J. V. Tyrrell (Chelsea College) for laboratory facilities.

REFERENCES

1 Chapman, D., Williams, R. M. and Ladbrooke, B. D. (1967) Chem. Phys. Lipids 1, 445–475
2 Chapman, D., Urbina, J. and Keough, K. M. (1974) J. Biol. Chem. 249, 2512–2521
3 Trudell, J. R., Payan, D. G., Chin, J. H. and Cohen, E. N. (1975) Proc. Natl. Acad. Sci. U.S. 72, 210
4 Cater, B. R., Chapman, D., Hawes, S. M. and Saville, J. (1974) Biochim. Biophys. Acta 363, 54–69
5 Jain, M. K., Wu, Y-M. N. and Wray, L. V. (1975) Nature, 255, 494–495
6 Hill, M. W. (1974) Biochim. Biophys. Acta 356, 117–124
7 Oldfield, E. and Chapman, D. (1972) FEBS Lett. 23, 285–297
8 Bitman, R. and Blau, L. (1972) Biochemistry 11, 4831–4839
9 de Gier, J., Mandersloot, J. G. and van Deenen, L. L. M. (1968) Biochim. Biophys. Acta 150, 666–675
10 Chapman, D. (1975) Quart. Rev. Biophys. 8, 185–236
11 Trauble, H. and Haynes, D. H. (1971) Chem. Phys. Lipids, 7, 324–335
12 Ladbrooke, B. D. and Chapman, D. (1969) Chem. Phys. Lipids, 3, 304–367
13 Hinz, H-J. and Sturtevant, J. (1972) J. Biol. Chem. 247, 6071–6075
14 Rand, P., Chapman, D. and Latsson, L. (1975) Biophys. J. 15, 1117–1124
15 Tardieu, A., Luzzati, V. and Reman, F. C. (1973) J. Mol. Biol. 75, 711–733
16 Papahadjopoulos, D., Jacobson, K., Poste, G. and Shepherd, C. (1975) Biochim. Biophys. Acta 394, 504–519
17 Papahadjopoulos, D., Moscarello, M., Eylar, E. H. and Isac, T. (1975) Biochim. Biophys. Acta 401, 317–335
18 Jacobson, K., Wenner, C. E. and Kemp, G. (1975) Cancer Res. 35, 2991–2995
19 Shimshik, E. J. and McConnell, H. M. (1973) Biochemistry 12, 2351–2360
20 Jacobs, R. E., Hudson, B. and Anderson, H. C. (1975) Proc. Natl. Acad. Sci. U.S. 72, 3993–3997
21 Hui, F. K. and Barton, P. G. (1973) Biochim. Biophys. Acta 296, 510–517
22 Fourcans, B. and Jain, M. K. (1974) Adv. Lipid Res. 12, 148–226
23 Jacob, J., Marszlak, I., Epsztein, R., Olomucki, M. and Guermont, J. P. (1953) Compt. Rend. 237, 1289–1295
24 Craig, L. E. (1948) Chem. Rev. 48, 285–410
25 Devaux, P. F., Bienvenue, A., Laugin, G., Brisson, A. D., Vignais, P. M. and Vignais, P. V. (1975) Biochemistry 14, 1272–1280