Biomembrane Phase Transitions

STUDIES OF LIPID-WATER SYSTEMS USING DIFFERENTIAL SCANNING CALORIMETRY*

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SUMMARY

Differential scanning calorimetry has been applied to the study of some simple model biomembranes, i.e. lipid-water systems, and the endothermic phase transitions which they exhibit when transforming from the gel to liquid crystalline phase have been studied.

These studies show that:

1. When lipids of the same class, e.g. lecithins with dissimilar chain lengths C₁₄ and C₁₆ are mixed, a continuous series of solid solutions are formed below the Tc line and co-crystallization occurs. No great change in the range of the thermal transition occurs as a result of the mixing.

2. When lipids of different classes, i.e. lecithin and phosphatidylethanolamine classes but of the same chain length, are mixed, a considerable increase in transition range occurs. Clusters of gel and liquid crystalline lipids can coexist within this temperature range.

3. Small amounts of 1,2-dimyristoyl phosphatidylethanolamine present in a lecithin bilayer cause pronounced effects associated with the head group transition of the lecithin. This appears to be an example of a more general effect, i.e. the presence of small amounts of a “foreign molecule” in a membrane surface can sometimes affect the packing of the polar groups of many other lipid molecules in the surface.

4. Interactions of divalent cations with the polar groups of the lipids raise the gel to liquid crystalline transition temperature. This behavior parallels the properties observed in monolayer systems.

5. Electrostatic binding of proteins and basic polypeptides to the (anionic) polar groups of phospholipids shifts the temperature of the gel-liquid crystal transition of the lipid and also affects the energy barrier for reorientation of a spin label in the hydrocarbon phase. This behavior may be relevant to the interpretation of thermal transitions observed with some biomembranes.

6. Admixture of the ionophore gramicidin A with dipalmitoyl lecithin at low concentrations affects the lipid polar group packing and at higher concentrations causes a marked decrease in the energy associated with the lipid endothermic phase transition. This effect may occur because of intercalation of gramicidin A molecules among the lipid chains.

In previous studies Chapman and co-workers (1–3) have shown by means of the techniques of differential thermal analysis and differential scanning calorimetry the existence of thermotropic phase transitions with simple liquid systems when they are present in the anhydrous form (1, 2) and also in aqueous systems (3). Heating curves reveal the existence of marked endothermic transitions which, using a range of physical techniques, have been shown to be associated with “melting” of the lipid chains (2). Chapman and Salsbury (4) were the first to show, using NMR spectroscopy, that in the liquid crystalline phase there is a distribution of motion along the lipid chain, i.e. translational and rotational movements of the methylene groups of the lipid chains occur which are particularly marked at the methyl end.

The temperatures at which these endothermic phase transitions occur have been shown to be dependent upon the head group, the hydrocarbon chain length, and the degree and type of unsaturation present (5). The presence of cholesterol at sufficient relative concentration has the effect of removing these phase transitions (6, 7). Those biomembranes which contain large amounts of cholesterol, e.g. myelin membranes and also their total lipid extract, do not show these marked endothermic transitions except after some dehydration (8). Some biomembranes and total lipid extracts, which contain little or no cholesterol, however, show similar endothermic transitions to those observed with the simple lipid systems (9–11).

Only a few thermal studies of membranes or total lipid extracts from membranes (12) or on simple lipid mixtures in model systems (13, 14) have to date been carried out. Some, such as mitochondrial membranes, show a phase transition which is complete before the growth temperature is reached, (10) whilst...
others, such as *Acholeplasma laidlawii* membranes (9, 15), show transitions which are not complete until temperatures much higher than the growth temperature. Membranes and lipid extracts of membranes in water generally show very broad transitions (8, 9, 12, 15).

It is important to understand the parameters which contribute to these transitions in membranes and the ways in which these transitions can be shifted (19–18). We have, therefore, undertaken the calorimetric study of some model membrane lipid-water systems which may be useful for this purpose.

In this paper we examine the following situations.

1. The mixing behavior of simple lipid components of the type found in biomembranes. This is to enable us to see how different lipid classes or differing chain lengths of the same class mutually affect each other, both with regard to the packing of the polar groups and also of the lipid chains.

2. The effects of electrostatic interaction of ions, polypeptides, and proteins with the polar group of lipids to see how this affects lipid phase transitions. This should shed additional light on the interpretation of the heating curves observed with some biomembranes (11) and is relevant to trigger mechanisms involving metal ions and amino acids.

3. The effect of the ionophore polypeptide gramicidin A on the phase transition of dipalmitoyl lecithin. This is to enable us to see the effect of a polypeptide molecule which is involved in a "hydrophobic interaction" with the lipid.

**MATERIALS AND METHODS**

1,2-Dipalmitoyl lecithin and 1,2-dimyristoyl phosphatidyl ethanolamine were products of Fluka (Buochs). They were purified by column chromatography. 1,2-dimyristoyl lecithin was obtained from Koch-Light Ltd.

Phosphatidylethanolamine, from ox brain, grade 1 (mannoside salt) was purchased from Lipid Products Ltd., when necessary, it was further purified using the method of Long et al. (19). Salt-free cytochrome c (ferri form) was a product of C. F. Boehringer and Sohne GmbH, Germany. Crystalline lysozyme, crystalline bovine serum albumin and low molecular weight polylysine (PM-800) were purchased from British Drug Houses (BDH) Chemicals Ltd. High molecular weight (PM-100,000) polylysine was a Koch-Light product. All other reagents were of analytical reagent grade.

*Acholeplasma laidlawii* B phospholipids were obtained from freeze-dried membranes (the growth of the organism was carried out in tryptose medium). The separation and purification of the membranes was carried out according to Razin et al. (20); the membranes were extracted three times with chloroformmethanol (2:1). The total lipids were fractionated using silicic acid column chromatography (21).

The lipid-metal ion complexes were prepared by taking the pure solid dipalmitoyl lecithin and mixing it with an equal weight of a nonbuffered water solution of uranyl acetate containing the correct amount of the salt to make the desired proportion of uranyl equivalents to phosphate groups. The mixture was brought to ~50°C and shaken in a whirlmixer. This procedure was repeated several times to ensure that the interactions were complete and the samples then transferred to the calorimeter pans. The interaction was monitored by following the change in the infrared spectra of the complex.

The lipid-protein complexes were prepared as follows. The lipid was precipitated as a thin film from a chloroform-methanol solution by eliminating the solvent with a nitrogen stream; final solvent residues were eliminated under vacuum for several hours. The lipid residue was then suspended in a volume of 10 mg per ml of solution of the corresponding protein, sufficient to make a 1:1 w/w preparation of lipid-protein. The complex was continuously shaken during one hour under nitrogen atmosphere. The complex was finally obtained as a twice-washed pellet prepared by centrifugation at 10000 × g for 20 min. They were resuspended in distilled water for the ESR experiment, or used directly for differential scanning calorimetry.

Protein was determined by the Folin method, according to Lowry et al. (22), except for cytochrome c, which was measured spectrophotometrically. Lipids were determined by phosphorus assay, according to Bartlett (23).

Molecular mixtures of lipids were made in chloroform-methanol solutions. Solvent was removed under dry N₂ followed by vacuum desiccation for approximately one hour, and glass distilled water was added to 50% of the total weight. Samples were heated to approximately 15°C above the gel to liquid crystalline transition temperature of the highest melting component, and the lipid-water mixtures were dispersed using a vortex mixer. Known amounts of sample were weighed into Perkin-Elmer pans using a microbalance.

Pans were sealed and the samples analyzed using a PerkinElmer DSC1-B calorimeter operating in the low temperature mode with liquid N₂ as the coolant. Recrystallized fatty acids were used to standardize the apparatus for temperature and quantitative heat determinations. Heating and cooling rates of 8°C per min were generally used. All runs were repeated on at least two to four separate samples. Peak areas were measured using a Planimeter.

The interpretation of differential scanning calorimetry curve has been discussed in previous papers (12, 13). In infrared spectroscopic experiments, lipid-water mixtures, and lipid-protein complexes were prepared by introducing the N-ethyl-4,4'-dimethoxyazolidine derivatives of ketogenic acid methylester (12NS), prepared according to Kenna et al. (24). The molar proportions of spin label to lipid were always 1:100, corresponding to a proportion of normal hydrocarbon chains and to chains containing the nitroxide side group 1:200. The introduction of this spin label did not affect the thermotropic mesomorphism of the lipids. The ESR experiments were carried out in a Varian E.3 X-band spectrometer operating at 9.15 GHz and at a field of 3240 gauss, equipped with a variable temperature probe which allowed the temperature of the sample to be adjusted to an accuracy of ±0.5°C.

For the study of the activation energy for the movement of spin probe in the lipids and lipid-protein complexes, the experimental values of (log) correlation times against T⁻¹ were fitted by the linear regression program 70803 of a Hewlett Packard model 9100B Calculator. Correlation times were obtained from the spectra, according to Wagliotto et al. (25).

Infrared spectroscopic studies were carried out with a PerkinElmer 257 grating infrared spectrophotometer. Pure lipids were in the form of a thin film prepared in chloroform-MeOH solutions evaporating the solvent over NaCl plates under nitrogen atmosphere. Lipid complexes were prepared as water suspensions, layered over AgCl plates and allowed to dry in the air.

**RESULTS**

The differential scanning calorimeter curve for a pure simple lecithin-water system (1, 2) (dipalmitoyl lecithin) is shown in Fig. 1. This shows the "pretransitional" peak (1.6 Cal per mole) and the main endotherm (8.7 Cal per mole). This is similar to our previous results obtained with a series of lecithin-water systems (3, 16).

**Mixing Characteristics of Lipid Phase Behavior**—The differential scanning heating curves of a number of lecithin-water mixtures of 1,2-dimyristoyl - 1,2-dipalmitoyl lecithin water systems are shown in Fig. 2. All heating runs were started at temperatures of ~25°C or below. Cooling runs were taken to below the water freezing exotherm. (The water peaks are not included in the diagram.) As the proportion of 1,2-dipalmitoyl lecithin in the mixtures is increased, the transitions shift to higher temperature, and slight broadening of the peaks occurs as the proportion of lipids tends toward 1:1. The transition endotherms remain relatively symmetrical throughout the whole range. Similar behavior was observed with the exotherms on the cooling runs. The onset temperatures of the
FIG. 1. Differential scanning calorimetry heating curve of 1,2-dipalmitoyl lecithin (50:50 mixtures)-water system.

main transitions from both heating and cooling runs, and similarly the temperatures of fastest melting and solidification, show only very slight deviations from linearity, suggesting that almost ideal mixing of the lipid components occurs at all concentrations. The range or widths of the main transition endotherms indicate that even in 1:1 lipid mixtures the range of the melting process is only increased by approximately 4° over that of the sharpest melting pure component.

The phase diagram of the mixed chain length lecithin-water system is shown in Fig. 3a, and it can be seen that with any mixture there exists only a small temperature range over which the hydrocarbon chains are present in coexisting gel and liquid

FIG. 2. Differential scanning calorimetry heating curves of 1,2-dimyristoyl lecithin-1,2-dipalmitoyl lecithin-water mixtures (lipid-water = 50:50).

FIG. 3. a, phase diagram of 1,2-dimyristoyl-1,2-dipalmitoyl lecithin-water mixtures showing $T_c$ heating and $T_c$ cooling temperatures. b, the heats of transition for 1,2-dimyristoyl-1,2-dipalmitoyl lecithin-water mixtures. Main transition heating run.
crystalline forms. This result is similar to that obtained earlier for 1,2-dipalmitoyl-diestearoyl lecithin-water mixtures (13). One point of note in the phase diagram is that the onset temperatures obtained from the heating and cooling runs for the two pure components do not coincide. Those from the cooling runs are higher than those of the heating runs. The difference in onset temperatures does not display any straightforward relationship with programming rate. Even when programming rates of 16° per min were used, onset temperatures were consistently higher on cooling runs. The differences tend to become smaller with slower programming rates but even at the lowest rate possible with the instrument (0.5° per min) a substantial difference remained (1–3°). Difficulty in establishing thermal equilibrium in the samples because of their size and geometry may be the explanation for this behavior.

The heats of the main transition are shown in Fig. 3b. (The vertical bars on this and all other graphs are not error bars but represent the absolute values measured.) The difficulty of determining the exact point of departure from the base-line of the endo- and exotherms has been previously pointed out (12). With the mixed lecithin systems the heats of the pretransitional peaks have to be considered. The shapes of the curves indicate that the maximum heats of the main transitions are reached when approximately 50 to 60% of the lipid mixture is dipalmitoyl lecithin.

In Fig. 4 the differential scanning curves of the heating runs of 1,2-dimyristoyl lecithin-1,2-dimyristoyl phosphatidylethanolamine-water mixtures are presented. (Here the chain lengths are the same but the polar groups differ.) Both sets of these onset temperatures show considerably more curvature than those obtained with the simple lecithin-water mixtures, with the effect being pronounced in the heating runs, Fig. 5a. The plots of temperatures of fastest melting and freezing are markedly sigmoidal, a reflection of the asymmetry in the endo- and exo-
Lipid-metal Ion Complexes—The interaction of different divalent cations with 1,2-dipalmitoyl lecithin and ox brain phosphatidylserine has very strong effects on the thermotropic mesomorphism of both lipids. Fig. 6 shows thermograms corresponding to a titration of 1,2-dipalmitoyl lecithin with UO$_2^{2+}$ monitored by the effect of this interaction on the transition temperature of the lipid. The original transition temperature is at 41.5$^\circ$, which is finally shifted in the 1:1 complex to 46$^\circ$. With ox brain phosphatidylserine the binding of Ca$^{2+}$ at 1:1 proportion, as shown in Fig. 7, shifts the transition from 17$^\circ$ to 22$^\circ$. The 1:1 complex with UO$_2^{2+}$ moves the transition to 40$^\circ$. A comparison of the infrared spectrum of egg-lecithin and the UO$_2^{2+}$ lecithin complex shows that the strong binding of this ion is specific to the phosphate groups. This is shown in Fig. 8 where the interaction of the UO$_2^{2+}$ shifts the characteristic $\nu = O$ stretching band of lecithin at 1250 cm$^{-1}$ to 1170 cm$^{-1}$. The same shift was observed with UO$_2^{2+}$-phosphatidylserine complex.

Lipid-protein Complexes—The gel-liquid crystalline transition temperature of Acholeplasma phospholipids is lowered as a consequence of the ionic interaction with protein. The differential scanning heating curve of this lipid in water and its lipid complex with cytochrome $c$ are shown in Fig. 9. The complex shows a single reversible transition, characteristically broad and centered around 55$^\circ$, attributable to denaturation of the cytochrome $c$.

Similar effects are also observed with other lipids and lipid-protein complexes. In Fig. 10 a series of calorimetric scans of a natural ionic phospholipid is presented, phosphatidylserine (PS) from ox brain, and its complexes with several basic proteins; cytochrome $c$, lysozyme, and a positively charged polypeptide, polylysine (low molecular weight 8000). The interaction with the basic proteins shifts the transition down by 7$^\circ$. The polylysine lowers the transition by 3$^\circ$. High molecular weight polylysine (100,000) lowers the transition by 5$^\circ$. The results of the spin label study shown in Fig. 11 show that a logarithmic plot of the experimental correlation times against $1/t$ is linear. This allows the calculation of an “activation energy” related to the energy barrier encountered by the probe molecule for reorientation in the hydrocarbon part of the lipid.
Fig. 9. Differential scanning calorimetry heating curves of: A, Acholeplasma laidlawii B phospholipids-water = 40:60 (312.5 K = 39.5°C); B, Acholeplasma phospholipids-cytochrome c complex-water = 40:60 (304 K = 31°C). The marks in the temperature scale indicate the center of the transition, defined as the temperature of the maximum rate of heat absorption. Proportions expressed in weight by weight.

Fig. 10. Differential scanning calorimetry heating curves of: A, phosphatidylserine-water = 50:50 (301 K = 28°C); B, phosphatidylserine-cytochrome c complex-water = 50:50 (294 K = 21°C); C, phosphatidylserine-lysozyme complex-water = 50:50 (294.5 K = 21.5°C); D, phosphatidylserine-polylysine complex-water = 50:50 (298.5 K = 25°C).

The values obtained from four separate experiments are 5.75 ± 0.25 Cal per mole for the system phosphatidylserine in water, and 4.05 ± 0.50 Cal per mole for the complex phosphatidylserine-lysozyme in water. Although there was some variability in the value of the activation energy with the lipid-protein (presumably as a consequence of small variations in the process of formation of the complex) the logarithmic plots for each separate experiment were linear with a regression coefficient $R = 0.994$.

Gramicidin A and Lipid Phase Transitions—The pretransitional endotherm of 1,2-dipalmitoyl lecithin is removed by gramicidin A even at the lowest concentration examined (1 mole of gramicidin A to 200 moles of lipid). At higher concentrations the peak maximum shifts a few degrees to lower temperatures and the energy of the main transition endotherm is lowered markedly, see Fig. 12, a and b.

DISCUSSION

Many membranes contain mixtures of lipid classes as well as associated fatty acids of various chain lengths. The lecithins are often the most predominant of the lipids present in animal membranes (5). The lecithin-water system used in the present experiments is known to consist of a lipid bilayer over a wide range of concentration in water (3).

Previous studies of pure phospholipids have delineated their thermal behavior pointing to the quasi-crystalline nature of the lipid chains below the transition temperature in the so-called gel condition, and their more fluid nature in the liquid crystalline condition above the main transition temperature (1-4).

Quantitative estimates of the heats involved in these phase transitions and the related entropy changes have been discussed (16). Volume changes associated with the transition have also been discussed (26). Theoretical studies relevant to molecular motion of chains in the hexagonal phase have been reported (27). Theoretical studies have also been reported by Whittington and Chapman (28) on the nature of the phase change from the gel to liquid crystalline form and of the motion of the chains involved. This study emphasized the cooperative nature of the twisting and movement of the CH2 groups of adjacent chains in the liquid crystalline phase.

More recent studies have been reported by other workers which also emphasize the cooperative nature of these chain movements (29, 30). Membrane permeability is known to differ below and above the phase transition temperature (31).

The lower transition or pretransitional peak (see Fig. 1) has been associated by Ladbrooke and Chapman (12) with an increase in mobility of the polar head portion of the lipid. This is supported by NMR spectroscopic studies showing that modification of motion of the polar group occurs prior to the main endothermic transition (32, 33). Veksl et al. (32) concluded that the first 4 to 5 molecules of water form a “primary” hydration layer which has a major effect in loosening the polar group lattice. A modification or rearrangement of the water associa-
is not observed with the phosphatidylethanolamine lipids. This transition peak. A change of chain tilt may occur from the orthorhombic phase at temperatures below the pre-transition temperature almost 30° higher than the corresponding lecithin (see Fig. 1 and Fig. 4). The most straightforward explanation of this appears to be that the bulkier trimethylammonium group and the associated water structure of lecithin prevents the lipid chains from packing as effectively as can occur with the phosphatidylethanolamine lipid. This thermotropic behavior has its parallel in the monolayer behavior of these compounds. Thus, monolayers of phosphatidylethanolamine molecules are much more condensed than are monolayers of corresponding phosphatidylcholines with the same hydrocarbon chains (37).

The condensation of monolayers of long chain acids with an increase of the pH of the subphase is another example of this effect of polar group packing affecting lipid chain packing (38).

**Mixture Characteristics of Lipids—Pure lipids, particularly saturated ones, in excess water give relatively sharp transitions which extend only a few degrees (3, 16, 35) corresponding to a highly cooperative transition. This is the case even with a lipid containing different fatty acid chains, e.g. stearoyl and elaidoyl chains (13). Mixtures of lipids containing a range of unsaturated fatty acid chains (egg yolk lecithin) show, however, a wider range of transition. The mixture of 1,2-dimyristoyl lecithin and 1,2-dipalmitoyl lecithin which we have examined in water, co-crystallise below Tc to give a series of solid solutions (Fig. 3a). This is similar to the behavior of mixtures of 1,2-dimyristoyl and 1,2-distearoyl lecithin which we have previously examined (13). In both cases ideal mixing of the two lipids is occurring and monolayer studies are consistent with this. When the difference of chain length between lecithins is increased to four methylene groups, monotectic behavior without solid solution becomes apparent (13). Thus, whilst the lattice arrangement can accommodate small chain length differences so that co-crystallization occurs; with greater differences, as the system is cooled, migration of lecithin molecules within a given bilayer occurs to give regions corresponding to the two separate components. Mixtures in excess water of dioleoyl lecithin with a number of long chain saturated lecithins also exhibit monotectic behavior with two well defined transitions corresponding to the phase changes of the individual components (13). These properties are important for the interpretation of the behavior of cell membrane systems where two different fatty acids are incorporated biosynthetically.

The mixtures of two different lipid classes of the same chain length, the 1,2-dimyristoyl lecithin-1,2-dimyristoyl phospho-

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2 K. Larsson, private communication.
tidylyethanolamine mixtures, show very different melting behavior than the simple lecithin mixtures. The first point to note is that the presence of only 5% dimyristoyl phosphatidylethanolamine within the lecithin bilayer causes the pretransition endotherm of the lecithin to be substantially broadened, and to disappear entirely at amounts of about 10%. This shows that the admixture of relatively small amounts of foreign material (even of related lipids) into the head group region can affect the packing and hence the long range order of the polar group organization of the lipid. Similar effects are observed when cholesterol is included in lecithin bilayer systems and is also observed with gramicidin A. Such behavior may be of general biological significance, and underlie the way in which important signals can be transmitted from one part of the membrane surface to another.

The asymmetry and broadening which occurs at higher concentration of phosphatidylethanolamine (see Fig. 4) is particularly interesting. It shows that clusters of lipids in a gel and liquid crystalline forms can coexist within this transition range. The transition is of lower cooperativity than occurs with the individual components (see the reduction of enthalpy involved Fig. 5). Some clustering of the different lipid classes may be occurring.

Lipid-metal Ion Complexes—The effect reported here of divalent metal ions on the thermotropic mesomorphism of lecithin and phosphatidylserine lipids is a further confirmation of the influence of the polar group of the lipid on the organization of the hydrocarbon chains. The increase of the lipid transition temperature as a consequence of the interaction of lecithin with UO$_2^{2+}$ and of phosphatidylserine with Ca$^{2+}$, Mg$^{2+}$, and UO$_2^{2+}$ means that at a given temperature the interaction causes an increase of hydrocarbon chain packing and a decrease in permeability characteristics. This result is consistent with early studies of metal ion interactions with lipid systems and supports the correlation of bulk properties and monolayer properties of phospholipid systems (37).

Early studies of stearic acid monolayers (38, 39) showed that Ca$^{2+}$ increased surface pressure (condensation) and decreased the permeability to water (40). Many similar observations have been made with phospholipid systems.

Rojas and Tobias (41) observed that Ca$^{2+}$, but not Na$^+$ or K$^+$, produced condensation of phosphatidylserine monolayers. Later more extensive studies by Papahadjopoulos (42) showed that a variety of acidic phospholipids showed increase in surface potential and decrease in surface pressure in the presence of Ca$^{2+}$ and other bivalent cations. The order of cation effectiveness for both systems is Ca$^{2+}$ > Ba$^{2+}$ > Mg$^{2+}$ with phosphatidylserine showing more selectivity than phosphatidic acid. Papa hadjopoulos (42) proposed the formation of linear polymeric complexes to account for these findings.

These results are also supported by the technique of spin labeling as an index of molecular order of stacked lipid (beef brain) bilayers (43). It has been found that the degree of anisotropy of the cholestane nitroxide spin label and, hence, the extent of ordering (density of packing) of the phospholipid molecules is dependent upon the cation, Ca$^{2+}$ being very much more effective than univalent cations and La$^{3+}$ and Th$^{4+}$ being progressively more effective than Ca$^{2+}$.

Phosphatidylserine bilayers (black lipid membranes) formed in the presence of Ca$^{2+}$ are more stable and have a higher electrical resistance than those formed in the presence of Na$^+$ only (44, 45).

Bungenburg de Jong (46) and Blaustein (47) have studied the effects of a group of polyvalent cations on the binding of Ca$^{2+}$ to phosphatidylserine by determining their activities in inhibiting Ca$^{2+}$ transfer from an aqueous to an organic phase, La$^{3+}$, Al$^{3+}$, and Ce$^{3+}$ were found to be highly effective. Barton (48) determined the concentrations of cationic species to produce charge reversal in dispersions of phosphatidylserine and determined association constants. Good agreement was found between these values and the values determined by Blaustein for the inhibition of Ca$^{2+}$ transfer with the notable exception of the uranyl cation (UO$_2^{2+}$). According to Barton (48) the high affinity of UO$_2^{2+}$ for the bulk phosphatidylserine surface could be related to the contribution of lattice energy derived from the formation of an allotriomeric-like crystal structure. This is interesting in view of the fact that, in our experiments, UO$_2^{2+}$ binds to the phosphate group of lecithin. The much stronger effects on phosphatidylserine than occurs with other divalent ions probably correspond to the partially covalent character of the UO$_2^{2+}$ coordination bonds. The observation of two transitions in the titration of dipalmitoyl lecithin with the UO$_2^{2+}$ ions (see Fig. 6) is indicative of the formation of clusters, one containing unaffected lipid and the other containing the lipid-metal ion complex.

From monolayer and liposomes experiments uranyl ions UO$_2^{2+}$ are known to interact stoichiometrically with the polar groups of lecithin (40, 50) and to bind in the membrane surface of different biological membranes, presumably to phosphate groups blocking the transport of sodium and glucose through the membrane (51).

The manner in which electrostatic interactions of metal ions with the polar groups affect the hydrocarbon chain fluidity of lipids may be important for a number of biological situations, particularly where transient effects occur in biological membranes. A substantial amount of information derived from membranes and membrane models indicates that the binding to and displacement from the cell membrane of calcium constitutes a determinant step in the control of membrane potential, permeability, stability, etc. and for the action of membrane-bound enzymes present as lipoprotein complexes (52).

Lipid-protein Complexes and Lipid-polypeptide Interactions—The type of lipid-protein model system which we have studied in this work has also been studied by several authors (53–55) using x-ray diffraction. They consist of lipid bilayers with intercalated protein-water layers interacting with the polar group of the lipid. It was shown, by consideration of the thickness of the protein layer in the different lipid-protein phases and by the criterion of circular dichroism, that the protein largely retains the solution conformation and that the main interactions consist of electrostatic attraction between the charged groups of the lipid and the protein. The strongly shifted thermal transition of the lipid (to lower temperatures) is probably related to the reorganization of the polar groups of the phospholipid by the electrostatic interaction with the charged groups in fixed positions in the globular proteins or polypeptides. This reorganization leads to less efficient packing of the lipid chains, resulting in a lower thermal transition temperature. This shift of lipid transition temperature is also confirmed by the electron spin resonance experiments which show that the energy barrier encountered by the spin label for reorientation in the hydrocarbon phase is lower when the label is in the lipid-protein complex compared with when the label is in the lipid alone.

The "electrostatic" effect of polypeptides and proteins on lipid fluidity may also be important in transient and structural situations. It is clearly relevant where comparisons are made between transitions which occur with membranes where electro-
static interaction of lipid and protein is thought to occur, compared with those of the extracted lipids.

This effect on lipid fluidity by interaction of protein with the polar groups of lipids may also occur in other important systems, such as the concanavalin A-glycolipid system and antigen-antibody interactions and could be of considerable biological importance.

Gramicidin A is an ionophore which is thought to transport ions by means of a pore mechanism (56, 57). With this molecule, the pretransitional peak is affected at low polypeptide concentrations, suggesting that the packing of the lecithin polar groups has been affected, but in addition to this the heat involved in the main lipid endothermic transition is markedly reduced in a somewhat similar manner to that observed with cholesterol. It seems reasonable to conclude, therefore, that as with cholesterol the molecule is interdigitated among the lipid chains preventing chain crystallization from occurring. Consistent with this it has been postulated that gramicidin A forms channels bridging the lipid bilayers (56, 57). This result is also interesting in view of results on black lipid membrane systems. Krisne et al. (58) have shown that gramicidin A was able to mediate potassium ion transport above and below the transition temperature of the lipid forming a "black lipid film." It is possible that whilst the bulk of the lipid below the transition temperature was rigid, that the lipid immediately adjacent to the gramicidin A was fluid. Previous studies of antibiotics show that chlorothrin and polymixin B also remove the phase transition of a saturated lecithin 1,2-dipalmitoyl lecithin water system (17, 18).

Interpretation of Thermal Transitions in Biomembranes—We have in an earlier paper (11) commented upon the various assumptions underpinning interpretation of the thermal transition of biomembranes. In the case of the Acholeplasma laidlawii membranes we have interpreted such wide curves encompassing the growth temperature (9, 11) as indicating the presence of both fluid and rigid lipid regions at this temperature (59). This conclusion has been given further support by NMR studies (60). This raises a number of important questions which require further study concerning the protein distribution in this membrane. e.g. is the protein preferentially associated with the fluid lipid region or the rigid lipid region or with both?

A further point of interest is that the thermal transition of the lipid of Acholeplasma laidlawii membrane occurs at the same temperature as the isolated lipid (9). As our work shows that polar group interactions can affect lipid transition temperatures, the simplest interpretation of these results is that most of the lipids of the Acholeplasma laidlawii membranes do not interact via the polar groups with protein. This does not, of course, rule out the idea that the smaller phospholipid fraction present in the Acholeplasma laidlawii membrane interacts electrostatically with protein, but that the effect of this is not observed in the melting energy contribution from the major lipid material present.

With mitochondrial membrane the fact (10) that the transition appears to be complete before 37^oC suggests that at body temperature the lipids of this membrane are completely fluid. The small shift observed with the mitochondrial membrane compared with the extracted lipids may indicate some electrostatic interaction, perhaps with the phosphatidylethanolamine and phosphatidylserine or cardiolipin molecules present (61).

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