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PHYSICAL STUDIES OF PHOSPHOLIPIDS. VI. THERMOTROPIC AND LYOTROPIC MESOMORPHISM OF SOME 1,2-DIACYL-PHOSPHATIDYLCHOLINES (LECITHINS)

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The thermotropic and lyotropic mesomorphism of a series of saturated 1,2-diacyl-L-phosphatidylcholines (lecithins) has been studied by light microscopy, differential thermal analysis, X-ray diffraction, infrared and nuclear magnetic resonance spectroscopy. The phosphatidylcholines exhibit polymorphism and generally exist in the form of their monohydrates. Anhydrous phosphatidylcholines can be prepared by heating the monohydrates or, alternatively, by crystallisation from certain solvents. Three different liquid crystalline phases have been characterised; the higher homologues of the monohydrates exist in a cubic liquid crystalline phase within certain well defined temperatures. This cubic phase gives rise to a high resolution proton magnetic resonance spectrum showing peaks associated with the different proton groupings in the molecule.

The structures of the lyotropic phases formed by the addition of water to the phosphatidylcholines have been determined. The phase diagram of the 1,2-dipalmitoyl-L-phosphatidylcholine/water system has been determined as a function of water concentration and temperature. Calorimetric techniques show that approximately ten molecules of water are bound to each molecule of phosphatidylcholine.

Introduction

The membranes of cells are now considered to be a major basic organizational element determining both the structure of the cell and a variety of functions taking place within the cell. Therefore, at the present time, increasing attention is being given to the fundamental properties of membrane molecular structure. Phospholipids, together with protein and water, are major building units of many cell membranes. Each membrane type usually contains a variety of phospholipid classes; in most mammalian tissues the diacyl-L-phosphatidylcholines (lecithins) are the most numerous phospholipids present. Associated with each phospholipid class is a distribution of both saturated and unsaturated fatty acid residues. The unsaturated hydrocarbon chains are usually attached to the 2-position of the glycerol moiety of the lipid. In some biological tissues phospholipids are present which

contain two completely saturated chains, e.g. the major phospholipid in lung membrane is 1,2-dipalmitoyl-L-phosphatidylcholine.

During the last forty years many physical studies have been carried out on the diacylphosphatidylcholines. Attempts have been made to obtain insight into their behaviour and their interaction with water, ions, cholesterol and lipases[1]). Various types of model membranes have been made with natural lipid mixtures[2,3]). Until recently almost all of these studies have been made with material obtained from natural sources, such as egg-yolk lecithin. This material, although a single phospholipid class, contains a distribution of chain lengths and varying degrees of unsaturation among the hydrocarbon chains.

Recently, the improvement of synthetic techniques[4]) has increased the number of studies made on pure, simple phospholipids. We have recently studied, using a range of physical techniques, a series of pure synthetic diacylphosphatidylethanolamines[5]). In the present paper we discuss the physical properties of a series of pure phosphatidylcholines, using data obtained by a variety of physical methods including X-ray diffraction, thermal analysis and spectroscopic techniques. In this integrated approach we intend to provide a firm basis for the understanding of their solid state behaviour, their thermotropic and lyotropic mesomorphism, and to provide evidence to support reasonable speculation about their role in biological membranes. We also compare and contrast the physical properties of these two important phospholipid classes present in cell membranes, i.e. the diacylphosphatidylcholines and diacylphosphatidylethanolamines.

The nomenclature used for indicating the position of fatty acid residues on the glycerol moiety has been changed frequently in recent years. A recent recommendation[6]) intended to stabilize the position is to indicate the position of the phosphate group by the number 3. We have adopted this new nomenclature in the present paper. This differs from the earlier nomenclature using an α, β-notation, or the nomenclature adopted earlier[7]) by ourselves, and used in earlier papers in this series, of indicating the phosphate position by the number 1.

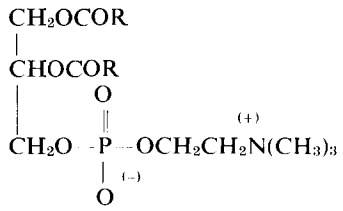
Experimental

Materials

Pure 1,2-diacyl-L-phosphatidylcholines were synthesised by a partial synthesis technique starting from pure egg-yolk lecithin using the following route:

Egg-yolk lecithin $\xrightarrow{\text{hydrolysis}}$ α-glycerylphosphorylcholine[8])
$\xrightarrow{CdCl_2}$ α-glycerylphosphorylcholine/$CdCl_2$ complex
$\xrightarrow[\text{Pyridine}]{RCOCl}$ 1,2-diacyl-L-phosphatidylcholine[9]).

The chemical formula of 1,2-diacyl-L-phosphatidylcholine is



where R is a hydrocarbon residue. Final purification was achieved chromatographically by elution with benzene/methanol (70:30) from an alumina column (Merck, neutral, Grade I). Purity was checked by means of thin-layer chromatography. Physical studies were also made using a sample of 1,2-dipalmitoyl-DL-phosphatidylcholine from Messrs. Fluka Ltd., Basle, Switzerland. Thin-layer chromatography indicated that this commercial material contained approximately 97% phosphatidylcholine. (The impurities found were lysoderivatives, palmitic acid and traces of unidentified material.)

Crystallisation of the lecithins from chloroform/methanol solution yielded two different hydrated forms (which we shall, at this stage, designate by α_1 and α_2). Precipitation from chloroform solution with diethyl ether yielded a third hydrated form(β^1). For 1,2-distearoyl-L-phosphatidylcholine we were able to obtain anhydrous crystals by very slow crystallization from dry diethyl ether/chloroform solution.

Lecithin/water mixtures were prepared by sealing weighed amounts of the components into glass ampoules, heating them to above the onset temperature for liquid crystal formation, and centrifuging each mixture many times through a narrow constriction in the centre of the ampoule. The concentrations were checked, wherever possible, by dry weight determination. All concentrations, c, are expressed by grams of lipid per gram of lipid/water mixture.

Thermal analysis

Thermal analysis was carried out using two separate instruments; a Perkin Elmer Differential Scanning Calorimeter and a Du Pont 900 Differential Thermal Analyser. The Scanning Calorimeter records the differential energy required to maintain a constant rate of change of the sample temperature and integration of the recorded peaks gives direct calorimetric data. The sample holder is an aluminium pan. The Dupont instrument is a conventional thermometric differential thermal analyser. The sample container in this case is a glass capillary tube.

Since the results obtained by both of these instruments are superficially similar, care is necessary in presenting results to maintain a distinction

between the two classes of data. This is the case in the diagrams presented in this paper. The differential scanning calorimeter (d.s.c.) curves are drawn with endothermic changes in an upward direction, while in the differential thermal analysis (d.t.a.) curves they are drawn in a downward direction. The optimum value for the transition temperature is where the trace leaves the base line on the d.s.c. recordings but corresponds to the peak maxima on the d.t.a. recordings.

Scan speeds used were 10°/min in the case of d.t.a. and 8°/min for the d.s.c. work. Lipid/water mixtures were examined in volatile sample holders which were hermetically sealed by cold welding an aluminium cover on to the sample pan. We shall discuss elsewhere[10] in more detail the procedures involved in the thermal analysis of phospholipids.

X-ray diffraction

A Rigaku-Denki low angle X-ray goniometer, operated *in vacuo* to eliminate air scattering, was used to record diffraction patterns, within the range $0.03 > \sin\theta/\lambda > 0.002$ Å^{-1}, on a photographic film. The incident X-ray beam was collimated by a slit system, and the sample to film distance used was of the order of 25 cm. The X-rays diffracted in the high angle region were recorded on a separate film placed 4.2 cm from the sample. The sample was held in a variable temperature cell. CuK_α ($\lambda = 1.5418$ Å) radiation was used.

Infrared spectroscopy

All spectra were obtained by the potassium bromide disc technique using a Grubb Parsons (GS4) double-beam grating spectrometer. The sample concentrations were 2 mg per 300 mg potassium bromide. The disc was held in a variable temperature cell allowing spectra to be obtained at temperatures between -186 °C and $+150$ °C.

Nuclear magnetic resonance spectroscopy

High resolution nuclear (proton) magnetic resonance spectra were obtained at various temperatures using a 60 Mc/s Perkin Elmer (R 10) instrument with a high temperature probe and a spinning sample tube.

Results

Microscope observations

Using a Köffler heating stage, a number of thermotropic phase transitions were observed with each phospholipid. All the lecithins are transparent, anisotropic solids, which transform at a particular temperature (T_1) to a transparent fluid anisotropic liquid-crystalline phase. At a higher tempera-

TABLE 1
Microscope observations on the 1,2-diacyl-L-phosphatidylcholines

Description of phase	Anhydrous		Monohydrate				
	distearoyl-	dipalmitoyl-	distearoyl-	dipalmitoyl-	dimyristoyl-	dilauroyl-	dicapryl-
Transition temperature T_1 (°C) Anisotropic, fluid liquid crystal	110–120	95–105	72–78	60–70	47–53	30–35	15–25
Transition temperature T_2 (°C) Isotropic, viscous liquid crystal	–	–	118	125	125	–	–
Transition temperature T_3 (°C) Anisotropic, fluid liquid crystal	–	–	149	175	180	–	–
Capillary melting point (°C) Isotropic, fluid liquid	231	230	228	229	227	200	213

ture (T_2), the higher members of the homologous series, when hydrated, exhibit a further phase change. This new phase is transparent, *viscous* and isotropic. At a higher temperature (T_3) a third transition occurs to a transparent, anisotropic liquid crystalline phase. The microscopic texture of this anisotropic phase differs from the texture of the lower temperature anisotropic phase. The final melting points are well defined and are characterized by a marked increase in the fluidity of the sample and a change from anisotropic to isotropic behaviour. The transition temperatures are listed in table 1.

The lecithin hydrates, when dried under vacuum at 90 °C for 4 hours, lose weight corresponding to approximately one molecule of water per molecule of lecithin. After this treatment they no longer exhibit the viscous isotropic phase. When the anhydrous phospholipid was placed over silica gel in a desiccator for thirty minutes, the *viscous*, isotropic phase re-occurred in about half of the sample. When the lipid has been left for one hour in the desiccator, all the lipid could exhibit this isotropic phase. (The anhydrous lecithins are found to increase in weight by absorbing water, even whilst over phosphorus pentoxide in a desiccator.) Single crystals of anhydrous 1,2-distearoyl-L-phosphatidylcholine do not exhibit such hygroscopic behaviour, but after heating to above the crystalline-liquid crystalline transition temperature (T_1), and leaving the sample to cool, hygroscopic behaviour is observed.

Various lipid/water mixtures were also examined by means of the microscope between 10 °C and 90 °C. The observations and the transition temperatures are summarised in table 2.

TABLE 2

Microscope observations on the 1,2-dipalmitoyl-L-phosphatidylcholine/water system

Concentration (*c*)	Appearance below transition temperature	T_1 (°C)	Appearance above transition temperature
0.86	one-phase, clear anisotropic, semifluid	51–53	one-phase, clear, anisotropic fluid
0.78	two-phase, cloudy, anisotropic, semifluid	40–43	–do–
0.70	–do.–	40–43	–do.–
0.60	–do.–	40–43	–do.–
0.52	–do.–	40–43	two-phase, cloudy, anisotropic, fluid
0.40	–do.–	40–43	–do.–
0.28	–do.–	40–43	–do.–

Thermal analysis (lipids)

A series of d.t.a. heating curves for the α_1 form of the lecithin monohydrates is shown in fig. 1. In each case a pronounced endothermic transition is obtained many tens of degrees below the capillary melting point of the lipid. As the hydrocarbon chain length of the fatty acid residue becomes shorter the principal endothermic transition temperature steadily decreases. The presence of *cis* unsaturated groups in the hydrocarbon chains also causes a decrease of the main endothermic transition temperature. The temperature of the main transition varies depending upon the actual water content of the sample. This is because the monohydrates are themselves hygroscopic and readily absorb more water.

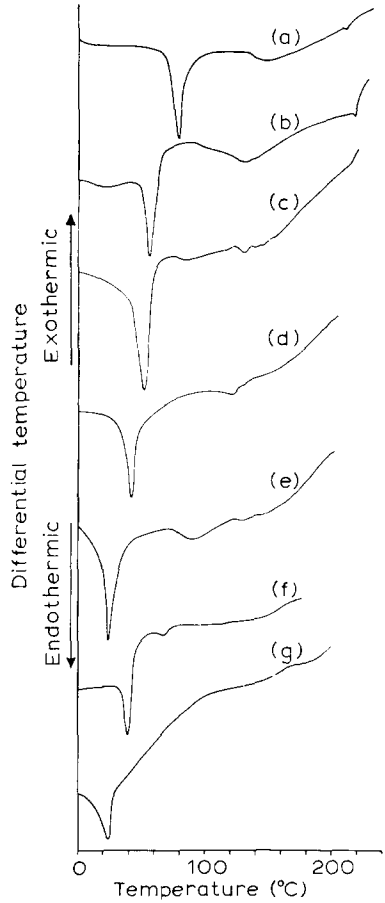


Fig. 1. D.t.a. curves of 1,2-diacyl-L-phosphatidylcholine monohydrates (α_1 form). (a) distearoyl, (b) dipalmitoyl, (c) dimyristoyl, (d) dilauroyl, (e) dicapryl, (f) 1-stearoyl-2-oleoyl, (g) egg yolk.

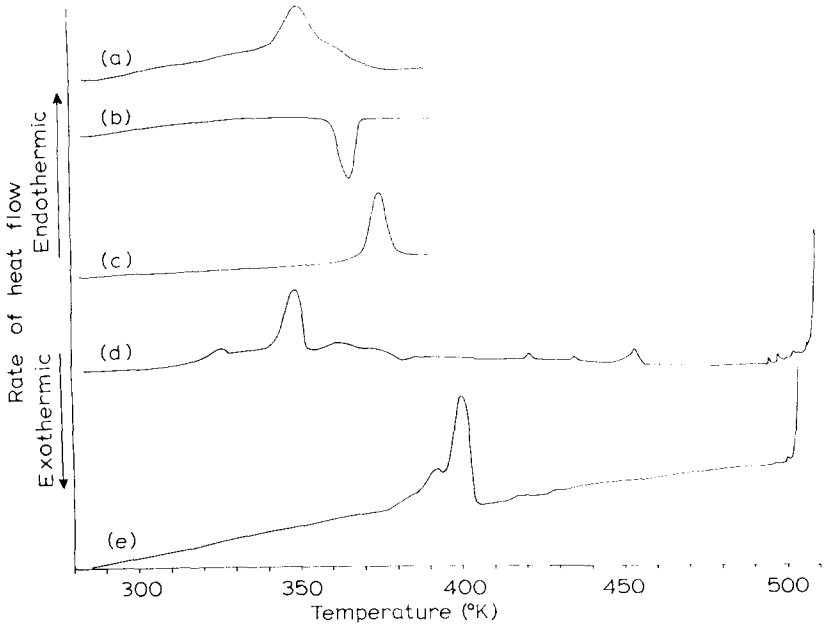


Fig. 2. D.s.c. curves for 1,2-dipalmitoyl-L-phosphatidylcholine. (a) heating, (b) cooling, (c) reheating curves for the α_1 form, (d) heating curve for the α_2 form, (e) heating curve for anhydrous, crystalline 1,2-distearoyl-L-phosphatidylcholine.

In fig. 2 are shown d.s.c. heating and cooling curves using an open pan for 1,2-dipalmitoyl-L-phosphatidylcholine. The first heating run on the α_1 form shows an endothermic transition at 65° but, on cooling, an exothermic peak is observed at 92 °C. (A weight loss of about 3%, corresponding to one molecule of water per molecule of lecithin, occurs during this temperature cycle.) On reheating the sample the endothermic transition occurs at 92 °C. If the sample is then exposed to the atmosphere there is an increase in weight and the transition temperature drops back to 65 °C. When the water content is less than that of the monohydrate both transitions occur rather than a single one at an intermediate temperature. The first heating run on the α_2 form shows an additional endothermic peak near 50 °C. After being heated once the sample behaves the same as the α_1 form. The other members of the fully saturated series behave similarly. The variation of transition temperature with chain length for anhydrous and hydrated forms is summarised in fig. 3.

The anhydrous, crystalline 1,2-distearoyl-L-phosphatidylcholine exhibits a complex endothermic transition at about 115 °C (fig. 2, curve e). If the crystalline material is heated above this temperature, cooled, and then re-

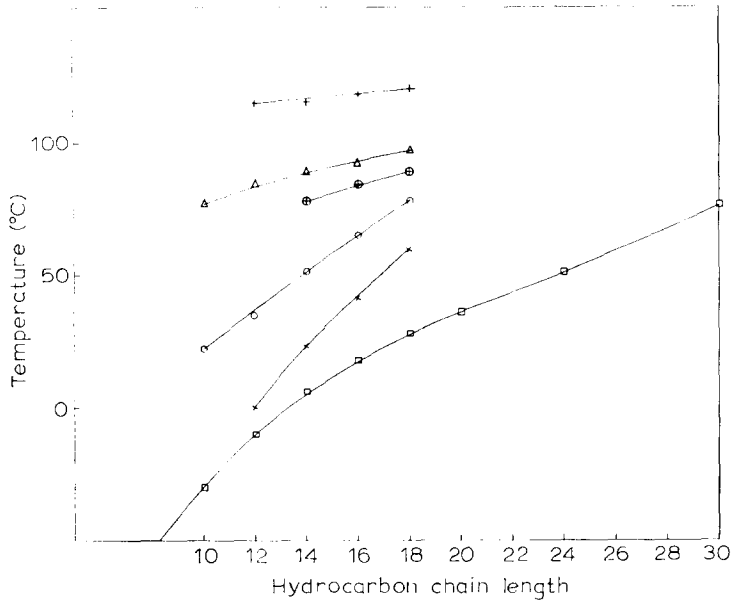


Fig. 3. Transition temperatures of various diacylphosphatidylethanolamines and diacylphosphatidylcholines, both in the anhydrous condition and also in the presence of water. The melting points of n.-paraffins of the same hydrocarbon chain length are also included for comparison. ⊦ anhydrous 1,2-diacyl-DL-phosphatidylethanolamines; ⊕ 1,2-diacyl-DL-phosphatidylethanolamines in water; △ anhydrous 1,2-diacyl-L-phosphatidylcholines; ○ 1,2-diacyl-L-phosphatidylcholine monohydrates (α_1 form); × 1,2-diacyl-L-phosphatidylcholines in water; □ melting points of normal paraffins.

heated, the transition is now found to occur at 97 °C, i.e. at the same temperature as is obtained for the α_1 form after drying. Approximate values for the heat changes involved in the thermal transitions are given in table 3.

In the region of their capillary melting points, in an atmosphere of oxygen-free nitrogen, the lecithins gradually decompose causing an unstable base line to be observed on the d.s.c. recordings. If the heating takes place in an atmosphere of helium, decomposition takes place sharply at a temperature

TABLE 3

Heats of transition for 1,2-diacyl-L-phosphatidylcholines

Acyl chain length	Anhydrous, crystalline	Anhydrous, non-crystalline	Monohydrate α_1 form	System with $c < 0.7$
C_{18}	115 °C	97 °C	78 °C	60 °C
	17.5 cal/g	8.0 cal/g	9.3 cal/g	13.5 cal/g
C_{16}	–	93 °C	65 °C	41 °C
		5.9 cal/g	6.2 cal/g	11.8 cal/g
C_{14}	–	89 °C	51 °C	23 °C
		5.0 cal/g	5.4 cal/g	9.8 cal/g

corresponding to the capillary melting point of the sample. For 1,2-dipalmitoyl-L-phosphatidylcholine, a weight loss of 11 % occurs at this temperature and a low-melting residue remains. The infrared and the n.m.r. spectra of this residue show that, as a result of the decomposition, a choline group is lost from the molecule, leaving the phosphatidic acid.

Thermal analysis (lipid/water systems)

The 1,2-dipalmitoyl-L-phosphatidylcholine/water system was studied over the concentration range $1.0 \leqslant c \leqslant 0.1$. In the range $1.0 \leqslant c \leqslant 0.8$, the first endothermic transition temperature (T_1) is observed to decrease steadily to a limiting value (T_1^*) of 41 °C. In this concentration range, despite the fact that an appreciable amount of water is present, no transition is observed in the heating or cooling curves due to any melting of ice or freezing of the water present. Over the concentration range $0.8 < c \leqslant 0.1$, the lipid endothermic transition temperature remains constant and a peak at 0 °C, due to the melting of ice, can now be observed in the heating curve. As the concen-

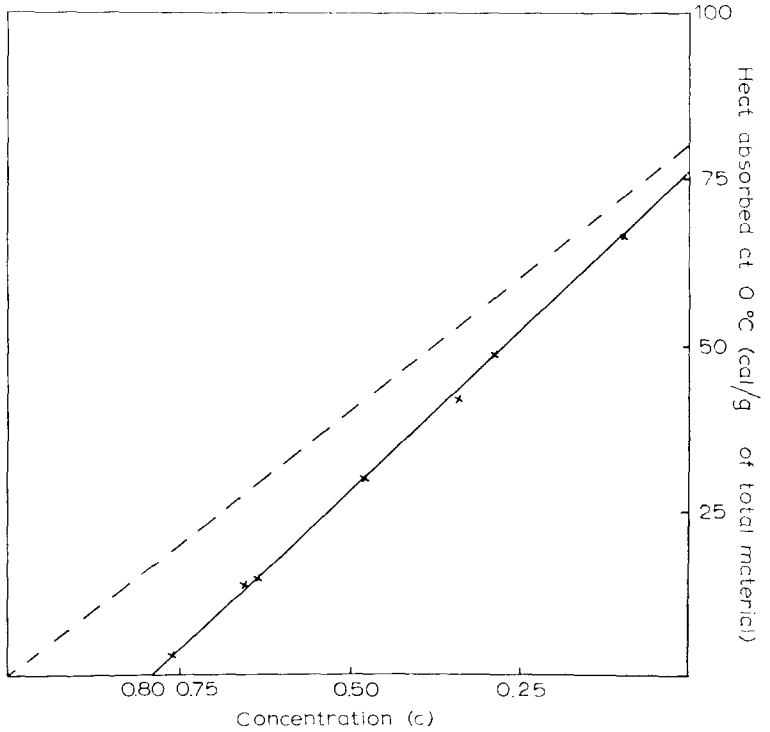


Fig. 4. Variation of heat absorbed at 0 °C with water concentration for the 1,2-dipalmitoyl-L-phosphatidylcholine/water system. ——— experimental results; - - - - - - - - theoretical curve assuming that no water is bound to the lipid.

tration of water in the mixture further increases, so does the size of this peak. A small additional endothermic peak appears below the main endothermic transition at 35°C.

The heat absorbed in the transition at 0°C, expressed as cal/g of total mixture, increases linearly from zero at $c=0.8$ to 77 cal/g at $c=0$ (fig. 4), indicating that a proportion of the water is associated with the lecithin in a fixed ratio of 1:4 by weight, and that this bound water does not form ice on cooling even to −100°C. The variation of heat change at the lipid transition temperature (T_1) with water content is shown in fig. 5. Repeated temperature cycling over the thermally active regions does not cause either the areas or the temperatures of the water peak at 0°C and the lipid peak at T_1° to vary. The thermal behaviour between 0°C and the transition temperature (T_1^*) is not precisely reproducible and consistent values for the small endothermic peak at 35°C could not be obtained. No additional thermal transitions were

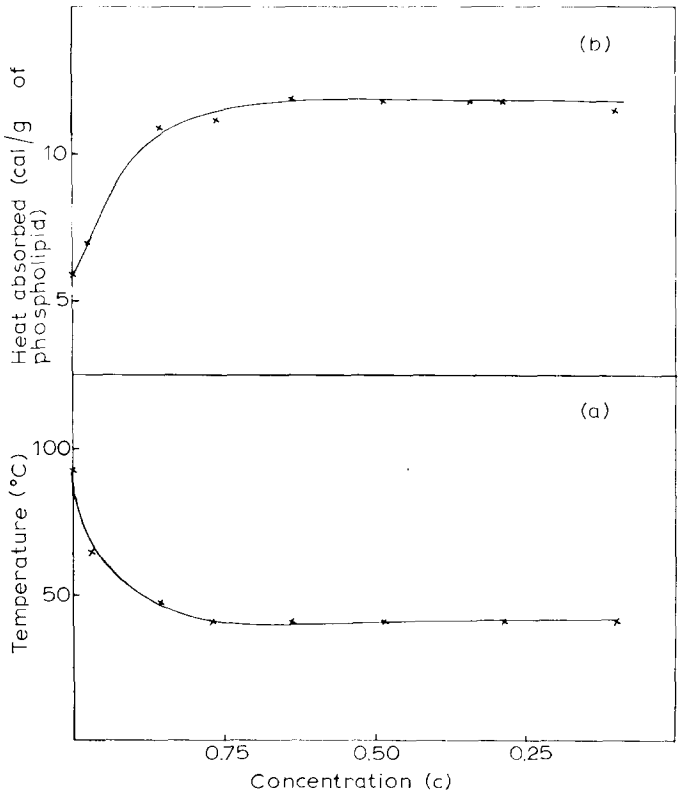


Fig. 5. The variation with water concentration of 1,2-dipalmitoyl-L-phosphatidylcholine/water system for (a) the transition temperature T_1 and (b) the heat absorbed at this temperature.

observed up to temperatures as high as ~150 °C, where the sample capsules usually burst.

The thermal properties of the commercial 1,2-dipalmitoyl-DL-phosphatidylcholine are similar to those of the pure material. When this lipid is dispersed in water no endothermic peak at 0° is obtained until more than 27% by weight of water has been added. At this concentration the transition temperature of the lipid reaches a value of 45 °C and remains constant on addition of further water. No transition is observed in the region between 0 °C and 45 °C, but the peak at 45 °C is followed by a broad endothermic peak extending from about 50 °C to 65 °C. The peak, due to ice formation, is depressed by some 10 °C. No such depression of the ice-melting point was observed with any of the pure materials.

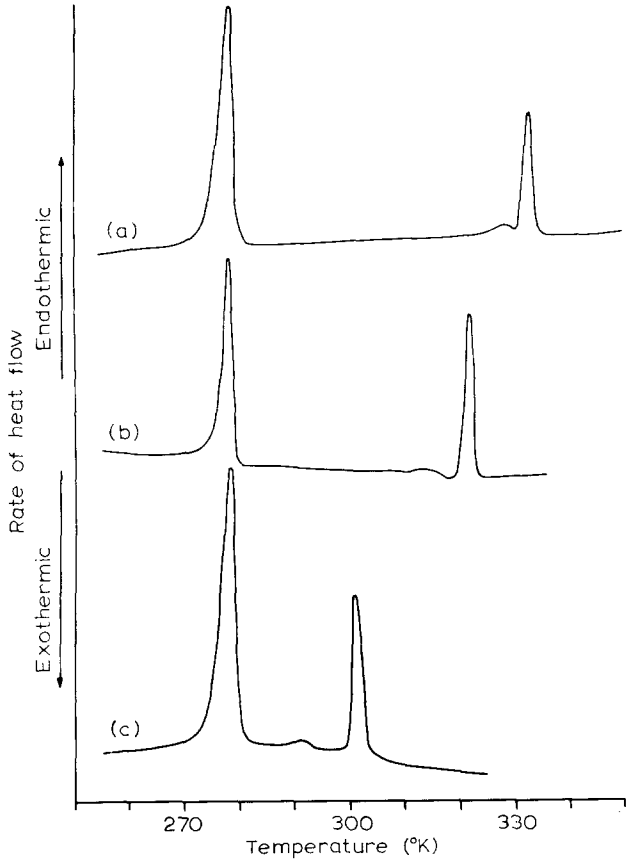


Fig. 6. D.s.c. heating curves for some 1,2-diacyl-L-phosphatidylcholine/water systems at $c \approx 0.5$ for (a) the distearoyl, (b) the dipalmitoyl and (c) the dimyristoyl derivatives. The peak due to ice melting at 0 °C is also shown.

The d.s.c. heating curves for phosphatidylcholines of different chain lenths in water at $c \approx 0.5$ are shown in fig. 6. It is seen that the endothermic transition temperatures occur at lower temperatures as the hydrocarbon chain length of the phospholipid becomes shorter. In addition to this the temperature interval between the main endothermic peak and the small preceding small peak increases. No endothermic peak arising from the lipid was apparent on the d.s.c. recordings with the 1,2-dilauroyl-L-derivative, but the ice-melting peak at 0° C is unusually broad, and we infer that some of this broadness is due to the lipid and that the limiting transition temperature for this lipid in water is close to 0 °C. The thermal behaviour of the dicapryl derivative is complex, several transitions occurring below 0 °C. Further work is being done on this and on other systems where the lipid transition temperature is below 0 °C.

X-ray studies (lipids)

The crystalline monohydrates are found to occur in three different polymorphic forms: two (α_1 and α_2) are obtained from chloroform/methanol solution, whilst the other (β') is obtained from chloroform solution by precipitation with diethylether. The crystals of anhydrous 1,2-distearoyl-L-phosphatidylcholine are obtained by very slow crystallisation from dry chloroform/diethylether solution. The principal X-ray diffraction spacings of the various forms are given in table 4.

Between room temperature and T_1 °C the diffraction pattern remains essentially the same (fig. 7). The crystalline, anhydrous 1,2-distearoyl

TABLE 4

X-ray diffraction spacings of the 1,2-diacyl-L-phosphatidylcholines at 23 °C (20 °C for the dicapryl-)

1,2-diacyl-L-phosphatidylcholine	X-ray spacings (Å)		X-ray spacings (Å)	
	long spacing	short spacing	long spacing	short spacing
	monohydrate α_1 form		anhydrous crystals	
Distearoyl-	63.3	4.13 (vs), 2.43 (w) 2.08 (mw, diffuse)	54.0	4.93 (m), 4.37 (s) 4.00 (m), 3.88 (m) 3.32 (w), 2.67 (w)
			monohydrate α_2 form	
Dipalmitoyl-	58.0	4.13 (vs), 2.40 (w) 2.08 (mw, diffuse)	55.5	4.13 (vs), 2.40 (w), 2.08 (mw, diffuse)
			monohydrate β' form	
Dimyristoyl-	53.6	4.2 (vs), 2.46 (vw)	54.3	4.11 (vs), 3.89 (m), 3.61 (m)
Dilauroyl-	49.9	4.2 (vs)		
Dicapryl-	44.5	4.2 (vs)		

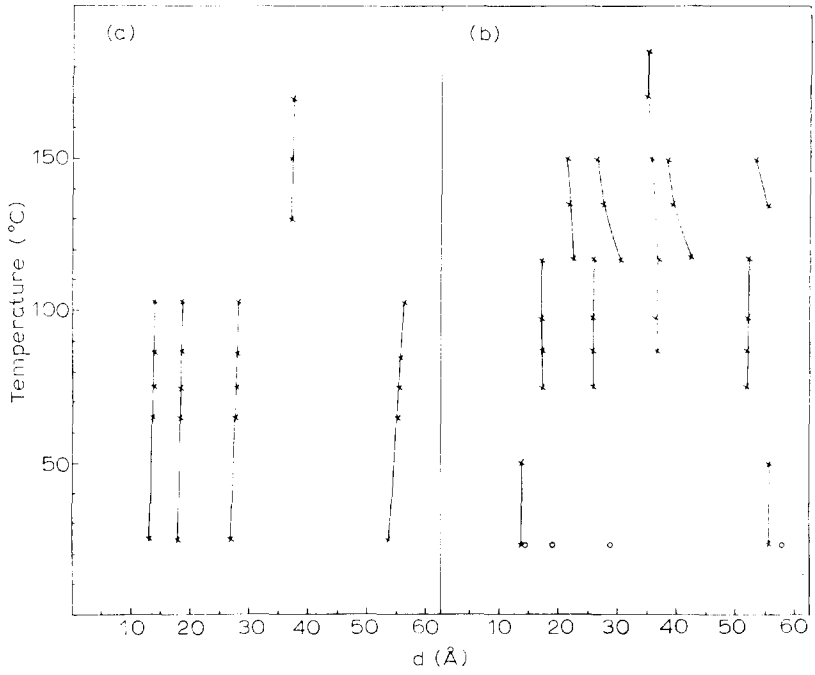


Fig. 7. Temperature dependence of the low-angle X-ray diffraction patterns of (a) anhydrous 1,2-distearoyl-L-phosphatidylcholine and (b) the monohydrate of 1,2-dipalmitoyl-L-phosphatidylcholine (○ form α_1, × form α_2).

derivative shows a slight increase in long spacing occurs from 54.0 Å at 23 °C to 56.4 Å at 103 °C. This is normal lattice expansion and is typical of a crystalline material. In contrast the monohydrates exhibit a small lattice contraction on heating up to T_1 °C. At this temperature a marked decrease occurs in the long spacing and, as well as this, the short spacings change to show only a broad diffuse line at 4.5 to 4.6 Å. Between T_1 and T_2 the low angle diffraction lines have spacings in the ratio of $1:\frac{1}{2}:\frac{1}{3}:\frac{1}{4}$. The diffraction patterns of the α_1, α_2 and β' forms are identical above the first transition temperature.

When the anhydrous, crystalline 1,2-distearoyl-L-phosphatidylcholine is heated to above T_1 °C and then cooled to room temperature, the long spacing is 63.0 Å, as compared to 54.0 Å before the temperature cycle. The short spacings also change to give a strong 4.2 Å line.

Above T_2 the ratio of the low angle diffraction lines is now found to be 1:0.708:0.500:0.374:0.356 for 1,2-dimyristoyl-L-phosphatidylcholine, and, 1:0.706:0.500:0.392 for 1,2-dipalmitoyl-L-phosphatidylcholine. These spacings can be indexed on a body-centred cubic lattice as the (110), (200), (220)

TABLE 5

Parameters of the cubic phase of the monohydrates of some 1,2-diacyl-L-phosphatidylcholines

	Dipalmitoyl-		Dimyristoyl-
T (°C)	130	150	130
Lattice type	I	I	I
a (Å)	78.6	77.6	74.8
Radii of water spheres (Å)	12	12	12
Number of molecules per primitive cell	≈180		≈180

(321) and (400) reflections for the former and as (110), (200), (220) and the (222) reflections for the latter.

The dimensions of the cubic phase are given in table 5. Very often large crystals are formed in the cubic phase, thereby making the indexing of diffraction lines impossible. For this reason it was not possible to obtain dimensions in the case of the distearoyl derivative. An individual sample of the 1,2-dimyristoyl-L-phosphatidylcholine gave a diffraction pattern consist-

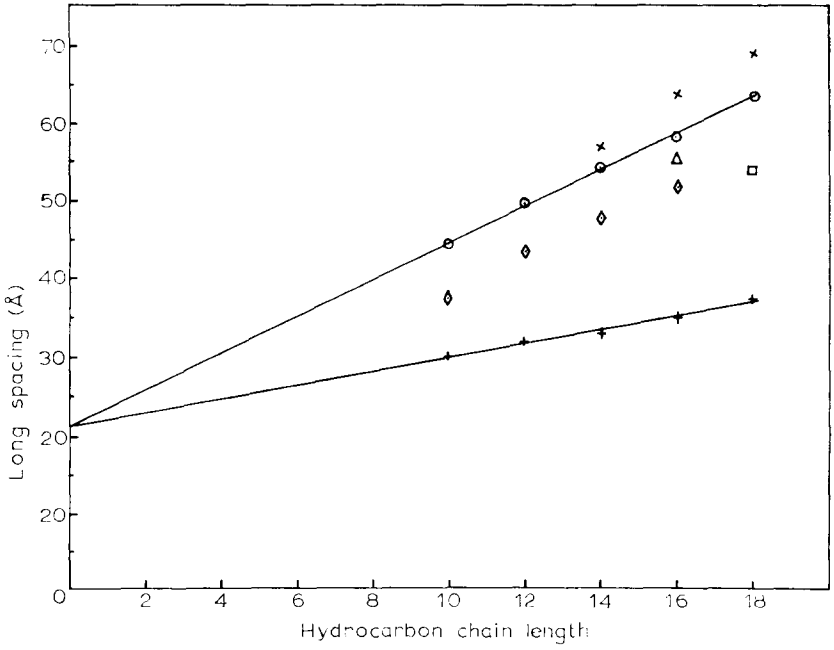


Fig. 8. Variation of long spacings with hydrocarbon chain length for a series of 1,2-diacyl-L-phosphatidylcholines. ○ monohydrate at 25°C (α_1 form); △ monohydrate at 25°C (α_2 form); □ anhydrous material at 25°C crystallized from chloroform/ether solution; ◇ liquid crystalline (lamellar) at a temperature just above the crystal to liquid crystal transition temperature, T_1°C; + liquid crystalline at 175°C; × phosphatidylcholine/water system at 25°C at maximum hydration.

ing of two lines which could be indexed on a face-centred cubic cell ($a = 58$ Å), but this observation could not be repeated.

Above T_3 the low-angle pattern consists of a single strong long spacing (of about 60% of the room-temperature value). No higher orders of this long spacing are observed. The high-angle diffraction pattern again consists of only a broad, diffuse band at ~4.6 Å.

Those lecithins which do not exhibit the cubic phase change directly at T_1 to a liquid crystalline phase having a long spacing of about 60% of the room-temperature value. The long spacing variation with chain length of the lecithins at 25°C and at 175°C is given in fig. 8.

X-ray studies (lipid/water systems)

The diffraction spacings were observed both as a function of concentration and temperature for the 1,2-dipalmitoyl-L-phosphatidylcholine/water mixtures. In all cases only integral orders of the principal long spacings were observed. On heating the sample from room temperature, the long spacing decreased abruptly at T_1°C. Below this temperature, the only high-angle diffraction is a sharp line at 4.27 Å whilst above this temperature this diffraction becomes diffuse and has a spacing of 4.5 to 4.6 Å.

At 25°C the long spacing increases as the water content increases, and reaches a limit at $c = 0.73$. At 50°C the long spacing increases until $c \approx 0.85$, where there is a sharp drop of some 5 Å followed by an increase until $c \approx 0.6$, after which the long spacing remains constant. The various changes in long spacing which occur as the lipid/water concentration varies are shown in fig. 9. The distearoyl and dimyristoyl derivatives were only examined at $c = 0.5$, where the long spacing is independent of the water concentration. The long spacings are plotted as a function of chain length in fig. 8.

An additional study was made of the commercial 1,2-dipalmitoyl-DL-phosphatidylcholine/water mixtures. At 25°C the low-angle diffraction lines are all integral orders of a principal spacing, indicating a lamellar structure, whilst the high-angle region is dominated by a sharp reflection at about 4.19 Å. The system is optically transparent.

At 25°C with $c < 0.77$ the long spacing increases linearly as $(1-c)/c$ increases. This, together with the single high-angle diffraction spacing of 4.19 Å, indicates that the system is in a gel phase[11]). A plot of long spacing against $(1-c)/c$ gives a straight line with an intercept at $(1-c)/c = 0$ of 46 Å, the thickness of the lipid layer. From the slope of this line the ratio of the density of the lipid to that of the water is found to be 1.1.

The area, S, occupied by a polar group at the phospholipid/water interface is given by $S = 2M/d\rho N_0$, where M is the molecular weight of the lecithin, d the thickness of the lecithin layer, and ρ its density and N_0 is the Avo-

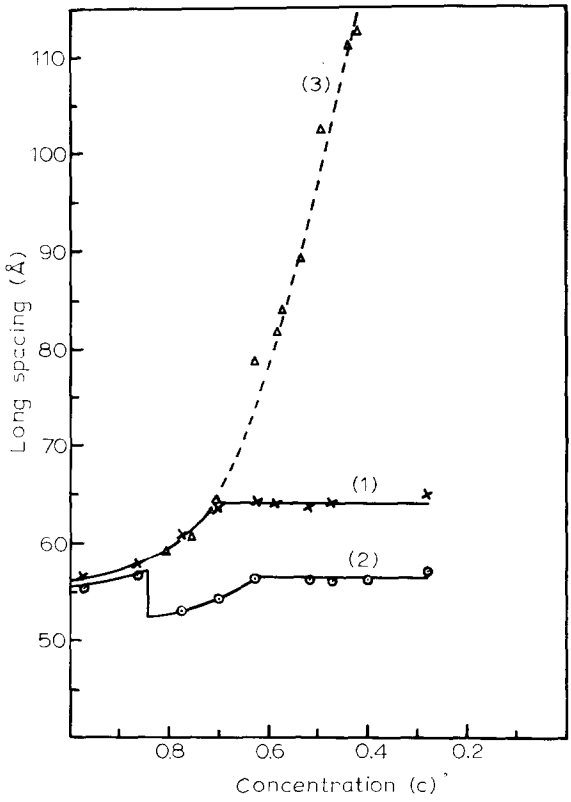


Fig. 9. Variation of long spacing with concentration (c) of (1) 1,2-dipalmitoyl-L-phosphatidylcholine/water system at 25 °C, (2) 1,2-dipalmitoyl-L-phosphatidylcholine/water system at 50 °C, (3) commercial 1,2-dipalmitoyl-DL-phosphatidylcholine/water system at 25 °C.

gadro number. At 25 °C the value of S is 48 Å^2. The single, intense diffraction line of 4.19 Å at high Bragg angles indicates that the hydrocarbon chains of the lecithin molecules are packed in a two-dimensional hexagonal lattice. Assuming an hexagonal packing of the hydrocarbon chains, the lateral space, S_0, occupied by each hydrocarbon chain in a plane perpendicular to the chain axes, will then be 20.3 Å^2. From simple geometry it can be shown that S_0 must be related to S by the expression $2S_0 = S \sin\tau$, where τ is the angle between the hydrocarbon chain axes and the phospholipid/water interface. For the commercial 1,2-dipalmitoyl-DL-phosphatidylcholine/water system τ is 58°. Over the concentration range $1.0 < c \leqslant 0.73$ the variation in long spacing with water content is very similar for both the pure 1,2-dipalmitoyl-L-phosphatidylcholine/water system and for the commercial dipalmitoyl lipid/water system. From this we may infer that the pure

material also forms a gel phase. However, the commercial material, unlike the pure phospholipid, seems to be able to incorporate unlimited amounts of water (fig. 9).

Infrared spectroscopy

The effect of temperature on the infrared spectrum of the anhydrous crystalline 1,2-distearoyl-L-phosphatidylcholine is shown in fig. 10. Cooling to $-186\,^{\circ}C$ increases the amount of fine structure present. In particular, the band near 720 cm^{-1} splits to give several components. On rewarming

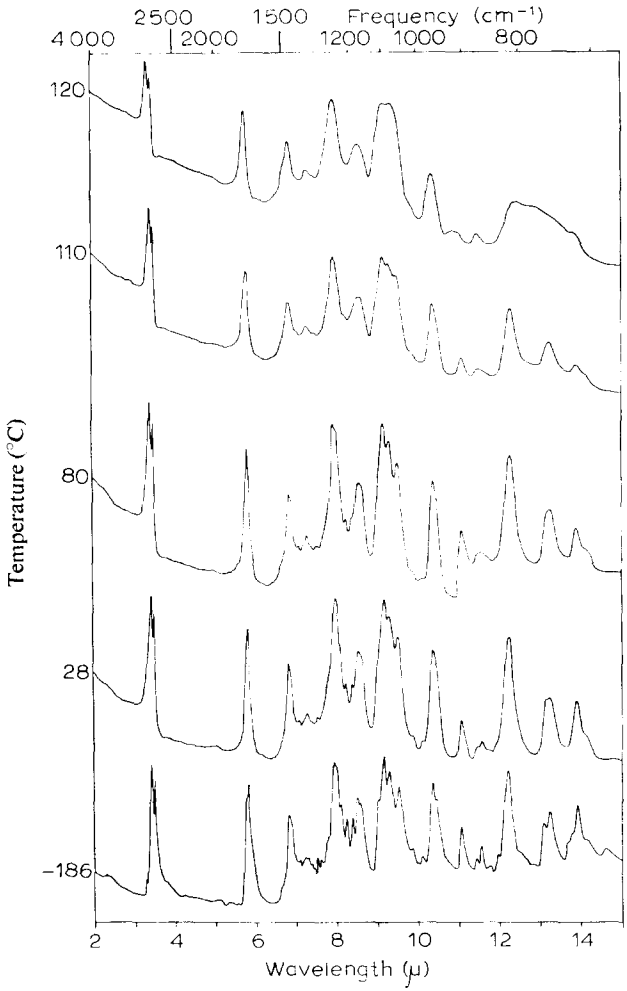


Fig. 10. Infrared spectrum of anhydrous 1,2-distearoyl-L-phosphatidylcholine at different temperatures.

the disc to room temperature, this additional fine structure is lost. On further heating, the band near 720 cm^{-1} drops markedly in intensity and, between 110° and 120 °C, bands in the regions 700 to 820 cm^{-1} and 1000 to 1150 cm^{-1} become smeared out. Raising the temperature above 120° produces no further significant change in the spectrum. On cooling a disc which has been heated to 120 °C, the crystalline spectrum is not recovered, even after prolonged cooling at −186 °C.

Lecithins crystallized from chloroform/methanol solution do not show detailed fine structure in the infrared spectra at room temperature. They contain a band at 3413 cm^{-1} indicating the presence of bound water. The water peak is lost on heating the disc to about 85 °C *in vacuo*. In the presence of a molecule of water, the band at 1252 cm^{-1} shifts to 1238 cm^{-1}. Addition of excess water does not produce any further shift. The spectrum at room temperature of 1,2-distearoyl-L-phosphatidylcholine, from which the water has been removed by heating *in vacuo*, is identical with that of the crystalline, anhydrous material which has been heated to above 110 °C, and cooled to room temperature.

Cooling the monohydrates to liquid nitrogen temperatures for several hours produces a slight increase in fine structure. In particular, the band associated with the main CH_2 rocking mode near 720 cm^{-1} splits to give two asymmetric components at 731 cm^{-1} and 719 cm^{-1}. Also, the series of small shoulders on the band near 1250 cm^{-1} become more pronounced. These shoulders appear to form part of the regular series of bands often observed in this region with long-chain compounds[7]). The band frequencies in this region observed for the phosphatidylcholines of different chain lengths are listed in table 6.

The infrared spectrum of 1-stearoyl-2-oleoyl-L-phosphatidylcholine has been studied at room temperature and a "liquid-like" spectrum is observed[7]). In this case the spectrum at liquid nitrogen temperature shows a marked increase in fine detail. It seems that a certain amount of supercooling can occur with these phospholipids when crystallized from solvent because

TABLE 6

Infrared band progression for the monohydrates of some 1,2-diacyl-L-phosphatidylcholines

1,2-diacyl-L-phosphatidylcholine (α_1 form)	Frequency of bands in 1250 cm^{-1} region
Distearoyl-	1340, 1330, 1311, 1284, 1267, 1252, 1232, 1211, 1190
Dipalmitoyl-	1344, 1330, 1304, 1284, 1277, 1242, 1220, 1195
Dimyristoyl-	1337, 1325, 1299, 1277, 1245, 1232, 1202
Dilauroyl-	1337, 1319, 1280, 1266, 1238, 1200
Dicapryl-	1337, 1319, 1282, 1235, 1208

this lipid has to be heated to ~40°C before all the fine structure again disappears.

When the monohydrates are heated, the intensity of the 720 cm^{-1} band

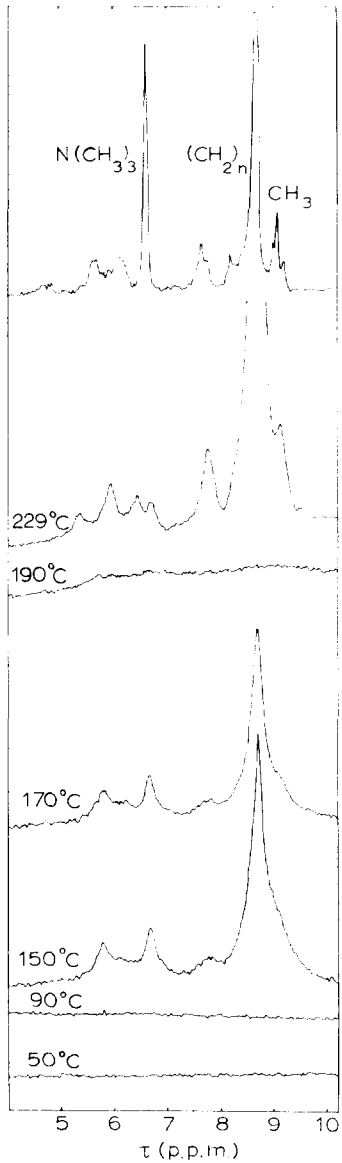


Fig. 11. High resolution proton magnetic resonance spectrum of 1,2-dipalmitoyl-L-phosphatidylcholine monohydrate at different temperatures below and above the capillary melting point. The spectrum (top) of a solution of the phospholipid in deuterochloroform is shown.

decreases, and the remainder of the spectrum becomes a little more smeared out. The temperature at which the 720 cm^{-1} band finally disappears becomes lower as the chain length of the lipid becomes shorter. For each phospholipid it occurs at about the same temperature, T_1, as the main endothermic transition observed by d.t.a. methods.

Nuclear magnetic resonance spectroscopy

Only a very broad band (in high-resolution terms) is observed with 1,2-dipalmitoyl-L-phosphatidylcholine at all temperatures up to 125 °C (T_2). At this temperature, however, the line width narrows sufficiently so that a high-resolution n.m.r. spectrum is observed. This spectrum shows lines corresponding to various chemically shifted proton groupings present in the phospholipid molecule, and is similar to the spectrum of the phospholipid dissolved in a solvent[12]). This spectrum persists until a temperature of 175 °C (T_3). Above this temperature this high-resolution spectrum is suddenly replaced by a broad, featureless, spectrum. At 229 °C (the melting point of the sample) a high resolution spectrum is once again observed, although there are some differences in its appearance as compared with the solution spectrum (fig. 11).

Discussion

Crystalline properties

We have shown that the 1,2-diacyl-L-phosphatidylcholine monohydrates can exist in at least three different polymorphic forms. This phenomenon of polymorphism is widespread amongst long-chain molecules[7]) and it would not be surprising if other forms were found to exist with these molecules, dependent upon the purity and other solvent treatments, etc. The removal of water of hydration by heating, and the possibility of obtaining an anhydrous, crystalline form, even by solvent crystallisation is of some interest. The chemical formulae of the phosphatidylcholines are frequently written in the form of a monohydrate[13,14]). The anhydrous phosphatidylcholines, obtained by heating the monohydrates are, however, extremely hygroscopic. In contrast the anhydrous 1,2-distearoyl-L-phosphatidylcholine, obtained from solvent crystallisation is not hygroscopic.

The three hydrated forms all give a strong short spacing in the region 4.1 to 4.2 Å. The higher homologues of the α_1 form also give rise to weak diffraction at 2.41 and 2.08 Å. The ratio of these spacings ($1:1/\sqrt{3}:1/\sqrt{4}$) shows that the hydrocarbon chains are packed in an hexagonal array, usually designated[15]) as α, and similar to that first reported for the *n*-paraffins near their melting points[16]). The α_2 form has the same short spacings, hence

the same hydrocarbon chain packing, as the α_1 form, but the long spacing is 2.5 Å less.

The hydrocarbon chain packing appears to change when these forms are cooled to -186 °C, because the splitting of the 720 cm^{-1} band in the infrared spectra indicates an orthorhombic, $0\perp$, array[15,17]) often designated as β'. The third form, β', gave a diffraction pattern consistent with the hydrocarbon chains being packed in an orthorhombic, $0\perp$, subcell even at room temperature. We have not been able to deduce the hydrocarbon chain packing of the anhydrous, crystalline, 1,2-distearoyl-L-phosphatidylcholine from the observed X-ray short spacings.

From the variation of long spacings with hydrocarbon chain length of form α_1 (fig. 8), we can deduce that the hydrocarbon chains are approximately perpendicular to the crystallographic reflecting planes determining the long spacing. This long spacing corresponds to the length of two molecules. The glycerylphosphorylcholine "end-group" has a dimension of 10.5 Å in the direction of the hydrocarbon chains. These results are in agreement with some earlier results[18]).

Assignments for the absorption bands in the infrared spectrum of anhydrous, crystalline 1,2-distearoyl-L-phosphatidylcholine are given in table 7. In assigning absorptions associated with the charged choline moiety, we used the results of Van Senden[19]) on N-alkyltrimethylammonium halides.

TABLE 7

Infrared spectroscopic assignments for anhydrous 1,2-distearoyl-L-phosphatidylcholine crystallised from chloroform/diethylether

Frequency (cm^{-1})			Assignment
-186 °C	28 °C	120 °C	
3021	3021		νC—H sym. of $\overset{+}{N}$—CH_3
2941	2941		νC—H antisym. of R—CH_3 and $\overset{+}{N}$—CH_3
2924	2924	2932	νC—H antisym. of —CH_2—
2849	2849	2857	νC—H sym. of —CH_2— (and νC—H sym. of R—CH_3)
1733 (sh)	1733 (sh)	1733	ν>C=O of 1-glyceride residue
1721	1721	1733	ν>C=O of 2-glyceride residue
1464	1466	1460	δ—CH_2—; —R—CH_2— scissors deformation
1447	1447	1439	δC—H; R—CH_3 antisym. bend
1414	1414	1416 (sh)	δC—H; $\overset{+}{N}$—CH_3 antisym. bend
1385/1373	1377	1370	δC—H; R—CH_3 sym. bend
1355	1355 (sh)		δC—H in general associated with various ≡CH sites in the molecule, the splitting only showing up at low temperatures. Probably due to degeneracy losses associated with crystal field effects.
1344		1340(sh)	
1331	1331		

TABLE 7 *(Contd.)*

Frequency (cm^{-1})			Assignment
-186 °C	28 °C	120 °C	
1315	1315 (sh)		band progression due to $(CH_2)_n$ wagging motions superimposed upon $\nu P=O$ or $\nu PO_2^{(-)}$ antisym. absorption.
1292	1292 (sh)		
1282	1282 (sh)		
1262	1258	1255	
1251			
1233	1235 (sh)		
1212	1214		
1189	1192		
1172	1172	1166	νC—O; C—O—C
1162	1162		
1105	1105 (sh)		νC—O—(P) antisym. stretching (out of phase)
1093	1093	1087	νC—O—(P)
1075	1075		$\nu PO_2^{(-)}$ sym.
1048	1048	1064	νC—O—(P) sym. (in phase)
1012	1012 (sh)	1010 (sh)	νC—C of alkyl chains (sym.)
989	989 (sh)		νC—C of alkyl chains (antisym.)
963	963	962	
955	955 (sh)		νC—$N^{(+)}$ vibrations
902	902	905	
873	873	870	
864	864		
849			
835	837 (sh)		
815	815	~815	νP—O—(C) antisym.
763	761	~760	νP—O—(C) sym.
754	754		
729 (sh)			
724 (sh)			
716	716		—(CH_2)—rock
705 (sh)	705 (sh)		

ν denotes stretch
δ denotes deformations, bends

As with the 1,2-diacyl-DL-phosphatidylethanolamines[5]), the absorption due to $\nu P=O$ vibration and the band progression associated with CH_2 wagging or twisting vibrations in the 1250 cm^{-1} region are superimposed.

Thermotropic mesomorphism

The X-ray diffraction, d.t.a., microscopic, infrared spectroscopy and also nuclear magnetic resonance data are consistent in showing that marked phase transitions occur many degrees below the capillary melting points of the diacylphosphatidylcholines. We have noted three phase transitions (at temperatures which we indicate by T_1, T_2 and T_3).

At T_1 the d.t.a. heating curves show a large endothermic transition. The

temperature at which this transition occurs (fig. 3) and the amount of heat absorbed at this transition (table 3) increase with lengthening of the chains. These facts confirm that this transition involves the hydrocarbon chains of the molecules. There is also a marked reduction in the X-ray long spacings and the sharp short spacings give way to a diffuse line at 4.6 Å, similar to that observed with liquid hydrocarbons. Broad line n.m.r. studies[20]) show appreciable molecular motion below T_1, but also show a considerable reduction in the line width at this transition. The infrared spectra (fig. 11) confirm that there is considerable motion below this transition as is evident from the loss of fine structure as the temperature increases. In particular the 720 cm^{-1} band begins to decrease in intensity well below T_1, in contrast to the phosphatidylethanolamines[5]) where this band remains sharp right to the transition temperature.

All the physical evidence is consistent with the formation of a lamellar liquid crystalline phase. All three polymorphic forms give the same phase. (Cooling a phosphatidylcholine from this phase always yields form α_1). In this phase there is considerable molecular motion of the hydrocarbon chains with flexing, twisting and individual rotation of CH_2 groups about the C—C bonds of the chains. The hydrocarbon chains can be said to be in a "liquid-like" state and the phospholipid lamellae are probably able to move relative to each other. Individual molecules may also be able to diffuse relative to each other within the bilayers.

The heat absorbed at T_1 for the anhydrous, crystalline 1,2-distearoyl-L-phosphatidylcholine (17.5 cal/g) is very much larger than that for the anhydrous, non-crystalline form (8 cal/g). The smaller heat absorption for the non-crystalline form probably reflects greater disorder of the chain packing below the transition temperature. The heat of transition for the crystalline phosphatidylcholine is of the same order as that found for the corresponding transition in the phosphatidylethanolamines.

Egg-yolk lecithin gives a much broader endotherm at T_1 than do the phosphatidylcholines of a single discrete chain length. This shows that egg-yolk lecithin is behaving as a mixture of chain lengths at this transition, not as an homogeneous phase as is often assumed.

The monohydrate of the higher members of the homologous series change at T_2 from the lamellar liquid crystalline phase to a liquid crystalline phase displaying body-centred cubic symmetry. At least one molecule of water *must* be present for each phosphatidylcholine molecule in order for this phase to be formed. A cubic liquid crystalline phase was first described by Luzzati *et al.*[21]) for soap/water systems. The Bravais lattice is, however, face-centred for the soap/water systems whereas we find it to be body-centred for the lecithins.

The organisation postulated for the cubic phase in the soap/water systems was a structure of spherical lipid particles embedded in a matrix of water. A different structure has recently been proposed[22]) in which a cubic face-centred lattice of water spheres is embedded in a matrix of lipid. Assuming that the cubic phase of the phosphatidylcholine monohydrate is composed of water spheres embedded in a lipid matrix, we find the radii of the water spheres to be 12 Å. The closest approach of the centres of the water spheres is half the length of the body diagonal of the cube. For the 1,2-dipalmitoyl-L-phosphatidylcholine monohydrate at 130 °C, the centres of the water spheres are 68 Å apart. The thickness of the lipid matrix is, hence, some 44 Å in this direction. This distance is quite consistent with the length of two phospholipid molecules with hydrocarbon chains in the fluid state. There cannot, however, be an absolute boundary between the water and the phospholipid as the phospholipid headgroups must be immersed in the water spheres.

A phase having this highly symmetrical structure can be expected to have a high viscosity since relative translation of the water spheres would destroy the symmetry and, hence, will be energetically unfavourable.

In view of the high viscosity of this cubic phase, it is somewhat surprising that it is possible to obtain a high resolution n.m.r. spectrum showing chemically shifted proton resonance lines, similar to the spectrum obtained when the same phospholipid is in solution in an organic solvent or ultrasonically dispersed in water[12,23]). We have observed similar high resolution n.m.r. spectra with soap/water systems under conditions of temperature and concentration corresponding to their existence in a cubic phase. High-resolution n.m.r. spectra are also obtained from soap micelles in water. Recently analogous results to ours have been reported with a dimethyldodecylamine oxide/water system as the concentration of water to detergent varies[24]).

Why does this cubic phase show a high-resolution n.m.r. spectrum, whereas the other liquid crystalline phases do not? One possibility is that, as a consequence of the chain organisation, there may be a reduction in the dispersion forces between the chains in this phase leading to a reduction in dipole-dipole interactions. We are presently studying nuclear relaxation times τ_1 and τ_2 in order to provide further information about the unusual properties of this phase.

At T_3 the structure changes from that of a liquid crystalline cubic lattice to a phase of unknown structure. This structure gives rise to a single strong X-ray diffraction long spacing of 60% of the spacing at 25 °C. The occurrence of only a single high-angle diffraction line precludes a determination by crystallographic means, of the detailed organisation of this phase, although

its appearance, when viewed between crossed nicols, is similar to that observed by Rosevear[25]) in the neat phase of soap/water systems which have been shown to be lamellar[26]). From fig. 8 we see that the dimension, in the direction of the chains, of the glycerylphosphorylcholine "head group" of the molecule is similar at 175 °C ($> T_3$) to that at 25 °C. The long spacing observed for this phase is identical to that shown by the anhydrous materials above T_1. Since the anhydrous material does not exhibit a cubic phase, we infer that the structure change from cubic to the high-temperature phase is a direct result of loss of water of hydration.

The fact that the occurrence of the cubic phase depends on the presence of water causes difficulties in the interpretation of the thermal analysis data in the region of T_2 and T_3. In the d.t.a. curves, broad, ill-defined heat absorptions are found which do not correspond with expected transitions to or from the cubic phase. The shorter chain homologues give similar d.t.a. curves, yet do not exhibit a cubic phase. We ascribe these heat absorptions to loss of water vapour from the sample. There is a further complication in that loss of vapour causes the thermocouple to be forced out of contact with the sample, resulting in an apparent exothermic baseline drift. If the sample is enclosed in a sealed capsule and examined with the d.s.c. instrument, these complications do not arise and distinct endotherms corresponding to T_2 and T_3 can be observed (fig. 2, curve d). Furthermore, the heat change at T_3 is much larger than that at T_2 which is consistent with elimination of water from the lipid matrix at T_3.

Lyotropic mesomorphism

In fig. 12 we show the phase diagram for the 1,2-dipalmitoyl-L-phosphatidylcholine/water system. It is seen that on addition of water the transition temperature, T_1, of the phospholipid is lowered to a certain limiting value (T_1^*). This transition temperature is the minimum temperature required for the water to penetrate between the layers of the lipid molecules. We have shown elsewhere[27]) that distearoylphosphatidylcholine spontaneously forms myelin tubes in excess of water only when heated to 60 °C, i.e. only when T_1^* is exceeded. A similar situation occurs with the soaps where the ability to disperse in water only occurs above a temperature, referred to as the Krafft temperature, which is nearly constant over a wide range of concentration.

Above the T_1 line, the phosphatidylcholine/water system exists in a mesomorphic lamellar phase. This phase consists of bimolecular layers of lipid molecules separated by layers of water. The long hydrocarbon chains of the lecithin molecules are in a fluid state and the hydrophilic groups lie on the surface separating the lipid and water layers. The composition of the system at maximum hydration is ~40 wt % water, a result in agreement

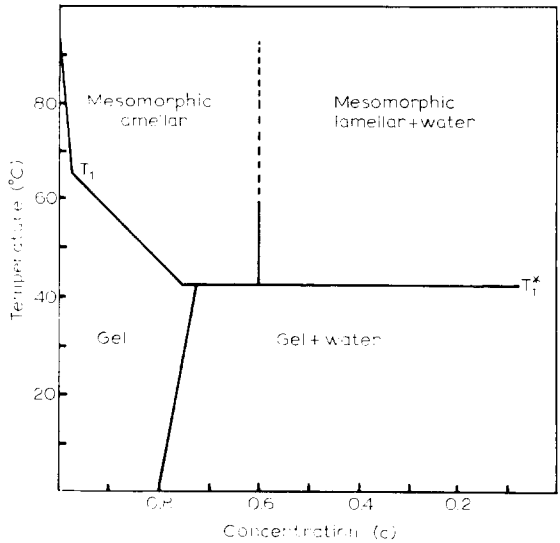


Fig. 12. Phase diagram of the 1,2-dipalmitoyl-L-phosphatidylcholine/water system.

with that recently determined for the egg-yolk lecithin/water system[28]). On addition of more than 40 wt % water the system dissociates into two phases: the mesomorphic lamellar phase and water.

When the phosphatidylcholine/water system is cooled below the T_1 line, the hydrocarbon chains adopt an ordered packing. The structure of this phase, the gel, is lamellar. The hydrocarbon chains are packed in an hexagonal subcell with the chain axes inclined at 58° to the lipid/water interface. Tilting of the chains may take place so that the glycerol moiety of the molecules can interact favourably with the water layer. The magnitude of the tilt will be controlled by a balance between the need for efficient hydrocarbon chain packing and the hydrophilic character of the polar head group. An angle of tilt of approximately 60° produces identical hydrocarbon subcell chain packing to that obtained with a tilt of 90°. A gel phase has been observed with other lipid/water mixtures, e.g. potassium soaps[11]) and monoglycerides[29]).

The large amount of bound water taken up by the phosphatidylcholines (some 20 wt %) which does not form ice on cooling the lipid–water mixtures below 0 °C is probably due to the formation of a hydrate structure associated with the polar group. The heat absorbed at the lipid transition temperature (T_1) increases (fig. 5) over the range $c=1.0$ to $c\approx 0.7$. As the transition temperature decreases some increase in the heat of transition ΔH is expected if the entropy change Δs is to remain constant. However, this is not sufficient

to account for all the observed increase. The reason for this may be due to the heat required to melt the lipid/water complex of the gel phase. We also note that the transition becomes sharper in water (cf. figs. 2 and 6).

The commercial 1,2-dipalmitoyl-DL-phosphatidylcholine/water system behaves differently from that of the pure lipid/water system. The "ice" peak is depressed by up to 10°C for the commercial material and it seems to be able to incorporate unlimited amounts of water. These effects are probably due to the presence of ionic impurities, such as palmitic acid and phosphatidic acid, which would lead to a net negative charge in the lipid bilayers. These charged bilayers would then repel each other almost indefinitely.

General discussion and biological implications

The transition temperatures of the lipids in water are an important factor in determining their physical behaviour, e.g. their dispersability, formation of myelin forms, etc. We see that, even with the fully saturated lecithins of chain length C_{14} or C_{12}, at temperatures close to body temperature (37°C) they are already in a mesomorphic state with the hydrocarbon chains in a highly mobile condition. This reinforces the idea that, at 37°C, the hydrocarbon chains of the unsaturated phospholipids present in natural tissues will be in a highly mobile condition, unless there is some inhibition of this motion due to special effects such as interaction with other molecules, e.g. cholesterol or protein. It is therefore necessary to provide a reason why the unsaturated phospholipids present in membranes should *not* be in a mobile condition rather than the reverse. To imagine that the chains of unsaturated lipids are mainly static with only slight transient departures of relatively small amplitude does not seem at all reasonable. This is an important point because, in some theories and speculations about membrane organisation, the latter philosophy of a mainly static structure, is often adopted even where highly unsaturated lipids are present in the membrane under consideration[30]). The limiting transition temperature (41°C) for the dipalmitoyl lecithin, a major lipid in the lung membrane, is close to body temperature. This may be of biological significance in the action of the lung membrane and requires further study.

The fact that the transition temperature can differ from one class of phospholipid to another, even where the chain length is the same, is also of some importance since it implies that one cannot necessarily discuss the fatty acids and their behaviour independently of the polar groups attached to them. We have commented earlier[31]) about the fluidity of the chains in natural membranes, and about the possibility that the variation of fatty acid distribution which occurs with lipids in membranes of the poikilothermic organisms as the temperature varies, may be part of some feed-back

mechanism related to a need for constant fluidity. It may be that the fluidity requirements of the membrane can be satisfied either by variation of chain length or by variation of the phospholipid class.

There are a number of questions which remain to be resolved by future study, e.g. how are the transition temperatures of the lipids affected by ions or cholesterol? Are the transition temperatures which one observes with lipids immediately relevant to biological membrane behaviour, i.e. should one observe similar transition temperatures in biological membranes?

The appreciable amount of water bound to the lecithin molecule is also of biological significance. It suggests a limiting value to the water content of a biological membrane beyond which it should not be possible to dry the membrane without disturbing severely its structure. The variation of this bound water in the presence of cholesterol or ions may have significance in the macromolecular complex of membranes. In model membrane systems[32]) the bound water will provide an additional layer which needs to be considered in its effect on the flow of ions or water through these model membrane systems. This is also relevant to calculations of the refractive index of these films, since layers of associated bound water need to be taken into account in this type of calculation.

Our observation showing the large amount of water incorporated between the bilayers of the commercial 1,2-dipalmitoyl-DL-phosphatidylcholine/water system is of interest in the light of some recent studies on mitochondrial lipid/water systems[33]). These workers have shown that in the lamellar phase of this material, almost unlimited amounts of water can be incorporated between the lipid layers. The mitochondrial lipid extract is known to contain a proportion of ionic lipids.

The fact that lecithins of different chain length in water adopt a lamellar or bilayer type structure over a wide variation of concentration is interesting. At first sight this may appear to lend some support to the idea that a natural membrane may be built up on this bilayer type organization. This need not, however, necessarily be the case. The influence of the membrane protein may be quite considerable on the resultant structure, and the organization of the membrane will depend upon the mode of interaction of the lipid and protein. Both the lipid and protein separately take up stable configurations to minimise hydrophobic and maximise hydrophilic interactions with the surrounding water. The important question for membrane organization is whether the combined lipid/protein complex attempting to balance these interactions will adopt an entirely different arrangement. The way in which membranes are synthesised naturally may also be relevant to this point.

The occurrence of a cubic phase with the lecithins emphasizes the degree of freedom of movement which can occur with these molecules even in the

bulk phase. To give rise to the high resolution n.m.r. spectrum considerable diffusion and relative movement of the molecules must be occurring. It is 'micellar' in type and this is interesting since there have been suggestions that lipids may occur in structures other than the normal lamellar type, and furthermore, that micellar type structures may be important in membrane organization[34]). However, we have only observed this cubic structure with the lecithins at high temperatures and low water content so that its importance for biological membranes is still uncertain. Krog and Larsson[35]) have found that for monoglyceride/water systems addition of ions greatly increases the range in which the cubic phase exists. Similar effects might occur in the lecithin/water systems.

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