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PHYSICAL STUDIES OF PHOSPHOLIPIDS

VIII. NUCLEAR MAGNETIC RESONANCE STUDIES OF DIACYL-L-PHOSPHATIDYLCHOLINES (LECITHINS)

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SUMMARY

Proton magnetic resonance spectra of anhydrous polycrystalline 1,2-distearoylphosphatidylcholine (lecithin) have been obtained at temperatures between —196° and 150°. The decrease of line width and second moment with increasing temperature indicates increasing rates and amplitudes of reorientation up to a sharp transition point. The motion is probably rotatory and oscillatory in nature, but with a wide distribution of correlation frequencies. The mesomorphic phase above the transition gives a narrow nuclear magnetic resonance absorption line corresponding with rapid molecular reorientation which is not isotropic. Similar spectra are found for egg yolk lecithin, and the discussion of molecular motion in phospholipids may therefore be relevant for the reorientations of many lipids in biological membranes. Preliminary studies of the influence of water (^2H_2O) on the molecular motion of phospholipids are also described.

INTRODUCTION

Phospholipids are involved at the molecular level in many biological processes affecting membranes. A variation of the fatty acid distribution and class of phospholipid occurs. Previous nuclear magnetic resonance (NMR) studies of some 1,2-diacylphosphatidylethanolamines[1] have shown that considerable molecular motion of the long chains and head group protons occurs above the transition temperature, corresponding to the formation of mesomorphic phases. The diacylphosphatidylcholine (lecithin) molecule is a major component of cellular membranes, and in order to assist the understanding of phenomena observed in biological membranes, we have examined the molecular motion of 1,2-distearoylphosphatidylcholine in the anhydrous state*. Preliminary studies of other lecithins and the modifications introduced by the presence of water are also reported.

Abbreviations: NMR, nuclear magnetic resonance; PMR, proton magnetic resonance.
* Preliminary report given at B.R.S.G. meeting, University of Kent at Canterbury, 1966.

EXPERIMENTAL

Pure 1,2-distearoyl- and 1,2-dipalmitoyl-L-lecithin were synthesised in this laboratory and their purity demonstrated by thin-layer chromatography. Crystallisation of the lecithin was made from chloroform–methanol and resulted in the monohydrate. Removal of water by vacuum drying over a week resulted in an anhydrous sample which was then sealed into the Pyrex sample tube. Egg yolk lecithin was prepared in the laboratory.

The experimental procedure was directly analogous with that described previously[1]. Where both broad and strong narrow components were observed, the experimental parameters (*e.g.* r.f. field, audio-frequency field modulation) were optimised for second-moment measurements on the broader components. The narrow component may be somewhat saturated and modulation broadened in the composite spectra obtained below the transition temperature. Above 25° the composite spectra were decomposed following the method of WILSON AND PAKE[2] and the second moment of the broad component, corrected for the effects of the finite modulation amplitude and frequency, was calculated by numerical integration[3,4] on an IBM 360 computer. Below 25°, the second moment of the total line shape was computed.

The error of temperature measurement was less than $\pm$ 2°, but the stability at a given temperature was constant to $\pm$ 0.5°.

RESULTS

Typical proton magnetic resonance (PMR) derivative absorption spectra of distearoyl-L-lecithin are shown in Fig. 1 at various temperatures in the range —196° to 140°. The line widths and second moments as a function of temperature are shown in Fig. 2. The standard error of at least four spectral measurements is also given for the major broad component.

At the lowest temperature, a broad line (I) of width 16.4 $\pm$ 0.3 Gauss is observed. The second moment of 29.0 $\pm$ 0.5 Gauss^2 does not appear to correspond with a rigid lattice condition due to a rather weak narrow component (II) of width of about 3 Gauss. With increasing temperature both the second moment and line width decrease with sharp reductions near —150° and —40°. Above —50°, a narrow component (III) of width 3.5 Gauss intermediate between the two other components is observed and obscures the resolution of Line II.

At higher temperatures, a very narrow component is resolvable and, particularly above 25°, becomes very intense. The second moment was obtained from decomposition of the composite line shape for lines I and III. Above about 65–75°, line III merged into the wings of the overmodulated narrow component, and resolution of the remaining broad component was imprecise (see Fig. 1 at 83°). From this temperature up to the transition temperature an additional broad component (7 Gauss) was observed, and another line of width 3.2 Gauss was resolved. The second moments were obtained from line shape decomposition based on the 3.2-Gauss line width. These features may be evidence of a poorly defined phase change centred near 75°, but lack of resolution hindered further analysis. At 100° no broad component is observed, although the wings of the overmodulated narrow line may conceal a line of width $\lesssim$ 1 Gauss.

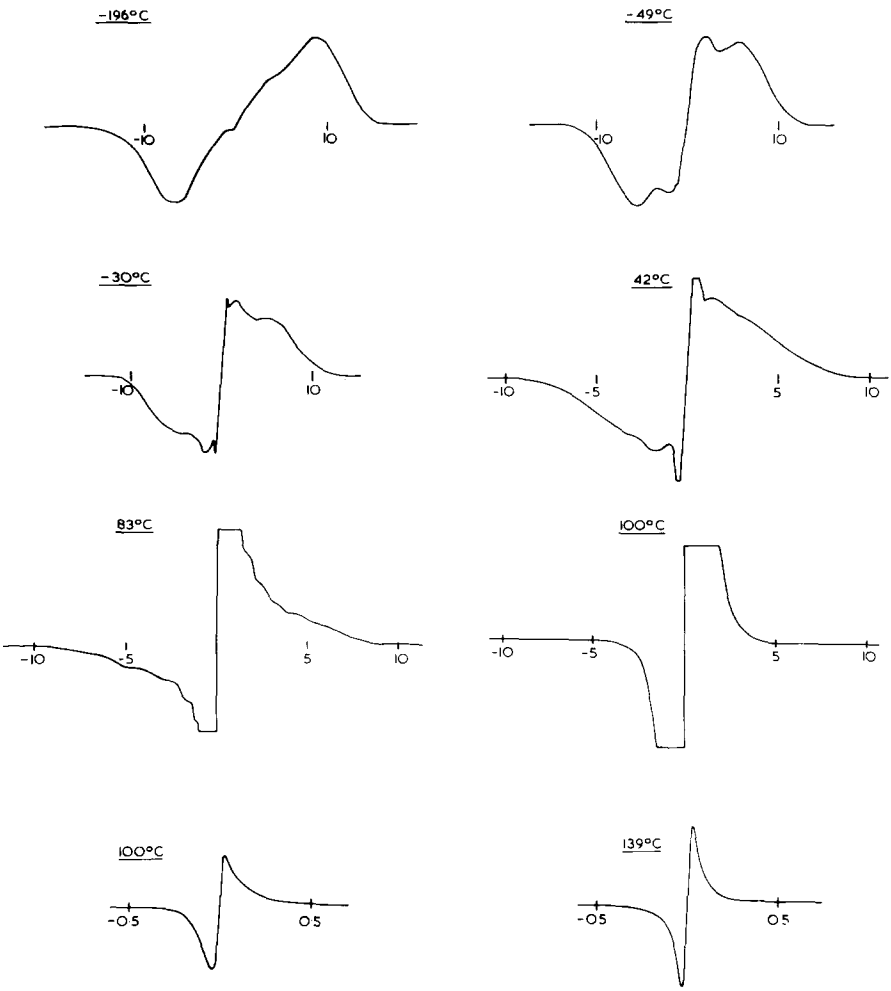

Fig. 1. PMR derivative absorption spectra of 1,2-distearoyl-L-phosphatidylcholine at various temperatures. The abscissae are calibrated in Gauss.

The transition of line width and second moment leads to only a strong narrow line of width 0.07 Gauss, as measured on the derivative absorption spectra. This width is not determined by the inhomogeneity of the magnet. Asymmetry of the derivative spectrum is noticeable; a greater intensity lies in the low-field part of the spectrum at temperatures just above the transition. (This is rather reminiscent of an anisotropic chemical shift.)

High-resolution NMR studies on this sample at temperatures just above the transition temperature show what is normally described as a broad structureless line of width 520 cycles/sec at half-height, that is $\Delta h_{\frac{1}{2}} = 0.12$ Gauss. With increasing temperature, a weak shoulder 2 ppm downfield from the main resonance became apparent after a small gradual reduction of line width to 0.09 Gauss at half-height.

The introduction of water to phosphatidylcholine systems causes the transition temperature in the resulting gel phase to be reduced to a limiting value at a certain

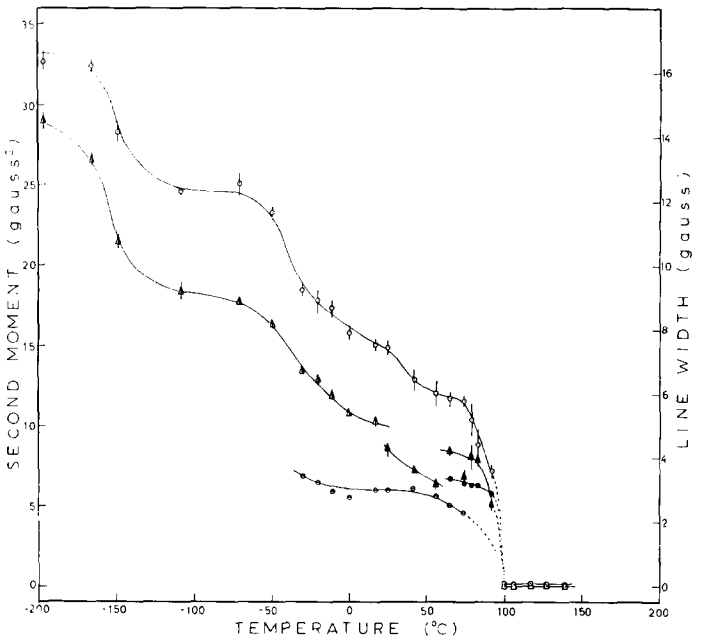

Fig. 2. Second moment (△) and line width (o) data as functions of temperature for 1,2-distearoyl-L-phosphatidylcholine. The second moment of the broad component only is shown thus (▲), and decomposed to include line III (⊖) up to 56°. The additional line width (●) at higher temperatures was used at higher temperatures for the decomposed second moment (▲).

concentration value[5]. The NMR line shapes at temperatures below the transition are also modified, as shown in Fig. 3. A reduction of line width for the medium component (III) of the anhydrous sample (Fig. 3a) reveals only a single broad component of width 4.8 Gauss and second moment 6.4 Gauss^2 at 23° for the distearoyl derivative (Fig. 3b).

A similar behaviour is also found for the dipalmitoyl derivative. The intermediate line width (III) is observed more clearly for the anhydrous sample than for the corresponding distearoyl sample. Similarly this component is narrowed following hydration, as shown in Figs. 3c and d, and resolution is obscured by the very strong narrow component.

With increasing temperature, a corresponding transition of line width (3.1 Gauss) and second moment (3.7 Gauss^2) occurs just above 50° for the distearoylphosphatidylcholine, and only a narrow line is resolved, chemically shifted from the H^2HO signal. Similar behaviour is found for the dipalmitoylphosphatidylcholine/^2H_2O system, as shown in Fig. 3e, where $\Delta h = 0.08$ Gauss. Egg yolk lecithin at 23° shows a similar structureless narrow line of width 0.28 Gauss when anhydrous, and of width 0.09 Gauss with 20% ^2H_2O at 23°, as shown in Figs. 3f and g.

DISCUSSION

Differential scanning calorimetry of the distearoyl-L-phosphatidylcholine shows, for the sample used here, a large endothermic transition at $T_c = 93°$, and a smaller

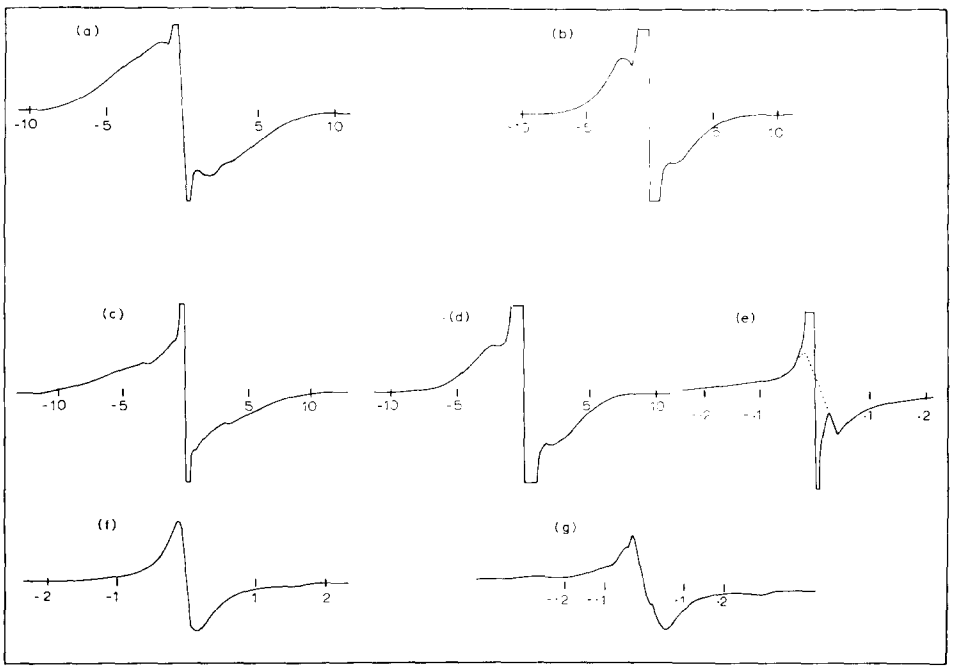

Fig. 3. PMR derivative absorption spectra of: (a) 1,2-distearoylphosphatidylcholine at 40°; (b) 1,2-distearoylphosphatidylcholine + ^2H_2O at 40°; (c) 1,2-dipalmitoylphosphatidylcholine at 23°; (d) 1,2-dipalmitoylphosphatidylcholine + 19% ^2H_2O at 23°; (e) 1,2-dipalmitoylphosphatidylcholine + 19% ^2H_2O at 50°; (f) egg yolk lecithin at 23°; (g) egg yolk lecithin + 20% ^2H_2O at 23°. The abscissae are calibrated in Gauss.

transition at 110°. Immediate reheating of the sample gives the large transition at 110°, but in a sealed NMR tube thermal hysteresis effects were noticeable for only short periods. The transition at 92° is reproducible after 24 h, but to ensure reproducibility, the results above 30° were obtained from one continuous heating run.

At the transition temperature there is a marked reduction of the X-ray long spacings and the sharp short spacings are modified to give a diffuse 4.6-Å spacing. Infrared evidence also indicates a modification of structure and mobility in the hydrocarbon-chain region[5].

It is well known that narrowing of the measured nuclear magnetic resonance dipolar broadened line width is possible when the rate of reorientation of the magnetic nuclei is comparable to, or greater than, the rigid lattice line-width expressed in frequency units (ref. 6). Thus, in this study, reorientational rates of about 60 kHz are required for line narrowing to occur. In general, slower rates of reorientation may occur at lower temperatures in the same crystalline phase, but the spectrum remains unaltered.

The theoretical rigid lattice second moment for a polycrystalline powder can be obtained by the use of the formula of VAN VLECK[7], when the structural data are available. These data are not yet available for the phosphatidylcholines, and we have therefore attempted to make an estimate of the rigid lattice second moment to aid the interpretation of our data.

The intra-molecular contribution for the fatty acid residues may be estimated by comparison with the *n*-paraffins[8] and long-chain fatty acids[9]. Assuming orthorhombic packing of the hydrocarbon chains and that the chains are perpendicular to the ionic head group lamellae[5], we estimate for the hydrocarbon-chain contribution to the second moment 19.7 Gauss^2 and 12.0 Gauss^2 for the intra- and inter-molecular contributions, respectively. These values can be compared with those calculated for the *n*-paraffins[8], silver stearate and stearic acid[9].

The contribution from the protons in the polar head group is more difficult to ascertain. If we assume the methyl protons to be randomly distributed on a circle determined by the three hydrogen atoms in each methyl group, then the intra-molecular contribution of any two neighbours will be about 1 Gauss^2, and will not depend greatly on their relative orientations[10]. The contribution of a fixed methyl group is 22.4 Gauss^2, assuming the accepted dimensions[10], and hence the intra-molecular contribution for the polar group is $\gtrsim$ 23.4 Gauss^2; this may be an underestimate due to the protons of the two remaining $-CH_2$ groups in the choline fragment. The five protons of the glycerol residue make a small contribution which is difficult to estimate. The inter-molecular contribution is also difficult to estimate, but is usually much smaller. For methyl-substituted compounds[11] having three methyl groups, the extra-methyl contribution is about 6 Gauss^2, and the dipolar fields near the trimethylammonium protons may be somewhat similar. Hence the inter-molecular contribution for the polar head group second moment is $\gtrsim$ 5 Gauss^2, since the inter-methyl contribution has been considered as intra-molecular.

Finally, our estimate of the total rigid lattice second moment for distearoyl-L-phosphatidylcholine, neglecting zero-point motions, is $S_0 = S_{\text{intra}} + S_{\text{inter}} = 20.5 + 10.5 = 31.0$ Gauss^2. A maximum error of about 10% is anticipated for what is likely to be an underestimate of the true value. Comparison of the estimated value with experimental values can be useful in understanding major line-narrowing molecular processes and such interpretations can be valuable. Minor changes must clearly be interpreted here with caution. For example, the measured second moment at $-196°$ is 29.0 Gauss^2, and the difference from the estimated rigid lattice value may be accounted for by the two aliphatic terminal methyl groups rotating about their C_3 axes at a sufficiently rapid rate. This model would then predict $S = 28.6$ Gauss^2. As the estimate is likely to be a minimum value, the experimental value indicates free rotation of the majority of these methyl groups.

If, in addition, rapid rotation or oscillation of the methyl groups of the $N^+(CH_3)_3$ head group occurs about their C–N bonds, then we estimate the second moment to be further reduced to $\lesssim$ 24.5 Gauss^2. Such rotation may be co-operative, due to steric considerations. In addition, some oscillation of the three methyl groups about the CH_2–N bond, together with motion of the remaining four protons in the $-CH_2$ groups of phosphorylcholine residue could occur as a further means of reducing the observed second moment.

A large reduction is observed near $-150°$, and the assignment to the $N^+(CH_3)_3$ group is consistent with the absence of a similar reduction of second moment and line width for the phosphatidylethanolamine[1] in which the N^+H_3 group is found.

The appearance of a somewhat stronger narrow component (III) of width 3.5 Gauss at temperatures above $-150°$ may further justify this interpretation, but the presence of a narrow component is not always a proof of a high molecular mobility.

Such lines can be the central part of the dipolar line shape from groups of three identical protons with heavy dipolar broadening in the rigid lattice, but which, in the presence of increasing motion of the neighbouring protons, is sufficiently narrow to be resolved[11].

Above $-110°$, the line widths of the narrow components (II and III) appear to merge, and a weaker and narrower line is observed to gain intensity with increasing temperature, particularly above 20°. Pencil-like rotation of the fatty acid residues about their long axes with the methylene groups rigidly held in a zig-zag conformation, as is found for the *n*-paraffins[8], is considered to be unlikely, due to the restrictive effect of the polar group lying in an ionic lamellar sheet. This is sufficiently effective to produce a high melting point for these molecules of over 200° (ref. 5). Oscillation of most of the $-CH_2$ groups about the C–C bonds or the long-chain axes, that is, out of plane motion, is a more probable mechanism with, in addition, rotation of an increasing number of $-CH_2$ groups at higher temperatures. The gradual decrease of line width and second moment observed between $-50°$ and 74° can be assigned to increasing amplitudes of such oscillations at a sufficiently rapid rate to result in line narrowing, as was proposed for the phosphatidylethanolamine[1]. Further, from steric considerations, increasing amplitudes, and rates of such oscillations are envisaged for the $-CH_2$ groups at increasing distances from the restraining influences of the glycerylphosphorylcholine head group*. Such distributions of correlation frequency are difficult to quantify for second-moment purposes.

If we discuss a mean amplitude of oscillation, albeit with some uncertain distribution about the mean, then consideration of the second-moment reduction as a function of small-angle oscillation amplitude, calculated for pairs of like nuclei[8], may account for the observed gradual reduction. This reduction will occur in the presence of the two motional processes already described. The first temperature at which oscillation becomes a source of line-narrowing appears to be $-130°$, at which an amplitude of almost 20° would be required to produce the obserbed second moment. An increase of oscillation amplitude of 40° is required to account for the observed second moment at 25°, compared with the modified rigid lattice second moment of 24.7 Gauss[2]. For greater angles, the small-angle approximation may no longer be valid. At higher temperatures the composite spectra may indicate a two-phase system, the protons responsible for the narrow component rapidly increasing in concentration with temperature.

The overall behaviour is comparable with the fully saturated phosphatidylethanolamine previously described, but the measured second moments, although similar at $-196°$, are reduced much more rapidly for the fully saturated phosphatidylcholine, particularly above 20°.

Line width changes are related to the reorientation frequency of the appropriate molecular motion occurring. GUTOWSKY AND PAKE[12] have related the line width ΔH to the correlation frequency of the motional process at a given temperature by the following expression:

$$2\pi\nu_c = a\frac{\gamma\Delta H}{\tan\left[\frac{\pi}{2}\left(\frac{\Delta H^2 - B^2}{A^2 - B^2}\right)\right]} \qquad (1)$$

* Preliminary studies of the spin-lattice relaxation time in the rotating frame ($T_{1\varrho}$), in collaboration with Dr. G. P. JONES, University College of North Wales, confirm these assignments of line-narrowing reorientations to the three groups of protons described.

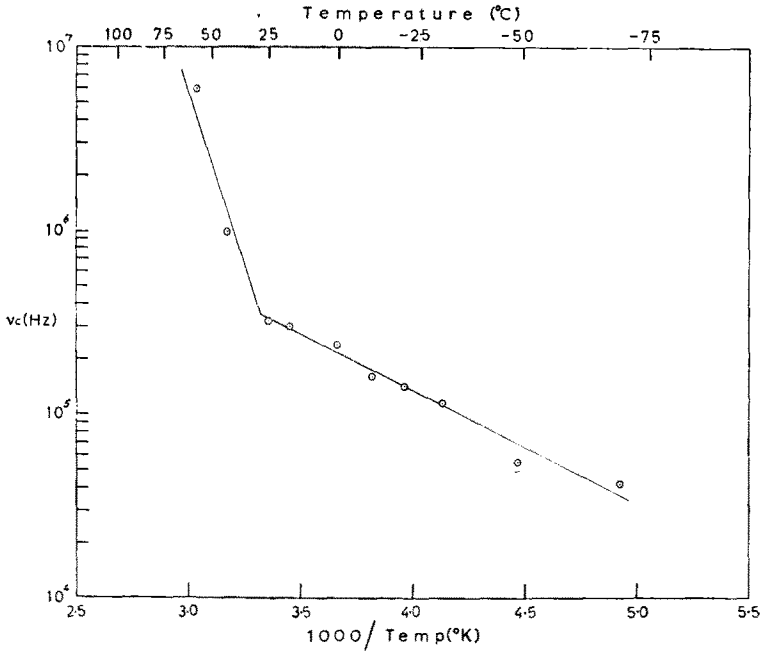

Fig. 4. Semi-logarithmic plot of the correlation frequency ν_c as a function of $10^3/T$ (°K^{-1}) for 1,2-distearoylphosphatidylcholine.

Here ΔH is the line width in the transition region, B is the value above the transition region, and A is the rigid lattice line width (where $\nu_c \to 0$), and α is a constant necessary to indicate the inadequate description of line shapes. Since $\alpha = 2S_0^{\frac{1}{2}}/\Delta h$, $\alpha = 1$ for a Gaussian line shape, and for a Lorentzian line shape α is infinite[13].

If the motions up to 92° can be adequately described by one correlation frequency (ν_c) and are hindered by a potential barrier (or activation energy) of height V per mole, then from the theory of rate processes

$$\nu_c = \nu_0 \exp\left(-\frac{V}{RT}\right) \tag{2}$$

where R is the gas constant per mole.

The variation of $\log_{10}\nu_c$ with $1000/T$ is shown in Fig. 4. Here we use A = 16.35 Gauss, but there is some uncertainty to the choice of B due to the absence of a well-defined region of static line width above the line narrowing region. Assuming B = 5.9 Gauss at 65°, two thermally activated processes are apparent from this treatment, although the calculation of ν_c may only be satisfactory to an order of magnitude.

Up to 25° we find $V = 2.8$ kcal/mole and $\alpha\nu_0 = 3.7 \cdot 10^7$ sec^{-1}, with α approx. 0.8. Above 25°, V about 19 kcal/mole. The lower value of V corresponds almost exactly with that found for dimyristoylphosphatidylethanolamine; the larger value is somewhat greater and is important at lower temperatures relative to the transition point. Values of $V \geqslant 12$ kcal/mole must indicate the onset of widespread large amplitude reorientation, presumably involving a translational mechanism on a molecular or segmental scale.

Above the transition temperarure

The observed line widths and second moments at temperatures above the transition point show that the change of structure must be accompanied by a large increase of molecular freedom, particularly for those protons of the two long chains. Such birefringent phases are described as mesomorphic, in which the hydrocarbon chains are "liquid-like" or "fluid", but with translation in the direction of the molecular axis being inhibited as in a smectic phase.

Let us examine the motional processes that may account for the observed drastic line-narrowing.

The second moment of an isotropic liquid is expected to be much less (10^{-4} Gauss^2) than the second moment obtained for the lecithin in its mesomorphic phase just above 92°. The measured values (10^{-2} Gauss^2) may be somewhat of an underestimate, since the accurate integration depends on the signal-to-noise ratio of the spectrometer, particular in the "skirts" of these rather Lorentzian line shapes (α about 3). A line-narrowing mechanism is required which will efficiently reduce the second moment of 7.5 Gauss^2 at 84° (with the estimated intra- and inter-molecular second-moment contributions, 4.7 Gauss^2 and 2.8 Gauss^2) to a total value $\gtrsim 10^{-2}$ Gauss^2 at 100°.

The reduction of the X-ray long spacings[5] to about 60% of their room temperature value and the increase of the short spacing to a diffuse 4.6 Å indicates some coiling of the long chains, accompanied by an increased cross-sectional area per chain, and that the observed molecular positions are maintained for a considerable period of time. No change of the intra-head group dimensions is observed.

The increased short spacing should result in a reduction of the barriers hindering reorientation about axes in the long repeat dimension of the structure. The high value of the activation energy (19 kcal/mole) found for the narrowing of the broad component just below the transition temperature must indicate a high-energy process, such as translation of the bulk of the protons responsible for that line, and is comparable with the 22 kcal/mole found for "backbone" motions of $C_{94}H_{190}$ just below its melting point[14].

It would be pertinent here to make a comparison of the heat of transition ($\Delta H = 8$ kcal/mole) for the phospholipid with the heat of fusion, but the latter transition is accompanied by decomposition at 220°. However, for sodium stearate, the heat of fusion associated with polar group disorder is 3.7 kcal/mole (ref. 15), compared with a total change of enthalpy of 13.9 kcal/mole. A similar energy is expected to be applicable to the onset of polar group disorder for the phospholipids and therefore the major molecular disordering must occur at the transition point.

To reduce the second-moment values so efficiently, the required translational motion must occur over a distance of the order of the molecular dimensions and at a frequency of $\gtrsim 10^4$ cycles/sec. Molecular diffusion *per se* will, however, be hindered by the incorporation of a given polar head group in a sheet of zwitterionic charges and the size of the vacancies required for successful translation of the molecular centres of mass as is shown by the non-zero second moment.

Normally, $S_{\text{inter}} < S_{\text{intra}}$, and hence in the mesomorphic phase S_{inter} might be small, but, due to the anisotropy of the bilayer structure, cannot be negligible. One such viable mechanism for such an efficient reduction of S_{inter} of the chains could be molecular oscillation or rotation about the P–OCH_2–CH– bonds of the glycerol

residue, such that the overall structure of the head groups is maintained, but in which the long chains undergo essentially a translation to adjacent lattice positions. Many of their neighbours must undergo incoherently a similar reorientation. The potential energy barrier of this motion is expected to be almost comparable to the energy of vacancy formation and jumping found in simpler systems, that is approx. 10 kcal/mole (ref. 16). This figure is of the same order of magnitude as the high activation energy found for the lecithin just below their transition temperatures. The increasing concentration of molecules surmounting this barrier is reflected in the growth of the narrow component and culminates in the crystal–liquid crystal phase transition. Such motion, if sufficiently rapid ($> 10^4$ Hz) and incoherent, would result in an efficient reduction of the inter-chain and intermolecular dipolar fields. The intra-molecular interactions are further reduced by the motions previously described, and also by chain flexing and wagging.

Order is therefore maintained only in the polar head group region, and a residual line width from these protons might be observed (approx. 2 Gauss). This relatively weak line (III) may, however, lie unresolved in the wings of the intense narrow line.

The above remarks particularly apply to fully saturated phosphatidylcholines in the absence of water. One determining factor for the formation of the 'rotating' phase may well be the molecular dimension in the short repeat direction. The residual dipolar interactions (about 10^{-2} Gauss[2]) are not expected to be averaged to zero, unless the anisotropy imposed by the bilayer structure is reduced to a minimum, and an even greater incoherence of translation of the molecular segments is required for the observation of chemically shifted fine structure.

The introduction of water is expected to reduce interbilayer forces. Due to the polar characteristics of the glyceroyl phosphatidylcholine head group, the water is found[5] to be located between the lipid lamellae. Thus the transition temperature for the formation of the liquid-crystalline phase is reduced[5], and the line of intermediate width (III) observed for the anhydrous sample is narrowed, due to the greater rate and amplitudes of reorientation of the head-group protons in the presence of an aqueous environment. No corresponding fine structure in the high-resolution spectrum is, however, observed for these protons, and this reorientation cannot be isotropic.

The second moment of the narrow component of the phospholipid in the presence of ^2H_2O is comparable with that of the anhydrous sample. If we can assume that the rigid lattice dipolar fields are comparable in the two cases, the conclusion must be that the motions of the lipid in the gel phase are not very different from those of the anhydrous system. (Due to the reduction of dipolar interactions between head groups, when enclosed in a sheath of ^2H_2O molecules, that assumption may not be justifiable). Below the transition point the second moment of the broad component is somewhat reduced, due to the reduction of restraining influence provided by the polar head groups.

Comparison of these results for the distearoyl derivative can be made with the dipalmitoylphosphatidylcholine. The intermediate line (III) is observed more clearly for the anhydrous dipalmitoyl derivative for which the relative concentration of $-CH_2$ groups is reduced (Fig. 3c). The addition of ^2H_2O causes a narrowing of this line such that a broad component and the overmodulated narrow component are observed.

Egg yolk lecithin makes an interesting comparison with these results. Due to

its distribution of chain length and *cis* double bonds, its poorly defined transition temperature is near 10° with 20% water. At 23°, there may be a mixture of crystalline and liquid-crystalline phases[16]. The room temperature spectra therefore show somewhat broader narrow lines which are normally characteristic of the lamellar mesomorphic phase, chemically shifted fine structure being absent, as shown in Fig. 3f.

Once the sample temperature is above the transition temperature, the narrow lines from different phospholipids, with saturated or unsaturated fatty acid residues, are nearly comparable in width. Assuming similar structures and line-narrowing mechanisms, then the conclusion must be that the molecular motions are approximately comparable, and are probably indicative of the anisotropy remaining in the lamellar phase.

A comparison of these results with those reported previously for phosphatidylethanolamine show overall similarity. The second-moment data indicates that the motion in lecithin may be somewhat more rapid and random below the transition, and is comparable just above the transition point. Thus the discussion of molecular motion made here can be reasonably extrapolated to the saturated diacyl phosphatidylethanolamines, particularly above their transition temperatures.

Further exact studies to determine the effect of water upon molecular motion in these phospholipids are in progress. However, the similarity of the spectra of the natural lecithin and the fully saturated phosphatidylcholine may indicate that the motional behaviour is comparable, at equivalent temperatures with respect to the appropriate transition temperature. The rate and amplitude of the motion of phospholipids in biological systems may be somewhat similar.

Measurements of relaxation times in high fields T_1, T_2 and in low fields ($T_{1\varrho}$) are being initiated such that further aspects of the motional behaviour of phospholipids in the presence of water can be elucidated.

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REFERENCES

1 D. CHAPMAN AND N. J. SALSBURY, *Trans. Faraday Soc.*, 62 (1966) 2607.
2 C. W. WILSON AND G. E. PAKE, *J. Chem. Phys.*, 27 (1957) 115.
3 G. E. PAKE AND E. M. PURCELL, *Phys. Rev.*, 74 (1948) 1184.
4 K. HALBACH, *Phys. Rev.*, 119 (1960) 1230.
5 D. CHAPMAN, R. M. WILLIAMS AND B. D. LADBROOKE, *Chem. Phys. Lipids*, 1 (1967) 445.
6 E. R. ANDREW AND R. A. NEWING, *Proc. Phys. Soc. London, Ser. A*, 72 (1958) 959.
7 J. H. VAN VLECK, *Phys. Rev.*, 74 (1948) 1168.
8 E. R. ANDREW, *J. Chem. Phys.*, 18 (1950) 607.
9 R. F. GRANT AND B. A. DUNELL, *Can. J. Chem.*, 38 (1960) 359.
10 G. W. SMITH, *J. Chem. Phys.*, 42 (1965) 4229.
11 J. G. POWLES AND H. S. GUTOWSKY, *J. Chem. Phys.*, 21 (1953) 1695.
12 H. S. GUTOWSKY AND G. E. PAKE, *J. Chem. Phys.*, 18 (1960) 162.
13 N. BLOEMBERGEN, E. M. PURCELL AND R. V. POUND, *Phys. Rev.*, 73 (1948) 679.
14 D. G. DOUGLASS AND G. P. JONES, *J. Chem. Phys.*, 45 (1966) 956.
15 R. D. VOLD, *J. Am. Chem. Soc.*, 63 (1941) 2915.
16 E. R. ANDREW AND R. G. EADES, *Proc. Roy. Soc. London, Ser. A*, 216 (1953) 398.
17 D. M. SMALL, *J. Lipid Res.*, 8 (1967) 551.