# X-RAY DIFFRACTION STUDIES ON THE POLYMORPHISM OF PHOSPHOLIPIDS

by

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In X-ray diffraction studies of the myelin sheat of peripheral nerve[7] it was observed that the diffraction bands which appeared to represent mixed lipid phases in the dried nerve pattern underwent marked changes when the temperature of the specimen was varied. Most of these changes could be reproduced with specimens of the total lipid extract of nerve. The changes appeared to be associated with polymorphic transformations among the lipids, examples of which have been reported by previous workers[2,6,8,15]. In order to understand the observations more clearly a study has been made of the variations with temperature of the diffraction patterns of a number of isolated phospholipids.

## APPARATUS

*Source of X-rays.* The rotating anode X-ray unit of the Department of Chemistry, University of Birmingham. Unfiltered copper radiation was used in most of the exposures, but key patterns were checked using nickel-filtered Cu K$\alpha$ radiation.

*Diffraction camera.* The collimating system was designed according to the principles established by BOLDUAN AND BEAR[4]. Permanent lead slits were mounted on separate inserts, and these could be spring-loaded onto holders, one of which (the one nearest the window of the X-ray tube) was rigid, and the other two (carrying defining and guard apertures respectively) provided both lateral and vertical movement. Several sets of such slits, both horizontal and vertical, were provided, and each holder would carry two slits close together so that horizontal and vertical slits could be used simultaneously to provide "pin-hole" collimation. The slit-holders were mounted on an optical bench, which also carried the specimen-holder and plate-holder. The collimating system could thus be adapted to the requirements of any experiment.

*Observation cells.* The high-temperature cell has been described briefly in a previous publication[7]. The low-temperature cell was essentially a brass block (2″ × 1″ × 1″) in which channels had been cut to allow circulation of pre-cooled liquids or air. The X-ray beam was arranged to pass through a hole drilled through the centre of the block, and the specimen was located in the beam at the centre of the block by mounting it on a brass insert. The brass insert was interchangeable between high-temperature and low-temperature cells.

## MATERIALS AND METHODS

Samples of synthetic dipalmitoyl cephalin and linoleoyl-palmitoyl cephalin were obtained from Dr W. G. ROSE of the Western Regional Laboratory of the U.S. Department of Agriculture, Albany, California, and of synthetic dimyristoyl cephalin and erucoyl-stearoyl cephalin from Dr E. B. KESTER of the same laboratory. The preparations and properties of these compounds have been described[16,9]. Three of these samples were pure white microcrystalline powders, but the linoleoyl-palmitoyl cephalin, despite storage in nitrogen, was pale yellow in colour and slightly waxy, and may in fact have undergone some oxidation. Pure samples of hydrolecithin (from lung), sphingomyelin (from lung) and acetal phospholipid (from brain) were supplied by Dr S. J. THANNHAUSER of Tufts Medical School, Boston, Mass., U.S.A. The methods of preparation and properties have been described[17–21]. The hydrolecithin was a pure dipalmitoyl-lecithin, but the sphingomyelin was a mixture of palmitoyl and lignoceroyl. The acetal phospholipid contained only the palmitoyl chain. All three samples as used in these experiments were white powders.

*References p. 384.*

The specimens were sealed up in thin-walled glass capillary tubes and mounted on the brass insert. The diffraction patterns were examined at room temperature, and then in the heating experiments the temperature was raised in steps, exposures being made at intervals of 10° C up to 100° C, and then at approximately 120° C and 150° C. At each temperature level ten minutes were allowed for equilibration. The history of each specimen was carefully recorded so that a study could be made of the effect of repeated heating and cooling on the sequence of changes in diffraction pattern. Several samples of each type of specimen were examined. For the cooling experiments, the insert carrying the specimen was mounted in the low-temperature cell and diffraction patterns recorded at room temperature, 0° C, −15° C, and in some cases at about −40° C. The wide-angle and low-angle diffraction bands were recorded in the same experiment but on separate films placed at different specimen-to-film distances.

TABLE I

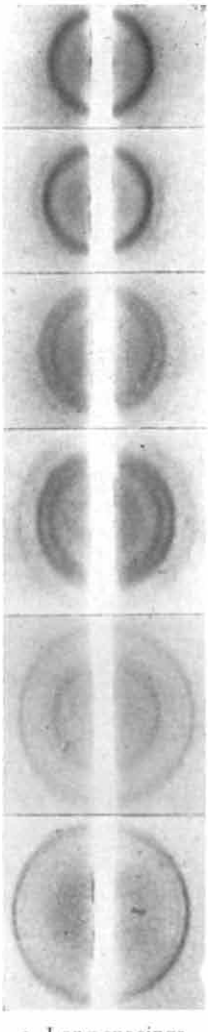
DIFFRACTION SPACINGS (In A)

| | *At 20° C* | | *80° C* | | *95° C* | | *110° C* | |
|---|---|---|---|---|---|---|---|---|
| Dipalmitoyl-cephalin | 57.0 A | VS | 55.5 | S | 55.0 | S | 54.0 | W |
| | | | 45.0 | S | 45.0 | S | | |
| | | | | | 36.5 | W | 32.5 | M |
| | 24 | VW | 28.8 | W | | | | |
| | 18 | W | 19.75 | W | 22.5 | W | | |
| | | | 16.85 | W | 16.85 | W | | |
| | 6.16 | W | 6.16 | M | 6.16 | M | | |
| | 5.61 | W | 5.61 | W | 5.60 | W | Diffuse | |
| | 5.10 | S | 5.10 | S | 5.10 | S | | |
| | 4.54 | S | 4.54 | S | 4.5 | W | | |
| | 4.15 | M | | | 4.2 | W | | |
| Dimyristoyl-cephalin | 51.5 A | VS | 50.0 | S | 49.7 | M | 36.0 | W |
| | | | | | | | 30.5 | W |
| | | | 5.99 | W | | | | |
| | 4.64 | S | 4.64 | M | 4.64 | W | | |
| | 4.21 | M | 4.20 | M | 4.20 | W | Diffuse | |
| | 3.93 | W | | | | | | |
| Erucoyl-stearoyl-cephalin | 54.5 | VS | 53.5 | S | 37.8 | S | 37.0 | W |
| | 26.5 | W | | | | | | |
| | 17.6 | S | 17.6 | W | | | | |
| | 5.58 | W | 5.58 | M | Diffuse | | Diffuse | |
| | 4.65 | S | 4.65 | S | | | | |
| | 4.07 | W | | | | | | |
| Linoleoyl-palmitoyl cephalin* | 38 A | S | 37.8 | S | 37.5 | S | 37.2 | S |
| | 4.5 | Diffuse | Diffuse | | Diffuse | | Diffuse | |
| Hydrolecithin | 59.5 A | VS | 55.5 | S | 50.5 | S | 45.5 | M |
| | | | 47.5 | M | 42.0 | S | 39.5 | S |
| | 32.5 | W | | | | | | |
| | 16.4 | S | | | | | | |
| | 5.10 | VW | 5.21 | M | 5.27 | M | Diffuse | |
| | 4.54 | S | 4.77 | S | 4.85 | S | | |
| Sphingomyelin | 65.5 A | VS | 62.0 | S | 59.0 | S | 55.5 | M |
| | | | | | 45.0 | M | 43.0 | S |
| | 17.8 | W | | | | | | |
| | 5.04 | VW | 5.10 | M | 5.24 | M | 5.30 | M |
| | 4.54 | S | 4.63 | S | 4.70 | S | 4.82 | S |

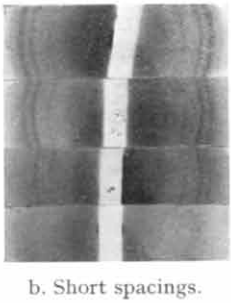
VW = Very weak. W = Weak. M = Medium. S = Strong. VS = Very strong.

* The principal long spacing of the freshly-prepared material is reported (W. GORDON ROSE, personal communication) to have been 55.2 A.

*References p. 384.*




a. Long spacings.




b. Short spacings.

Fig. 1. Examples of the effects of temperature on the diffraction pattern of synthetic dipalmitoyl-cephalin.

## RESULTS

The maximum experimental error calculated for the value of a principal long spacing in any diffraction pattern was $\pm$ 0.5 A. At least three series of experiments were carried out with each type of specimen. The types of changes in diffraction pattern with temperature varied considerably with the different phospholipids. Table I gives a comparison of the principal spacings recorded for these phospholipids at four key points in the temperature scale.

### *Dipalmitoyl cephalin*

Examples of both long and short spacing photographs are reproduced in Fig. 1. A diagram showing the variations of the principal long spacings with temperature is given in Fig. 2.

The fresh specimen (*i.e.*, the one with no previous heating or cooling history), when examined at 20°C, gave one long-spacing diffraction band at 57 A and three weak diffractions in the region of 15 to 40 A whose spacings could not be reliably estimated. There were also several short spacings which are listed in Table I. During the subsequent heating, clearly defined changes were observed in the long-spacing diffraction pattern. The band initially at 57 A remained practically unchanged up to about 70°C, but above this temperature its intensity began to decrease slowly and the spacing also dropped slightly to reach a value of about 54 A before the band became too weak to detect at a temperature of about 110°C. At about 70°C a second diffraction band appeared at 45 A. It reached its maximum intensity relatively quickly, and then started to fade. In some cases it disappeared well below 110°C at temperatures when the first band was still quite strong. The spacing of this second band appeared to be constant throughout. A third band appeared at a temperature of about 90°C. This was at first diffuse and the spacing was about 35 A, but it quickly gave rise to a very sharp diffraction band at 32.5 A which remained unchanged up to 150°C (the limit of the experiment). Cooling a fresh specimen to —15°C had no appreciable effect on the diffraction pattern.

The short spacing pattern also showed some interesting changes with temperature. Up to about 100°C the spacings represented in the pattern remained essentially the same, but there were marked changes in relative intensities. In general, the emphasis in intensity seemed to

*References p. 384.*

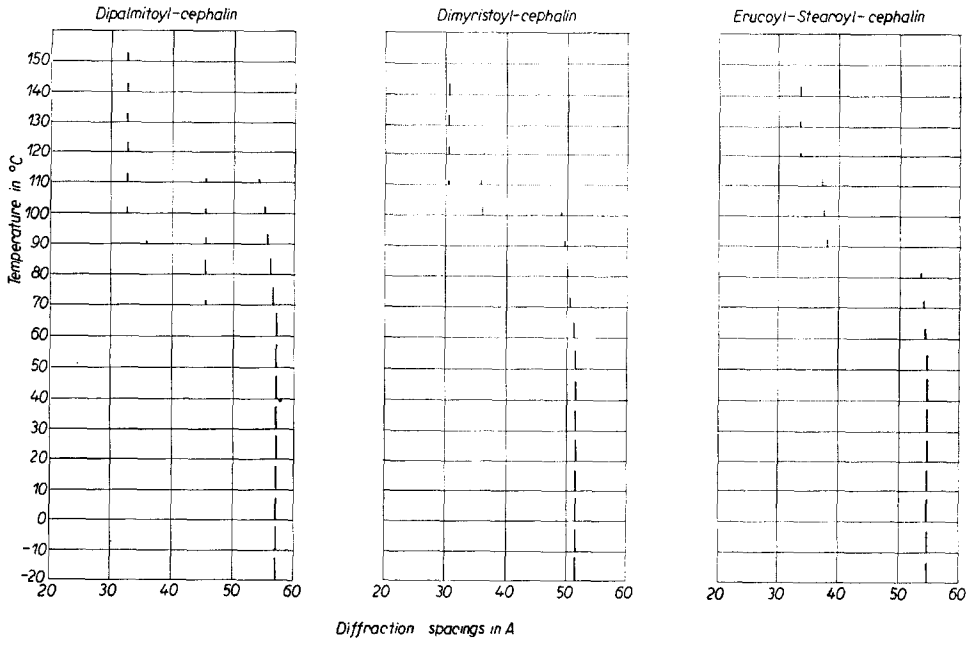



Fig. 2. Summary of changes in long-spacing diffraction patterns of synthetic cephalins.

shift towards the higher spacings with increase in temperature. No new short-spacing bands were observed to coincide with the appearance of the additional long spacings. Above about 100°C the short spacings fused to give diffuse haloes at about 4.5 A and 9 A.

On allowing a specimen to cool to room temperature after a heating experiment, the pattern obtained usually showed two long-spacing bands, one at about 45 A and the other in the region of 55 A (*i.e.* two or more Ångström units below that of the fresh specimen). When the specimen was re-examined a day or two later the 45 A band had usually disappeared, but it was often necessary to cool the specimen below 0°C before the longer spacing would return to its initial value of 57 A.

*Dimyristoyl cephalin*

The principal spacings at different temperatures are given in Table I, and the variations of the long spacings with temperature are represented diagrammatically in Fig. 2

The initial 51.5 A spacing of the fresh specimen remained unchanged up to about 60°C, and then showed a gradual shrinkage with accompanying decrease in intensity very similar to that observed with dipalmitoyl cephalin. A second diffraction band appeared at about 100°C. This was diffuse and corresponded to a spacing of about 36 A, but on increasing the temperature further it was resolved into two sharper bands at 36 A and 30.4 A. The former quickly faded but the latter increased in intensity and persisted at higher temperatures.

The short spacings showed changes in relative intensities with increase in temperature but no new spacings. These changes were similar to those observed for dipalmitoyl cephalin, but the spacings became diffuse at a lower temperature in the case of the dimyristoyl compound.

*References p. 384.*

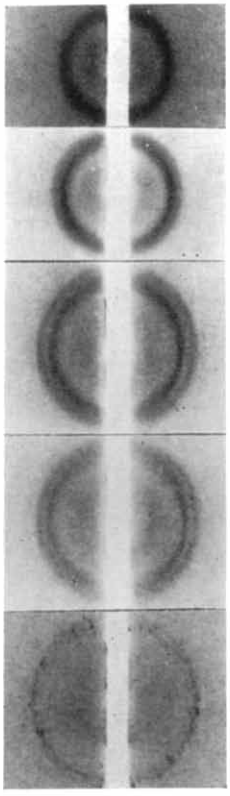
On allowing a specimen to return to room temperature after a heating experiment, only one long-spacing band was observed. This was initially at 49 to 50 A, but increased on cooling below 0°C.

*Erucoyl-stearoyl cephalin*

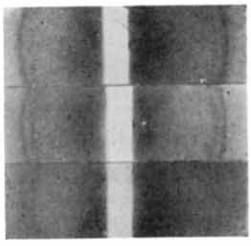
Details of results are given in Table I and Fig. 2. The initial long-spacing band was unchanged up to 50° to 60°C, but then showed a relatively rapid decrease in intensity accompanied by a much smaller shrinkage of spacing than was observed with the two saturated chain cephalins. At about 90°C, a diffuse band appeared at a spacing of approximately 38 A, and this remained diffuse over a temperature range of about 30°C before being replaced by a sharp band at 33.5 A. This latter band persisted to higher temperatures.

The diffraction pattern at 20°C showed two additional long-spacing diffractions which appeared to correspond to lower orders (second and third) of the principal diffraction band.

The short spacings showed little change before becoming diffuse below 100°C.




a. Long spacings.




b. Short spacings.

*Linoleoyl-palmitoyl cephalin*

At 20°C, the fresh specimen gave a diffraction pattern showing one well-defined long-spacing band at 38 A, and possibly a rather faint and diffuse band in the region of 80 A. In the short-spacing region only diffuse haloes at approximately 4.5 A and 9 A were observed. On heating to 150°C, the long spacing decreased to about 36 A and returned to 38 A on cooling. Cooling to —40°C produced no appreciable change in diffraction pattern.

*Hydrolecithin*

Examples of both long and short spacing diffraction patterns are given in Fig. 3, and a diagram showing the variations of the long spacings with temperature in Fig. 4. Spacings recorded at four different temperatures are given in Table I.

At 20°C, one sharp and intense long spacing was observed at 59.5 A and two principal short spacings at 1.84 A and 1.64 A. There was also a well-defined band at 16.4 A. This latter band disappeared before the temperature reached 70°C. At 50° to 60°C the 59.5 A spacing began to decrease steadily, and at about 70°C an additional band appeared at about 50 A. Successive diffraction photographs taken at 10° intervals up to 110°C all showed two closely spaced diffraction bands,

Fig. 3. Examples of the effects of temperature on the diffraction pattern of hydrolecithin.

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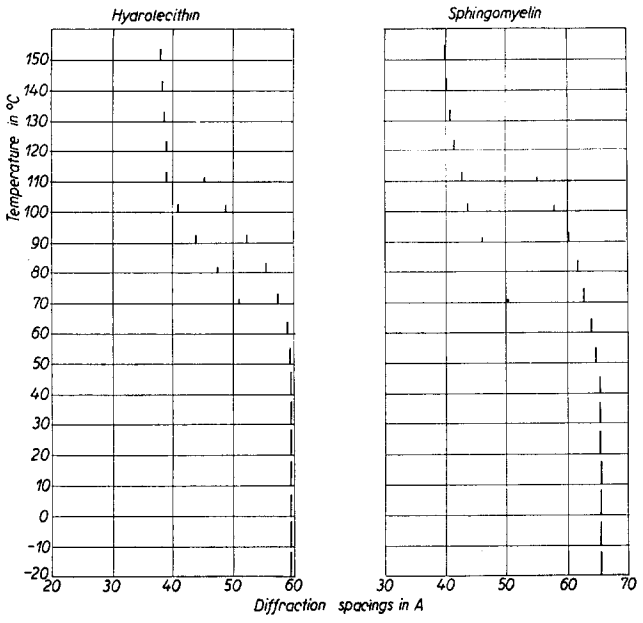
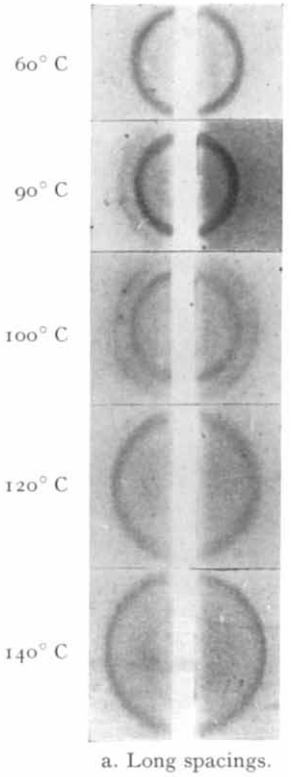
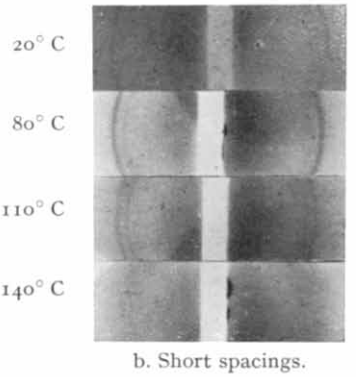



Fig. 4. Summary of changes in long-spacing diffraction patterns of hydrolecithin and sphingomyelin.




a. Long spacings.




b. Short spacings.

Fig. 5. Examples of the effects of temperature on the diffraction pattern of sphingomyelin.

the inner one being the more intense over most of the range. The spacings decreased steadily so that at 110°C the two bands were at 45 A and 40 A respectively. Above this temperature, the inner band became too faint to detect, but the 40 A band persisted up to at least 150°C. Parallel to the decrease in long spacings, the two short spacings showed gradual increases from 4.54 A and 5.10 A at 20°C to 4.85 A and 5.27 A respectively at 80° to 85°C. Above this temperature, only broad diffraction haloes were seen. When a specimen was reheated soon after finishing the first series of experiments, the changes in diffraction pattern were repeated but at somewhat lower temperatures. In one experiment, repeated heating and cooling led to the appearance of a diffraction band at 33.5 A above 100°C

*Sphingomyelin*

Results of observations made with sphingomyelin are given in Figs. 4 and 5 and in Table I.

The principal long spacing of the fresh specimen at 20°C was 65.5 A, and the corresponding short spacings 4.54 A and 5.04 A. There was also an additional well-defined long-spacing band at 17.8 A. Increase in temperature produced a steady decrease in the long spacing, commencing at about 50°C. This decrease seemed to be continuous, and

*References p. 384.*

the spacing was reduced to nearly 55 A before becoming too faint to detect (above 110° C). A second long-spacing band appeared at about 46 A when a temperature of about 90°C was reached. This spacing decreased steadily to 40 A at 150°C. When the specimen was allowed to return to room temperature, the diffraction pattern then obtained usually showed a long spacing of about 60 A, instead of the initial spacing of 65.5 A. On reheating such a specimen, the spacing decreased at a more rapid rate than in the initial experiment, and the second band appeared at a correspondingly lower temperature. The short spacing increased from 4.54 A and 5.04 A at 20°C to 4.82 A and 5.30 A, respectively, at 110°C. Above this temperature, the short spacings became diffuse.

*Acetal phospholipid*

This gave a very faint and poorly defined diffraction pattern at 20°C. It consisted of two bands at about 39 A and 65 A, and two fairly sharp rings at 4.50 A and 5.0 A. At about 50°C, the initial long-spacing pattern was replaced by one showing an intense and fairly sharp band at about 50 A. With further increase in temperature, this spacing decreased steadily to reach about 44 A at 110°C. The short-spacing diffraction associated with the appearance of this latter band was diffuse. On cooling to room temperature, the pattern lost definition and returned to its initial state. Cooling further to —40°C had no appreciable effect on the diffraction pattern.

## DISCUSSION

Details of the structures of the *n*-paraffins and of the long-chain fatty acids have been revealed by the studies of MÜLLER[10,11,12,13]. He established the zig-zag nature of the hydrocarbon chain, and gave a value of 2.54 A for the repeat distance along it (*i.e.* the distance between alternate carbon atoms.)

MALKIN and his collaborators have carried out X-ray and thermal studies on a number of additional long-chain compounds[2,3,6,8,15]. These include long-chain esters, diglycerides, triglycerides, and recently a number of synthetic $\alpha$ and $\beta$ (1:2 and 1:3) cephalins[3]. In some of these studies, two forms of a single compound were examined, one prepared for X-ray examination by pressing the material into a layer, and the other by melting and allowing to resolidify on a flat plate. Only general observations on the structures could be made from the diffraction data available. Several synthetic, saturated-chain cephalins were examined but only in the form of pressed layers. Distearoyl, dipalmitoyl, dimyristoyl, and dilauroyl cephalins of the $\alpha$ and $\beta$ series gave long spacings which when plotted against chain length (or number of carbon atoms in chain) were found to lie on two straight lines. From the slopes of these lines it was possible to deduce the angle of tilt of the hydrocarbon chain with respect to the crystallographic reflecting plane for the long spacing, which can also be considered as the plane of the bimolecular leaflet. By extrapolation of these lines to zero chain length, estimates could be made of the contributions of the end-groups to the diffraction spacings in the two series of compounds. In all cases the molecules were estimated to be arranged in the form of bimolecular leaflets in which the hydrocarbon chains were tilted with respect to the plane of the leaflet. The angles of tilt for the $\alpha$ and $\beta$ cephalins at room temperature were found to be about 77° and 55° respectively. The corresponding values for the contributions of the end-groups to the thickness of the bimolecular leaflets were approximately 15 A and 10 A.

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X-ray diffraction data on three synthetic enantiomorphic α-lecithins have been reported by BAER AND KATES[1]. No attempt was made by them to interpret these data, but if the results are treated as with the data obtained by BEVAN AND MALKIN[3] for the cephalins, it is possible to obtain an approximate value for the tilt of the hydrocarbon chains in the bimolecular leaflets, and for the length of the end-group. From the data it is clear that the hydrocarbon chains are approximately perpendicular to the plane of the leaflet, and that the end-group is about 10 A long in the direction of the chain.

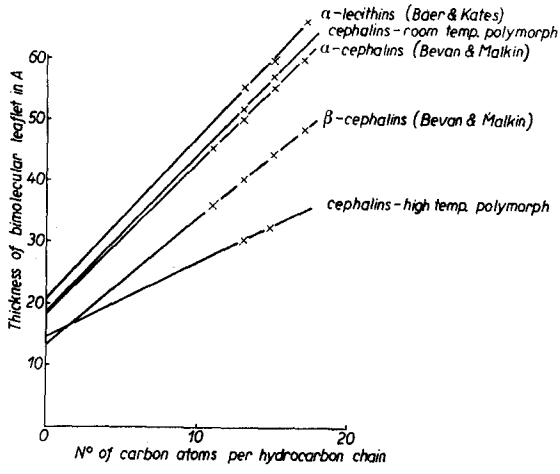


Fig. 6. Diagram showing relation between diffraction spacings (long spacings) and number of carbon atoms in hydrocarbon chains of phospholipids.

These previous results are referred to in order to facilitate a fuller discussion of the possible structural interpretations of the results reported in this paper.

Only two synthetic saturated-chain cephalins were available for this study, and these appear to correspond to the α cephalins of BEVAN AND MALKIN. In both cases the values of the long spacings at 20°C are slightly higher than those reported by the earlier workers for similar compounds. In these two samples of cephalin the hydrocarbon chains appear to be approximately perpendicular to the plane of the bimolecular leaflet. A line drawn through the two points obtained by plotting diffraction spacing against number of carbon atoms in chain (see Fig. 6) has a similar slope to that obtained by BEVAN AND MALKIN and cuts the C=O axis at about 19 A, indicating that the end-group contributes nine to ten Ångström units to the total length of each molecule. Construction of models show that this would be in keeping with a structure in which the chains are packed close together and the end-group is fully extended in line with the hydrocarbon chain (Fig. 7a).

At higher temperatures, the dipalmitoyl cephalin gives rise to two other, well-defined polymorphs in which the thickness of the bimolecular leaflet seems to be reduced to 45 A and 32.5 A, respectively. If this is considered as a simple tilting of the whole molecule with respect to the plane of the leaflet, then the angles of tilt would be of the order of 52° and 34°. In view of the sharp transitions from one form to another, these angles probably have some significance. The tilting of the molecules will result in a sliding of one

*References p. 384.*

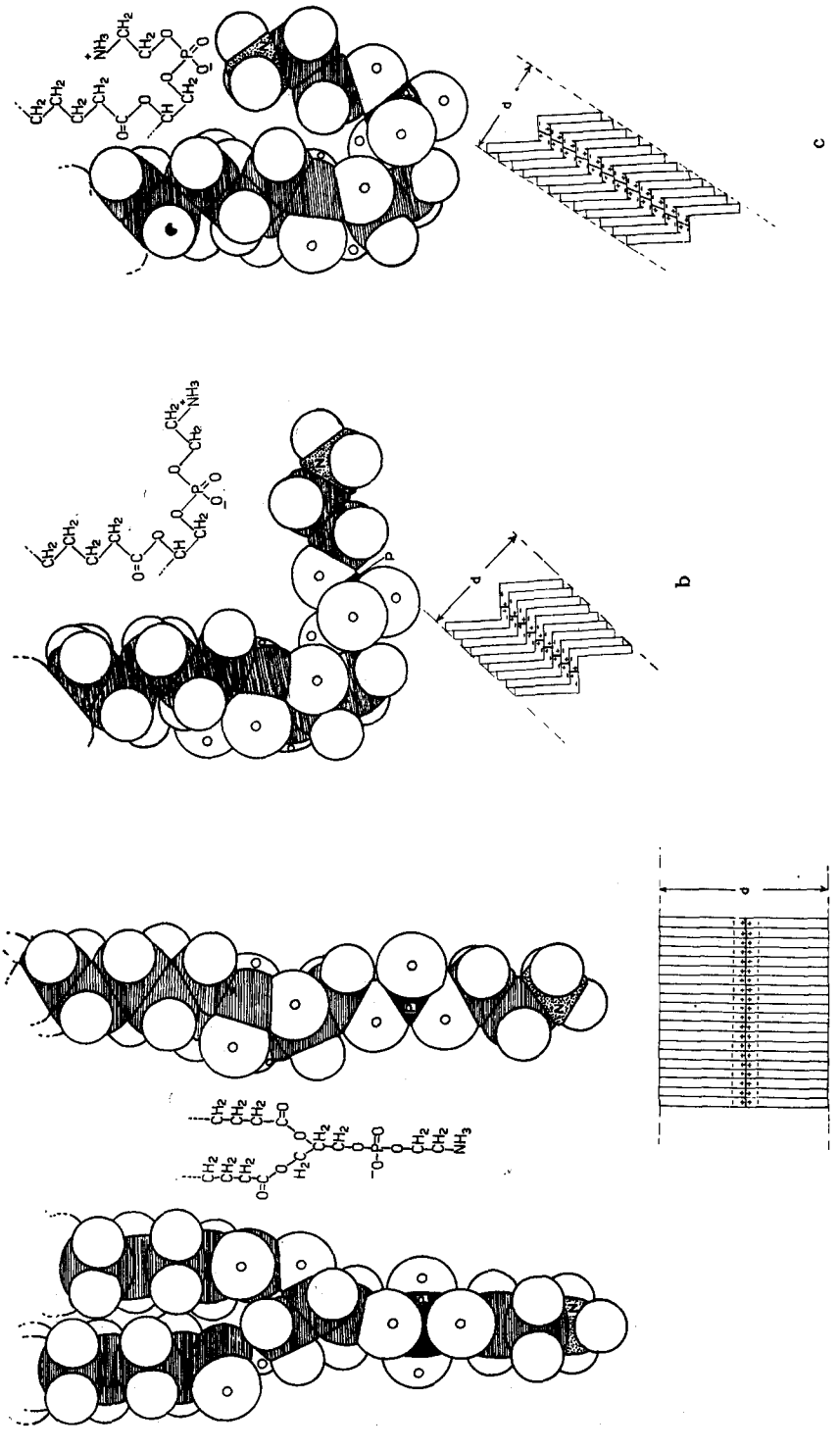


Fig. 7. Drawing illustrating possible modes of packing of phospholipid molecules into bimolecular leaflets.

molecule over the next in the direction of the length of the chain. This will change the relative positions of particular atoms or groups in adjacent molecules. It seems probable that the relative positions of the partially ionised groups ($-PO_4$ and $-NH_3$) in adjacent molecules will greatly affect the stability of any structural arrangement, and the significance of the two angles of tilt may lie in the consequent positioning of the two polar portions of the end-group.

These polymorphic transformations are reversible with temperature, which suggests competition between two types of intermolecular forces in the structure. These forces may well be on the one hand the VAN DER WAALS cohesion between hydrocarbon chains, tending to keep the chains perpendicular to the plane of the bimolecular leaflet, and on the other the tendency for the polar portions of the end-group to assume stable orientations towards each other. Increase in temperature would increase thermal motion and decrease the cohesive forces between hydrocarbon chains. The end-groups would thus gain more freedom of movement, enabling them to assume more stable orientations towards each other. Any relative movement of adjacent molecules in the direction of the long axis of the molecule would cause the hydrocarbon chains to slide over each other, thus changing the angle of tilt with respect to the plane of the leaflet. On cooling, the cohesive forces between chains would again increase, tending to pull the hydrocarbon chains back into line, and forcing the end-groups to resume their initial relative positions.

Dimyristoyl cephalin differs from the dipalmitoyl compound only in that it has two carbon atoms less per hydrocarbon chain, and therefore might be expected to give rise to a very similar series of polymorphic forms. However, the former does not show any polymorph which can be readily related to the form of diplamitoyl cephalin which is characterised by the 45 A spacing. The difference in chain length might mean a slight difference in the strengths of the cohesive forces between hydrocarbon chains in the two compounds, and this might in fact be reflected in the fact that the initial long spacing of the dimyristoyl cephalin begins to shrink at a somewhat lower temperature than is the case with the dipalmitoyl compound. This slight difference in inter-chain cohesive forces may enable the shorter-chain cephalin to go straight to the structure in which the most stable arrangement of end-groups is achieved without having to assume any intermediate position such as might be represented by the 45 A spacing in the case of the dipalmitoyl cephalin.

The polymorphs stable at high temperatures (*i.e.* above 100°C) are similar for the two compounds, and may represent the structure in which the most stable arrangement of end-groups is achieved. The arrangement would be expected to be the same for both compounds. The spacings of 32.5 A and 30.4 A, if considered to result from simple tilting of the fully stretched molecules (measuring 57 A and 51.5 A respectively), would give angles of approximately 34° 40′ and 36° 10′ for the respective angles of tilt. This difference is greater than can be accounted for by experimental error, and would appear to suggest that the relative positions of groups in adjacent molecules is different for the two structures. An alternative solution is to assume that the angle of tilt of the hydrocarbon chains is the same for the two structures, and in this case the tilt of the chain with respect to the plane of the bimolecular leaflet can be calculated from the slope of the line joining the points obtained by plotting the diffraction spacings against the number of carbon atoms in the hydrocarbon chain. The slope of this line gives the increase in thickness of the bimolecular leaflet per carbon atom increase in the hydrocarbon chain of the cephalin molecule. Comparing this with the value of 2·54 which (from MULLER's data) would be

* *References p. 384.*

the rate of increase in thickness if the chains were perpendicular to the plane of the leaflet, the angle of tilt of the hydrocarbon chain can be obtained. The value calculated from Fig. 6 is approximately 24° 40′. In order to reconcile this angle of tilt with the measured thickness of the bimolecular leaflet, it is necessary to conclude that the end-group is no longer in line with the hydrocarbon chain, and is in fact inclined to the plane of the leaflet at a much steeper angle. This deduction is supported by the fact that if the line joining the two spacings in Fig. 6 is extrapolated to zero chain length, the contribution of the end-group to the diffraction spacing is found to be about 15 A for the high-temperature polymorph as compared with 19 A for the low-temperature polymorph. If the end-group is considered to remain as a rigid rod hinged to the hydrocarbon chain, then the angle between the end-group and the plane of the leaflet would be of the order of 50°, and consequently the angle between end-group and hydrocarbon chain either about 90° or about 140°. Fig. 7b shows a model in which the end-group is rotated into a position approximately at right angles to the direction of the hydrocarbon chain. Such a structure would enable the $PO_4$ and $-NH_3$ groups of adjacent molecules to come into closer relationship than hitherto, but the $-NH_3$ groups of opposite molecules in the bimolecular leaflet would still be in opposition. At present there is no evidence to show that the end-group necessarily remains as a rigid rod hinged to the hydrocarbon chain, and Fig. 7c shows an alternative structure in which the end-group is curled round into a position in which the $NH_3$ groups of opposite molecules in the bimolecular leaflet are also separated. This would appear to be a relatively stable arrangement of the polar parts of the end-group, and is the structure in which the highest degree of tilt of the hydrocarbon chains is attained.

This diffuse band at about 35 A which was observed over a narrow range of temperature in the case of dimyristoyl cephalin may also reflect an intermediate degree of tilt, but probably not a very stable one. The slight contractions of the initial 57 A and 51.5 A spacings with increase in temperature may be due to a slight tilting of the molecules, or perhaps to a limited overlapping of the tips of opposed end-groups in the bimolecular leaflet.

The erucoyl-stearoyl cephalin shows two well-defined polymorphic forms (as indicated by the long-spacing diffraction patterns), and possibly an intermediate form which does not however give a sharp diffraction band. The high-temperature and low-temperature forms resemble those of the saturated-chain cephalins, but it was found impossible to relate them by simple comparison of diffraction spacings and chain lengths. The initial spacing of erucoyl ($C_{21}$)- stearoyl ($C_{17}$) cephalin, considered on the same terms as the saturated compounds, would appear to represent a structure in which the hydrocarbon chain already shows an appreciable angle of tilt to the plane of the bimolecular leaflet. The high-temperature polymorph would appear to exhibit a higher degree of tilting than was found in the case of the corresponding forms of the saturated-chain cephalins. To account for these differences in angle of tilt there is the difference in lengths of the two chains in the erucoyl-stearoyl cephalin, and the fact that one of the chains (the erucoyl) is unsaturated. The stability of the tilted form of structure at low temperatures must also be accounted for by the dissimilarities of chain lengths or the presence of an unsaturated hydrocarbon chain.

Linoleoyl-palmitoyl cephalin also contains dissimilar chains, one of which is unsaturated, but this particular sample only gave one form, which showed little change in spacing over the range of temperatures examined. In this form the chains would

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appear to be held at a low angle of tilt to the plane of the bimolecular leaflet, but with this specimen, in addition to the differences in the chain lengths and the unsaturation, the partial oxidation which has probably taken place may also be effective in stabilising the tilted form at low temperatures.

In the case of the dipalmitoyl lecithin examined in these experiments, the initial long spacing was 59.5 A. If the highest spacing (29.4 A) reported by BAER AND KATES for their synthetic α-lecithin is considered to be a second order diffraction, then the two results are in quite good agreement. The hydrolecithin isolated from lung can thus be considered to be an α-lecithin, and the structure at 20°C probably takes the form of a bimolecular leaflet in which the molecules are approximately perpendicular to the plane of the leaflet and the end-group fully extended in line with the hydrocarbon chain.The structure would thus be very similar to that of the α-dipalmitoyl cephalin at the same temperature, the difference in spacing being accounted for by the greater bulk of the terminal choline group of the lecithin as compared with the corresponding ethanolamine of the cephalin. The variations of the long spacings with temperature, however, follow quite different paths for the two compounds. The dipalmitoyl lecithin shows the same marked decrease in spacing with rise in temperature, and over some of the temperature range two distinct polymorphs are indicated, but there are not the same clear cut transformations as with the dipalmitoyl cephalin. With the lecithin, the changes appear to be more continuous, spacings decreasing by ten to fifteen Ångström units without a definite break. The explanation of these differences in behaviour between lecithins and cephalins with identical hydrocarbon chains is probably to be found in the nature of the end-group. It would appear (from previous considerations) that the effect of relative positions of the polar groups of adjacent molecules cannot be so marked in the case of the lecithin, as was considered probable for the cephalin. It has been suggested that the phosphate group of cephalin is much more polar than that of lecithin or sphingomyelin[5,14]. However, in the experiments in which this was demonstrated, the cephalin sample probably contained large amounts of phosphatidyl serine which would account for the high acid value. There would appear to be no good evidence to suggest that phosphatidyl ethanolamine is in fact more highly ionised than phosphatidyl choline, and the differences in behaviour reported in the present paper may have to be explained solely in terms of the difference in bulk of the end-groups.

The changes in diffraction pattern with temperature of the specimen of sphingomyelin resemble those observed with lecithin rather than those of cephalin. The initial long spacing probably represents the thickness of a bimolecular leaflet in which the hydrocarbon chains are approximately perpendicular to the plane of the leaflet and the end-group extended in line with the chain. The decrease in spacing is continuous as with lecithin but is less marked. The second long-spacing band, which is assumed to represent a different polymorph, appears at a higher temperature (90°C as compared with 70°C) and a correspondingly lower spacing (46 A for sphingomyelin and 51 A for lecithin). This second sphingomyelin also shows a continuous decrease in spacing with further rise in temperature, and shrinks to 40 A at 150°C. These decreases in spacing are probably due to tilting of molecules in the bimolecular leaflet, but the precise angle of tilt cannot be determined from the present results. The changes in the short-spacing diffraction patterns of lecithin and sphingomyelin with rise in temperature also show some similarities. They both showed gradual increases in the values of the principal short spacings, whereas with the cephalins the spacings remained constant and only the relative intensities

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changed. In no experiments were new short spacings observed when the long spacings indicated that a second polymorph had been produced, and in the case of the saturated-chain phospholipids no well-defined short-spacing bands were observed after the initial long spacing had disappeared. This latter effect may be largely a thermal effect, but in general the evidence seems to indicate that the short-spacing diffractions observed are associated with the initial low-temperature polymorph. The short spacings of lecithin and sphingomyelin increase as the long spacing decreases, indicating that the short spacings are not directly related to the long axis of the molecule (*e.g.* as high order diffractions), and suggesting that they are related to the portions of the end-group which are separated by the action of tilting. Thus for instance, from the considerations outlined above for the long axis, it is suggested that the tilting is produced by a separation of the polar portions of the end-group, and the short spacings may be related to the distances between these particular groups. In the case of the cephalins (dipalmitoyl and dimyristoyl) the short spacings do not change appreciably, but in this case the long spacing undergoes a relatively slight shrinkage, and this appears merely to shift the emphasis in intensity to the higher short spacings already present. The low-temperature polymorph of erucoyl-stearoyl cephalin appears to represent a structure in which the chains are tilted considerably with respect to the plane of the bimolecular leaflet, and it still gives well-defined short-spacing diffractions, which means that if tilting is partly responsible for the diffuseness of the short spacings it must be an extreme degree of tilting. Thermal motion probably plays a part too. The reason for the lack of definition of the long spacing pattern of acetal phospholipid at 20°C is not clear. It does not appear to be merely a crystallizing phenomenon, for even after heating, when a fairly sharp 50 A band is produced, the pattern again becomes indefinite on cooling.

In general it would seem that the long spacings of phospholipids tend to decrease with rise in temperature, the decrease being associated with a tilting of the long axes of the molecules with respect to the plane of the bimolecular leaflet. In the case of the cephalins, this tilting appears to take place in steps, giving rise to a set of distinct polymorphs, but with lecithin and sphingomyelin the process of tilting appears to be more continuous though these too show distinct polymorphic forms at certain stages of heating. These differences in behaviour may be associated with differences in the nature of the end-groups. In the case of the saturated-chain cephalins, there is some evidence to suggest that the whole phospholipid molecule does not behave as a rigid structure when tilting, but that the hydrocarbon chain and the end-group are tilted at different angles. This may also be the case with the lecithins and sphingomyelins. The changes in the short spacings are in keeping with this type of explanation.

Further studies of closely related series of phospholipids when these become available should lead to more detailed information on these structures.

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SUMMARY

The variations with temperature of the X-ray diffraction patterns of a number of phospholipids have been studied. The long spacings of these compounds tended to decrease with rise in temperature, often in well-defined steps which suggested the formation of distinct polymorphs. This decrease in long spacing is considered to reflect a tilting of the long axis of the phospholipid molecule with respect to the crystallographic reflecting plane (*i.e.* the plane of the bimolecular leaflet). The mechanism of tilting is discussed.

RÉSUMÉ

Nous avons étudié les variations avec la température des figures de diffraction des rayons-X d'un certain nombre de phospholipoïdes. Les longues distances réticulaires de ces composés tendent à décroître lorsque la température s'élève souvant en étappes bien définies qui suggèrent la formations de polymorphes distincts. Cette diminution des distances réticulaires est peut-être due au fait que le long axe de la molécule de phospholipoíde penche vers le plan cristallographique réflecteur (c. à d. au plan du feuillet bimoléculaire). Le mécanisme de ce phénomène est discuté.

ZUSAMMENFASSUNG

Es wurde die Temperaturabhängigkeit der Röntgenstrahlenbeugungsfiguren einer Anzahl Phospholipoide untersucht. Die langen Netzebenenabstände dieser Verbindungen neigen dazu beim Ansteigen der Temperatur, oft in wohldefinierten Stufen, die die Bildung bestimmter Polymorpher vermuten lassen, abzunehmen. Man vermutet, dass dieses Abnehmen der langen Netzebenenabstände das Kippen der Längsachse des Phospholipidmoleküls hinsichtlich der kristallographischen Spieglungsebene (d.h. der Ebene der bimolekularen Blättchen) widerspiegelt. Der Mechanismus des Kippens wird besprochen.

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