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Citation: The Journal of Chemical Physics **79**, 469 (1983); doi: 10.1063/1.445545
View online: http://dx.doi.org/10.1063/1.445545
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Transitions and molecular packing in highly purified 1,2-dipalmitoyl-phosphatidyl choline–water phases. II. The structures of DPPC phases with low water content

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The structures of the polar regions are established for a series of bilayer phases with less than four water molecules per lipid molecule and shown to be closely related. For this analysis, the size and symmetry of the unit cells, determined for three phases for which single crystals were grown, were compared with the size and shape of the phosphorylcholine group and the influence of the charges on the packing. The structures of two of these crystalline phases are almost identical with that of a dimyristoyl lecithin for which some details of a structure determination were reported recently. For other phases, reference is made to the chain subcells, which are obtained from x-ray diffraction data and to the polar subcells which can be identified. The polar structures can also be related, directly or indirectly, with those in the crystalline phases from evidence of the changes occurring at the transitions. A square packing of the polar groups is observed for an anhydrous phase and for a transient phase formed at higher temperatures from samples with higher water content. The choline groups are packed in a rectangular array with an hexagonal chain packing for a phase with from one to three water molecules per lipid. In this phase, the chain axes are perpendicular to the bilayer plane and the choline groups and phosphate nonester oxygen atoms are not coplanar. This arrangement is stabilized by the proximity of another bilayer. The crystalline monohydrate phase involves a rectangular packing with coplanar phosphate nonester oxygen atoms and choline groups. This very stable polar structure is found at higher temperatures for the dihydrate and at room temperature for phases with higher water contents.

INTRODUCTION

For this discussion, low water content is defined as molecular ratios r of water to lipid of less than seven. In this region, the influence of polar layers in different bilayers on each other is strong.

While the preparation and study of highly purified lipids has given much new accurate data, the analysis of molecular packing in these phases requires methods which differ from those currently favored in crystallographic and lipid research. The molecular packings in single crystals can be established by standard crystallographic procedures although there are severe problems in applying these to lipids. In the absence of a complete crystal structure analysis, unit cell dimensions and symmetry can be related to molecular size and shape, giving the general features of the packing. This procedure was highly successful for ionic crystals and oxides.[1] Alternative structures may be possible and other procedures must be used to confirm the conclusions. As noted in part I, structures requiring unusual bond lengths and atomic radii can be excluded.

Among methods of value for lipid structures, are the identification of the subcells which result from the packing of molecules with a regular structure. These are most evident in hydrocarbons, owing to the large number for long chains, and all DPPC phases show diffraction from hydrocarbon subcells. Weaker reflections from some DPPC phases can be identified with a two-dimensional subcell of the phosphorylcholine group. The subcells contain few atoms in each repeating unit and can usually be identified from powder data. However, the structure of the $0\perp$ subcell in DPG single crystals was established and refined with great accuracy by standard crystallographic methods.[2]

The subcells and unit cells are not simply related. This is because the packing between molecules differs from that in the molecule. In DPPC, the packing in the polar and diglyceride regions is different. For another example, in hydrocarbon crystals, the packing between the methyl groups differs from that between the bonded carbon atoms. In hexatriacontane,[3] the 1.270 Å spacing of the 0, 0, 2 subcell reflection corresponds to 0, 0, 74.9 of the main cell, so that both 0, 0, 74 and 0, 0, 76 are strong. The subcell and main cell axes are often inclined to each other, as in monoclinic hexatriacontane[4] and crystalline DPG. With the bilayer spacings, the chain subcell reflections are strongest from DPPC phases, an example being 0, 0, 98 from DPPC phase 2. Together with the presence of an unusually long axis, this gives problems in attempts to establish unit cell dimensions from powder data obtained from DPPC phases not available as single crystals. Good numerical agreement between the spacings may be fortuitous and should be confirmed by comparison of the measured and calculated densities. For this reason, these methods are not relied on here and confirmation of two-dimensional unit cells is even more difficult. Samples can be indexed by assuming that more than one phase is present, but evidence for coexistence of the phases is essential. Procedures to obtain single phase samples are advantageous.

DPPC phases in the presence of excess water often show good three-dimensional ordering in the diglyceride region by the presence of subcell reflections. This corresponds to a two-dimensional molecular ordering in the bilayer plane, but there is no strong evidence of molecular order between bilayers. The long spacing does indicate a regular stacking of the bilayer–water regions. For such systems, the subcell can be used

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for structural investigations and the value of molecular cells is doubtful.

Another procedure used here, especially for polar groups, is to relate their molecular dimensions and types of packing to molecular areas. Highly accurate data for this are given by the crystalline phases and their relation to the other phases. This method is based on the division of the bilayer into regions with different packing and on the planar arrangement of the polar groups. Whilst a planar arrangement of the molecules in the bilayer is tacitly assumed in many discussions, the structure of DMPC[5] shows that this is not correct for low water contents. However, results obtained which relate the structure of DPPC phase 6 with that of DMPC confirm the identification of the polar group packings which is made here.

The different packing of the polar and diglyceride regions is shown in the structures of DMPC[5] and dilauryl-dl-phosphatidylethanolamine.[6] This results in two molecular conformations and a displacement of the phosphorus atom by up to 2.4 Å from the line of chain 1. Each molecule occupies the same area in each region but the ester group permits packing into different subcells which arise because of the different shape and intermolecular forces. The flexibility of the ester group is indicated by a survey of crystallographic data,[7] this property is important for other vital biological systems.

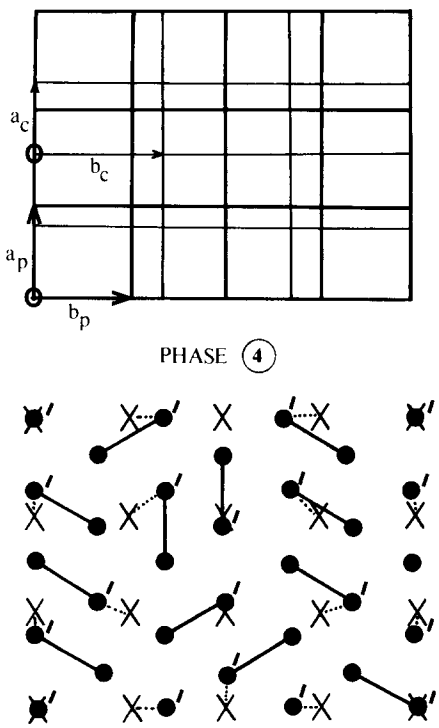

FIG. 1. Top: Superposition of subcells attributed to chains, $a_c = 4.90$ Å; $b_c = 8.48$ Å, and to polar groups $a_p = b_p = 6.56$ Å. [(Dimensions obtained by x-ray diffraction of oriented sample from single crystal in vacuum (anhydrous).] Axes of subcell parallel, as shown. DPPC phase 4. Bottom: Phase 4 structure obtained from subcells and molecular geometry. Relative displacement of the subcells would change details of the structure but general features would be the same. The chains are shown as solid circles joined by solid line when in the same molecule. Phosphate groups, X, are in square lattice joined to diglyceride groups by ester, shown as dotted lines. Projected on to a–b plane.

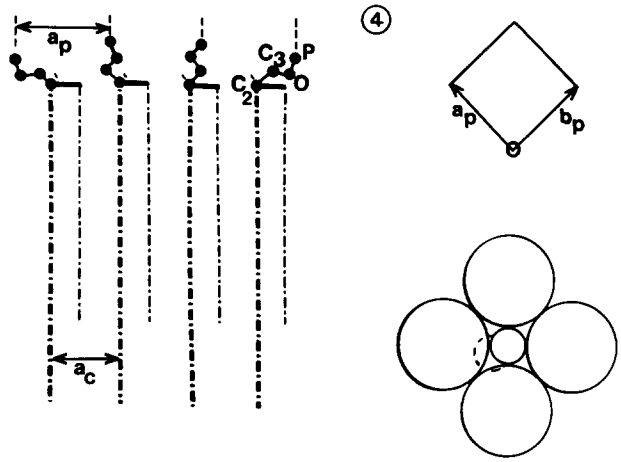

FIG. 2. Left: Diagram illustrating molecular variation resulting from different chain and polar lattices. The structure of phase 4 varies in three dimensions and is more complex. The chains are shown as broken lines in this figure. Right: Packing of choline groups (large circles) with oxygen atoms (small circles) into square polar lattice as observed in DPPC phase 4. Diameters are usual values. The orientation corresponds with that shown in subsequent figures.

Anhydrous DPPC phase 4

An oriented sample was studied in vacuum by x-ray diffraction. This is probably anhydrous and will be called phase 4. On dehydrating phase 2, the crystals crack when a small water loss occurs, but the chain packing is retained in the sample at room temperature although the transition temperature is lower for dry samples. In phase 4, two subcells are visible with parallel axes of different lengths as given in Table I of part I. This relation between the axes suggests that they are from the same phase. One of the subcells is square and the other rectangular and these can be assigned to the polar and chain regions. The chain subcell involves a slightly expanded hexagonal packing. A slight difference in the subcell areas indicates a small chain tilt. The square subcell does not correspond to any known packing of the chains and would require chain diameters smaller than those usually observed. If two phases are present, their orientations and lattices would also be related, but a more complex analysis would be required.

Superposition of subcells, as shown in Fig. 1 (top) gives a larger cell requiring 12 slightly differing molecular conformations, as shown at the bottom. The phosphate groups are displaced from the line of the axis of chain one by variable distances. There is a maximum lateral displacement of 2.4 Å as for other structures.[5,6] This displacement can be accommodated by the ester group which gives a variation in the angle between the line of the phosphate-ester and chain 1, as illustrated in Fig. 2 (left). Molecular lengths will differ by up to 0.9 Å which compares with a difference in length between molecules A and B in DMPC[5] of about 1.6 Å.

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The packing proposed for the polar layer is shown in Fig. 2 (right). A diameter of 6.56 Å for the choline group is in excellent agreement with the estimate of 6.34 Å for closest packing made in part I. The diagonal of 9.28 Å for the square lattice, gives a diameter of 2.72 Å for packing a single oxygen atom at the center of the square array of choline groups.

In the absence of water a choline group from the adjacent bilayer will be packed in contact with the four choline groups and above the oxygen atom to stabilize the square array. This is also a stable lattice for the charges.

It is evident from Fig. 1 (bottom) that there is a variation in alignment of the diglyceride groups and of the carboxy-ester groups (2). This is consistent with the small expansion of the chain subcell observed as compared with the usual values. The square polar lattice occupies a larger area than a rectangular array as observed in several other phases.

Monohydrate crystal phase 1

This phase will be discussed in more detail later, as the packing in the polar region is similar to that in phases with higher water content. The large area per molecule allows both phosphate oxygen atoms to be in the same plane as the choline group when tightly packed. This very stable polar layer structure stablizes loosely packed chains, giving a structure in this region which is very similar to that of potassium.[8] Because of the chain packing, the molecular volume is relatively large, as shown by Table III(b), part I.

Crystalline dihydrate phase 2

The crystals of this phase are well ordered and give high resolution x-ray data.[9] Highly purified samples and slow solvent crystallization contribute greatly to the ease of preparation of highly ordered DPPC phases. Discussion of the structures of phase 2 and closely related phases has been greatly advanced by the solution of the structure of dimyristoyl lecithin, DMPC, by Pearson and Pascher.[5] DPPC phase 2 and DMPC evidently have virtually identical packing in each bilayer. The chain tilt alternates in DPPC phase 2, doubling the C axis length so that d_{100}DMPC + 5.1 Å = $C/2$ DPPC. This conclusion is supported also by structural analysis and details of the Raman spectra,[10] although some conclusion from the latter differ from those in Ref. 6.

In DMPC, the phosphorylcholine groups are antiparallel, i.e., the $P-N$ axes in the molecules are all aligned in a direction close to the b axis but with opposite sense in adjacent rows. This arrangement will be used for the discussion of samples prepared directly from phase 2, but a structure with parallel polar groups can pack with the same area per molecule.

The $P-N$ axis tilt away from the bilayer plane and there are two molecular conformations in DMPC. In molecule B, using the nomenclature of Pearson and Pascher,[5] the nonester oxygen atoms are displaced from the nitrogen atoms by about 1.4 Å along the c axis. For molecule A, this lateral displacement is about 0.7 Å. As discussed in part I (see Fig. 5), this gives a more stable array of charges but reduces the forces between the polar layers.

In DMPC, adjacent molecular rows A and B in the same layer are displaced by 2.5 Å along the chain axes, which are nearly parallel to c. This enlarges the area occupied by the polar groups, in planes titled from the bilayer plane, while allowing close chain packing along a. If these molecular displacements were in the same direction, as in the crystalline phase of DPG and other glyceride phases, the polar layers would be closer in directions perpendicular to the layers. The packing found gives the minimum area in the bilayer plane and close chain packing. This constrasts with phase 1, where the polar packing is more stable; the extra water molecule in phase 2 slightly reduces the forces between the polar groups.

Water molecules separate phosphate groups along the axis for DMPC[5] and form a ribbon between the molecules along the b axis. The nature of the bonds between these molecules will be discussed in separate publications.

This structure shows the influence of intermolecular forces. The distance of 4.39 Å between the chains along the a axis gives an attractive force which is relatively large because of the number of CH_2 groups. This compresses the polar layer giving the lowest area per molecule in the bilayer plane for any DPPC phase. The area per molecule is 39.3 Å^2 for DPPC phase 2 in the bilayer plane. As the chains are tilted by 12° to this plane, this gives an area perpendicular to the chains of 38.37 Å^2. There are two planes of polar groups titled by ± 30° from the plane perpendicular to the chains, giving an area per molecule for these planes through the polar groups of 44.30 Å^2. The molecular displacement is required because the polar groups cannot be compressed. All the molecules are arranged in rows along the b axis so that all molecules A are in one set of planes and all molecules B in another set, both planes being parallel with the bilayer.

In the absence of the polar groups, the chains can be 4.2 Å from each other so that the greater distance of 4.39 Å observed in phase 2 is due to the forces exerted by these groups.

DPPC trihydrate phase 3

This forms excellent single crystals, similar in appearance and structure to those of phase 2. Their properties are consistent with the preceding discussion but detailed experiments with them have not been made, except for the determination of the cell dimensions and the main features of the diffraction pattern. There is a slightly expanded lattice, due to the addition of a third water molecule which is probably associated with the choline group as discussed earlier. As shown in Table III(b), of part I, there is precise agreement between the molecular volumes of phases 2 and 3.

DPPC phase 6

This is formed by heating the crystalline phase 2 above 64 °C. and cooling in a sealed container. The main fea-

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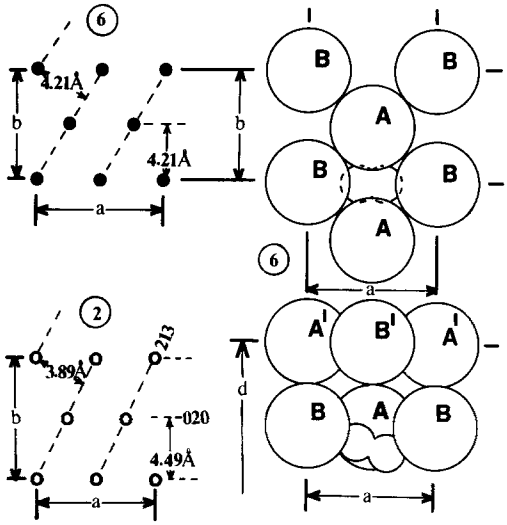

FIG. 3. Left: Chain packing and unit cells for phase 6 (top) and phase 2 (bottom). Projected on to bilayer plane. Chains as solid circles in phase 6 and circles in phase 2. Right: proposed packing of choline groups (large circles) in bilayer plane of phase 6 (top) a choline group diameter of 6.42 Å gives the same area as that occupied by the chains. Right (bottom): Packing of choline groups in a bilayer section for phase 6, the phosphate oxygen atoms cannot be in the same plane as the choline groups but will be closer to the diglyceride. A double layer of choline groups is shown, those in a different bilayer distinguished by a prime.

tures of the molecular packing can be established both from the chain packing and by reference to the structure of phase 2.

X-ray powder diffraction photographs of phase 2 show two strong lines from the chain subcell with spacings of 3.887 and 4.489 Å. These are the 213 and 020 reflections, the chain axes lying in these planes. For phase 6, the strong line with a spacing of 4.21 Å is observed, characteristic of the hexagonal chain subcell. The chain packing and unit cell for phase 2 are compared with the chain packing and a corresponding bimolecular cell for phase 6 in Fig. 3, at the top. The structure proposed for phase 4 has subcells for the chain and polar regions which differ in shape, giving a complex unit cell. However, the unit cell based on the chain subcell shown for phase 6 corresponds to a stable packing of the polar groups, The dimensions of this cell are $a_6 = 9.7$ Å which is longer than $a = 8.78$ Å for phase 2 and $b_6 = 8.4$ Å which is shorter than $b = 8.95$ Å for phase 2. The area in the bilayer plane is slightly greater, as shown in Table III of part I.

A planar packing of choline groups giving the same cell as for the chains is shown in Fig. 3 (middle). A rectangular cell with $a = 9.7$ and $b = 8.4$ Å gives a choline group diameter of 6.42 Å for the packing shown. This compares with 6.34 Å from standard atomic dimensions and 6.56 Å from DPPC phase 4. The packing of the choline groups is intermediate between square and hexagonal, the latter being the closest possible packing. A retangular cell provides a location of the right shape for the two phosphate oxygen atoms as shown in the dotted circles in Fig. 3 (middle). For the cell dimensions obtained for phase 6, this pair of atoms is too large to be placed in the same plane as the choline groups. A location between these groups and the diglyceride as in Fig. 3 (bottom), gives a stable array of charges for two polar layers in close proximity, as discussed in Part I (Fig. 5).

Transition from phase 2 to phase 6

The bilayer repeat distance for phase 2 is 2.54 Å longer that in phase 6. If this was the result of an increase in chain tilt, a density decrease of 4%–5% would be required, which is not observed. The accuracy of density measurements in these phases can be slightly affected by changes in water content but the good agreement between $d_{calc.} = 1.0854$ and $d_{obs} = 1.0915$ for phase 2 shows that these errors are small. These results indicate that when phase 2 transforms to phase 6, the molecular displacement along c disappears making the diglyceride groups in phase 6 coplanar.

The effect of this transition on the polar region is illustrated in Fig. 4. On the left, the packing of the choline groups in DPPC phase 2 is shown projected on the bilayer plane a–b and on the plane perpendicular to the bilayer. This is drawn from Fig. 1 of Pearson and Pascher.[5] On the right in Fig. 4 is shown the effect on this packing of an expansion to $a = 9.7$ Å, a contraction to $b = 8.4$ Å and a relative translation of alternate rows of molecules by 2.54 Å along the direction of the chain axes. Both the bilayer plane and a plane perpendicular to it are shown, as before. The molecular rows A–B' move relative to the rows B–A', where the prime indicates molecules in different bilayers, and the new axes are those obtained from the chain packing. This move-

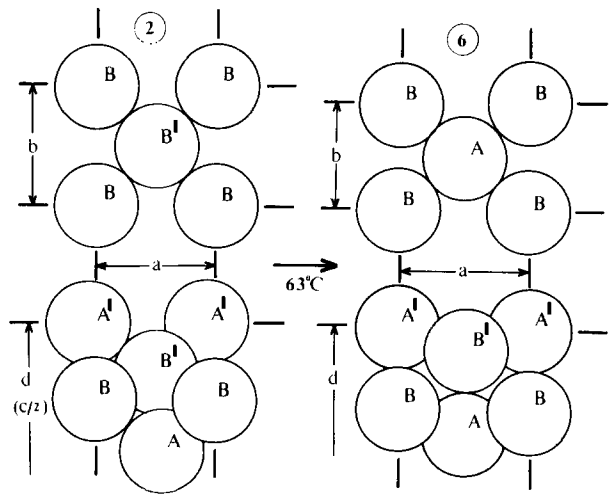

FIG. 4. Left: Packing of choline groups in bilayer plane of DPPC phase 2 (top) and perpendicular to bilayer plane (bottom). $a = 8.78$ Å, $b = 8.95$ Å, $C/2 = 60$ Å (Table I, part I). Packing as in DMPC, molecular conformations A and B indicated (Ref. 5). Groups in different bilyaers distinguished as in Fig. 3. Right: packing of choline groups in bilayer plane of DPPC phase 6 (top) and perpendicular to bilayer plane (bottom). $a = 9.7$ Å; $b = 8.4$ Å, and $d = 57.46$ Å (Table II, Part I). The packing is derived from that in phase 2 by a translation of 2.5 Å along the long molecular axis and a small change in chain packing as illustrated in Fig. 3 (left).

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ment shortens the bilayer spacing from 60 Å in phase 2 to 57.46 Å in phase 6.

In phase 2, the $a-b$ plane of the unit cell corresponds to a close packing of the choline groups B and B' from different bilayers in nearly square array, but the translation results in the packing together of choline group from molecules in the same layer in phase 6. The choline groups B and B' in phase 2 are in planes separated by nearly 2Å but, because of their large radii, this only reduces the area by 3.7 Å^2. This value was obtained by comparing the areas of phases 2 and 4 given in Tables IIIB and IIIC in part I.

From Fig. 1 in Ref. 5, the distance between choline groups in different bilayers along the c axis of DPPC phase 2 is estimated to be 5.94 Å. The molecular lengths L measured along c, were estimated from the same figure. A difference in length $L_B - L_A = 1.6$ Å was found made up of 0.8 Å from the increased tilt of the $P-N$ axis to the bilayer plane in B and of 0.8 Å from the change in angle of the phosphate-ester diglyceride link between the two molecules. The difference was attributed[15] to the stronger interaction of molecule B' in a different bilayer rather than with molecules A in the same layer. This is clearly illustrated in Fig. 4 (left). After the transition to phase 6, the forces on molecules A and B will be similar so that they will adjust towards the same length. This does not alter the bilayer spacing, but will make the choline groups coplanar, as shown in Fig. 3 (left).

There is a chain tilt of 12° in DPPC phase 2, but it was concluded from Fig. 2 of part I that the area per molecule for phase 6 does not vary with reduction in water content. The limit in area was identified with zero chain tilt. Data from an oriented DPPC phase[12] with a water content of 2% gives the same volume per molecule as phase 6 with 5% water when allowance is made for the water content. This requires a constant chain tilt, as the chain subcell is the same for both samples. From the volume per molecule for phase 6 with 5% water, calculated from the chain spacings and bilayers, bilayer spacings for lower water content were estimated. They are 56 Å for 2.48% and 55.7 Å for 2% water, the latter being the value observed.[11] Measurements for samples with lower water content are more precise that those made when more water is present.

Thus, at the transition from phase 2 to 6, there is an apparent change of tilt from 12° to zero. This is consistent with accurate bilayer measurements for the DPG $L\beta$ and crystalline phases which indicate slightly shorter chain lengths for the hexagonal packing.[12] The effect is due to the "disorder" or twist of the chain axes previously discussed in part I.

When two layers of choline groups as in Fig. 3 (left) are packed closely without water, the site which may be occupied by B' is slightly elongated. Close contact can only be made with two A groups and one B group in the other layer. A choline diameter of 6.42 Å gives a distance between choline groups along d of $A-B' = 4.85$ Å.

For phase 6 with a water content of $r=2$, a value of $A-B' = 5.9$ Å was estimated from Fig. 1 of Ref. 5 and the preceding discussion. This gives $A'-B' = 4.48$ Å for $r=1$ which supports the conclusion that close packing of the choline groups is possible with one water molecule present. A single water molecule is tightly bound to a choline group and can be located between this group and the phosphate oxygen atons of the next layer without appreciable separation of the layers.

The rectangular packing of the choline groups, as in Fig. 3 (left) will be stablized by a water molecule. In the absence of water, the choline groups can form a square array, as shown in Fig. 2 (right) for phase 4.

The structure proposed for the polar layer of phase 6 is therefore consistent with the diffraction data and the transition to phase 4. This study has also established the relation of phase 6 to other phases and the conditions required for the formation of this phase. In addition to the strong spacings of 4.2 Å from the chains and of 57.46 Å from the bilayer, weaker reflections at 4.07 Å and 4.93 Å are observed for phase 6 (Table II, part I). Diffraction at 4.85 Å is absent for the hexagonal chain subcell and these reflections are consistent with the proposed polar structure.

This analysis is based on the packing of the groups in the molecule which can be satisfied by conformations close to those found in crystals. Tilt of the polar group and change in relative position of the phosphate group and carbon atom C_2 of the glyceride involve rotations of th C–C–O bonds of the choline and the $O-C_3-C_2$ bonds of the glycerol.[5,7] The chains and the diglyceride group in phases 2 and 6 are nearly perpendicular to the bilayer plane with carboxy-ester group 2 parallel to it. As the C_3-C_2 bond is inclined to the bilayer plane, rotation about this bond will alter the distance between the phosphate group and the carboxy-ester group.

A complete structure for phase 6 involves both the polar and chain regions and requires more than one molecular conformation because of the difference in coordination number in these regions for the bylayer planes. The chain packing in phase 6 is in the hexagonal subcell with two chains for each molecule as shown in Fig. 5, left. This requires the glycerol C_2 atoms to be 4.85 and 8.4 Å apart. The most stable packing of the charged groups has a larger area but can be compressed for samples with lower water content($n<4$). This gives the rectangular cell with phosphate groups also about 8.4 and 9.2 Å apart which correspond with the C2 positions along alternate rows of molecules but not in the other rows. At least two molecular conformations are required as shown in Fig. 5 (right). These are similar to those in phase 2 and are the simplest way of relating the polar and chain regions in phospholipid bilayers.

When the water content is higher, the charged groups in each bilayer tend to become coplanar and the area per molecule increases rapidly with water content. The structures formed will be discussed in part III.

PHASES FORMED BY HEATING THE DIHYDRATE

As described in the experimental section of part I, on heating phase 2, there are a series of transitions as shown in Fig. 1(b), part I. For heating in a closed con-

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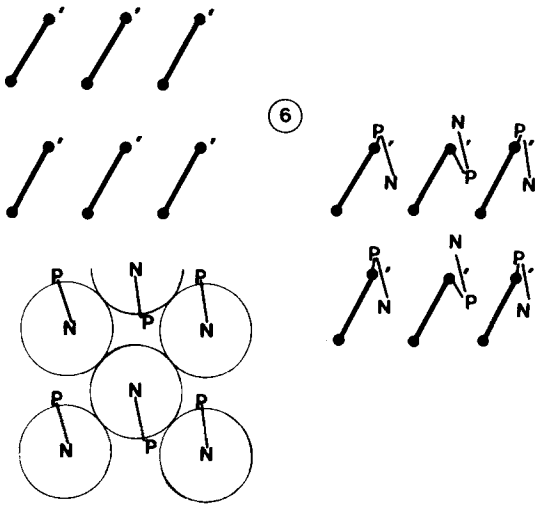

FIG. 5. Effect of chain and polar packing on molecular conformations of phospholipids. (Left) (top): Chain packing in DPPC phase 6. Same symbols as in Fig. 1. Carbon atom 2 of glycerol indicated by prime. Left (bottom): Polar packing in phase 6, choline groups shown as large circles packed in rectangular lattice. Right: Combination of chain and polar packing requires at least two different molecular conformations. The structure is not known precisely. In the illustration, planes parallel to the bilayer are shown.

tainer, which maintains the overall water content, the phases formed have been numbered 6, 7, 8, and 9 and are further characterized in Table II and Diagrams 3 and 4 of part I. Phases 6 and 8 resemble the $L\beta$ and $L\delta$ phases previously described.[13] For the dihydrate, phase 7 is only made visible by the doubling of the phase

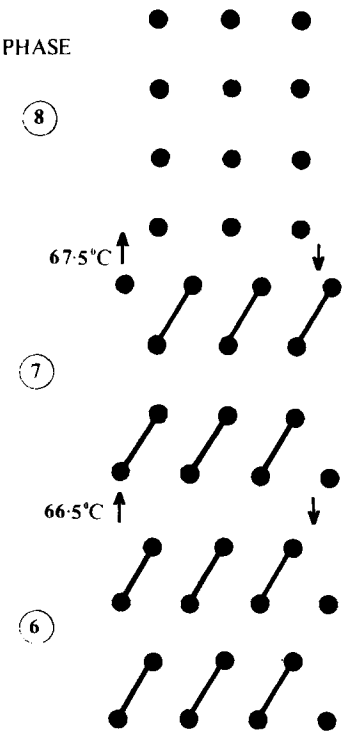

FIG. 6. Chain packing in planes perpendicular to the chain axes (bottom) phase 6; middle phase 7 and (top) phase 8. From x-ray data for 6 and 8, thermal data, 7. Symbols as in Fig. 1.

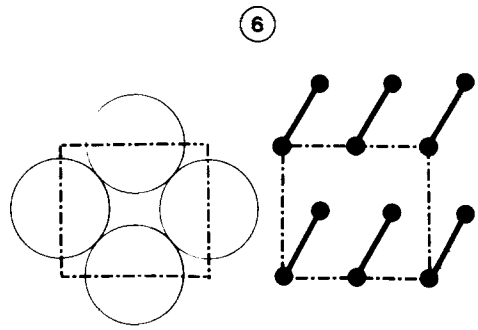

FIG. 7. Proposed packing of polar groups (left) and chains (right) in phase 6. A cell with two molecules per layer is shown, the relation between polar and chain layers is now known precisely. Area per molecule = 40.8 Å^2.

transition, but other experiments showed that this phase is more stable at lower water contents. In this section, the structures of phases 6, 7, and 8 will be discussed.

In phase 6, the chains are packed in the hexagonal subcell with their axes 4.85 Å apart and perpendicular to the bilayer plane, this is the same as in the DPG $L\beta$ phase.

The diffraction data for phase 8 indicates a square packing of chains 4.8 Å apart for which a helical configuration was proposed.[13] There appears to be no evidence that the chain conformations differ greatly in phases 6 and 8. The properties of all DPPC phases with ordered chains discussed here, can be derived without change in the overall shape in the diglyceride group as discussed in part I. An increase in the stability of phases with hexagonal subcells for glycerides. and DPPC can be attributed to the carboxy-ester groups. One of these groups(C_2–O–) is parallel to the bilayer plane and holds the chains attached to it about 4.8 Å apart, as in DPPC phases 6 and 8. Both carboxy–oxygen atoms can be packed between chain cylinders in phase 6.

The packing of the chains in phase 6 is shown in Fig. 6 (left) together with a packing for the diglyceride groups As the molecules have one dimension twice the other in the chain region; a distinct tendency towards parallel stacking, as shown, is probable. The shape and polarity of the carboxy-ester group also favors this arrangement.

In phase 6, each chain has six neighbors, which is reduced to five in phase 7 and to four in phase 8, giving the correct ratios for the enthalpies. The packing in phases 6 and 7 will be stabilized by the carboxy-ester groups and stacking of the diglyceride.

Having established the general features of the chain packings, the molecular packing of the polar groups in phases 6, 7, and 8 can be investigated. For phase 6, this was shown to involve a rectangular array of the choline groups, the relation between the polar and the chain regions being shown in Fig. 5 and 7.

For phase 7, area per molecule of 43.2 Å in the chain region corresponds with a square packing of choline groups with a diameter of 6.57 Å. Thus, the polar sub-

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cell is the same as in phase 4 but the chain structure is different. From the polar and chain subcells, a larger molecular cell as shown in Fig. 8 is obtained. The structure is consistent with an increased stability at lower water content and a significant effect due to stacking of the diglyceride groups.

The spacing of 4.5 Å observed for phase 8 corresponds with a polar cell, as in phase 1, with an axis of 9.0 Å. This suggests that the polar structures are similar and involve a stable coplanar array of charges. As shown in Fig. 9, this gives a cell with 3×9.0 Å polar subcells and 4×6.75 Å for the chains so that $b=27$ Å. Along the other axis, 2×10.8 Å $=3\times7.2$ Å involving a chain tilt of 20°. Thus, a complex two-dimensional cell similar to phases 7 and 4 is obtained, with 12 molecules. A disorder of the orientation of carboxy-ester groups C_2–O– and diglyceride is necessary but the chains are ordered despite the large volume per molecule and absence of hexagonal close packing.

The packing can be confirmed by a calculation involving the bilayer repeat spacing of 54 Å and the molecule volume. At 20 °C the molecular volume of phase 6 is 1.117 Å^3 at 20 °C. For DPG phase LB at 49 °C with the same subcell dimensions as DPPC phase 6 at 20 °C, the volume is 966 Å^3. This gives a volume of 205 Å^3 for the phosphorylcholine group. After allowing for the atoms common to both molecules and expansion of the diglyceride region to the volume indicated by the subcell of phase 8, a volume for the DPG groups in phase 8 of 1.085 Å^3 is obtained. This is an increase of 119 Å^3. The volume change in the phosphoryl group will be small and may be negative for effective packing, giving a volume per molecule of 1.290 Å^3 for phase 8. Dividing by $d/2$ gives an area per molecule of 47.8 Å^2, in good agreement.

The structures proposed are completely consistent with the observed x-ray spacings and do not require the assumption that a mixture of phases forms on heating. Whilst there is at present no diffraction data to support the analysis of the structure of phase 7, only small variations are required between the polar structures of phases 6, 7, and 8 and the chain packings are supported by x-ray and thermal data.

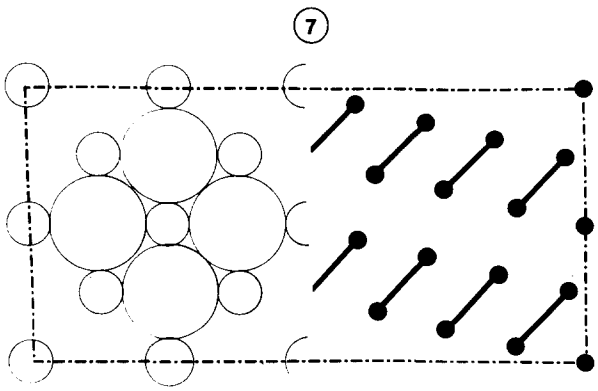

FIG. 8. Proposed packing of polar layer (left) and chains in phase 7. A square two-dimensional subcell with $a=b=6.57$ Å and an area per molecule of 43.2 Å^2 is shown. The smallest possible unit cell has $a=18.6$ Å, $b=37.2$ Å and contains 16 molecules.

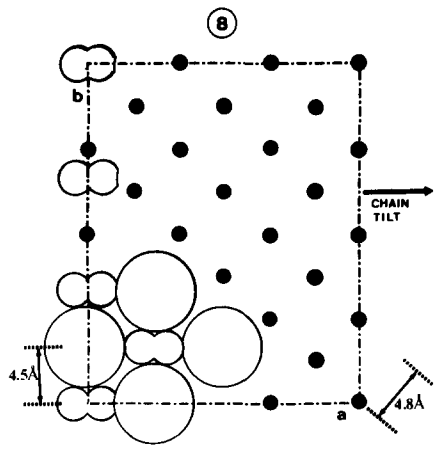

FIG. 9. Proposed packing of polar layer in phase 8 (bottom left), this gives a diffraction spacing of 4.5 Å, as observed (Table II, Part I). The chain packing proposed gives a diffraction spacing of 4.8 Å also observed. For this proposed structure, the area per molecule is 48 Å^2, giving a bilayer spacing of 54 Å with a chain tilt of 20°. The polar subcell is similar to that in crystalline phase 1. Smallest possible cell has 12 molecules.

DISCUSSION

The double transition is an interesting feature which has been observed for other DPPC phases and other Lecithins. For the transitions from phase 6 to phase 8, the conclusion given here is that an intermediate structure is stabilized by small forces between the diglyceride groups : A similar analysis may apply to other examples.

From the diffraction data, the volume expansion between phases 6 and 8 is 10% which compares with 11% for the melting of hydrocarbons and 7% for the transition from DPPC phase 10 to phase 12. The chains in the square subcell occupy the same volume as in liquid hydrocarbons, as noted by Vand.[14] Since the forces between the chains are weak, the structure of phase 8 is stablized by the double layered polar group packing over a large temperature range. Addition of water will allow greater motion of the polar groups as the layers seperate and a transition to phase 12 with a very small volume change will occur.

The space between the chains in the square packing will accommodate sodium or calcium ions but not potassium which may be significant with unusual properties. Removal of water or oxygen atoms around the ion is necessary.

In part III of this article, the structures of phases with increased water content are discussed and are shown to be closely related to the phases which have been described here.

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